Is the Response of Serum Lipids and Lipoproteins to Postmenopausal Hormone Replacement Therapy Modified by ApoE Genotype?

Anna-Mari Heikkinen, Leo Niskanen, Markku Ryyränen, Marja H. Komulainen, Marjo T. Tuppurainen, Markku Parviainen, Seppo Saarikoski

Abstract—Postmenopausal hormone replacement therapy (HRT) has favorable effects on the serum lipid profile, and it also decreases the risk of cardiovascular diseases. The apolipoprotein E genotype has influence on serum levels of lipids and lipoproteins; apoE allele e4 (apoE4) is associated with high total and LDL cholesterol levels. Genotype also influences the lipid responses to treatment with diet and statins, but the effect of HRT in different apoE genotypes is unknown. We studied the effects of HRT on the concentrations of serum lipids in apoE4-positive early postmenopausal women (genotypes 3/4 and 4/4) compared with apoE4-negative women (genotypes 2/3 and 3/3) in a population-based, prospective 5-year study. In all, 232 early postmenopausal women were randomized into 2 treatment groups: an HRT group (n = 116), which received a sequential combination of 2 mg estradiol valerate (E2 Val) from day 1 to 21 and 1 mg cyproterone acetate (CPA) from day 12 to 21 (Climen), and a placebo group (n = 116), which received 500 mg/d calcium lactate. Serum concentrations of total, LDL, and HDL cholesterol and triglycerides were measured at baseline and after 2 and 5 years of treatment. A total of 154 women completed the final analysis. During the follow-up period, serum total cholesterol and LDL cholesterol concentrations decreased in the HRT group in apoE4-negative women (8.1% and 17.1%, respectively; P < 0.001) but did not change in the HRT group in apoE4-positive women or in the placebo group. Serum HDL cholesterol concentrations decreased in the placebo group (apoE4-negative, 3.9%, P = 0.015; apoE4-positive, 8.1%, P = 0.004) but did not change significantly in the HRT group. Serum triglyceride levels tended to increase in both study groups and genotypes (15.1% to 36.2%, P < 0.038 to 0.001), but no differences were observed between the study groups or genotypes, respectively. Our finding was that in postmenopausal Finnish women LDL cholesterol levels in apoE4-negative subjects respond more favorably to HRT than those in apoE4-positive subjects. This finding has potential importance in postmenopausal women with hypercholesterolemia, if confirmed in other studies. (Arterioscler Thromb Vasc Biol. 1999;19:402-407.)

Key Words: ApoE genotype n postmenopausal hormone replacement therapy n LDL cholesterol n prospective study

A polipoprotein E (apoE) is a liver polypeptide that has an important role in lipid metabolism by serving as a ligand for the LDL receptor. In humans, there are 3 alleles of apoE (e2, e3, and e4) and hence 6 different genotypes (2/2, 2/3, 2/4, 3/3, 3/4, and 4/4). ApoE genotype distribution, in particular that of the apoE allele e4 (apoE4), is associated with total and LDL cholesterol levels and also with cardiovascular morbidity. The frequencies of apoE genotypes vary in different age, sex, and race groups. ApoE polymorphism is estimated to explain 4% to 15% of the variation in LDL cholesterol concentrations. In postmenopausal women, this variation has been reported to be greater than in premenopausal women. The response to cholesterol-lowering diet and statins has been shown to differ in subjects with different apoE genotypes. It is well known that postmenopausal estrogen therapy changes serum lipoprotein concentrations favorably, which may explain about 25% to 50% of the cardioprotective effect of estrogen, but the association between apoE genotype and lipoprotein responses to postmenopausal HRT is not known.

The aim of this placebo-controlled, prospective 5-year trial was to investigate the response of HRT (sequential combination of 2 mg estradiol valerate [E2 Val] and 1 mg cyproterone acetate [CPA]) with respect to serum lipids in apoE genotypes 3/4 and 4/4 (apoE4-positive subjects) compared with genotypes 2/3 and 3/3 (apoE4-negative subjects) in a population-based, randomized group of early postmenopausal women.

Materials and Methods

Subjects
The population of the present study is a subgroup of the Kuopio Osteoporosis Risk Factor and Prevention Study. In 1989 a postal inquiry was sent to all 14 200 47- to 56-year-old women in Kuopio...
Province, Eastern Finland, to investigate osteoporosis risk factors among perimenopausal women. \(^{14}\) The 464 voluntary postmeno-

Pausal women who had their last menstrual period within 6 to 24 months before the study were included in the clinical osteoporosis prevention trial. Exclusion criteria were restricted to general contra-

indicators for HRT, including history of estrogen-dependent cancer, thromboembolic diseases, and medication-resistant hypertension. The participants were randomized by a computer to 4 treatment groups: E\(_2\) Val/CPA group, vitamin D\(_3\) group, E\(_2\) Val/CPA + vitamin D\(_3\) group, and calcium lactate group (placebo). Random allocation to study groups was carried out by blocks using a computer, the block size being 4, 8, or 12. The study was not blinded as to HRT. The data analyses were done blind. Those women who wanted to change treatment groups were excluded from the analysis. The personnel involved were unaware of the group allocations. The study design, and in particular the adverse effect of vitamin D on the serum lipid profile, has been described elsewhere in more detail. \(^{15}\) Therefore, for this study, only the women using HRT or placebo without vitamin D\(_3\) were included: (1) HRT group (n = 116): E\(_2\) Val (2 mg) on cycle days 1 to 21, combined with CPA (1 mg; Climen, Schering AG) on cycle days 12 to 21, with a treatment-free interval on cycle days 22 to 28; and (2) placebo group (n = 116): calcium lactate (Rohto Ltd), 500 mg/d, equivalent to 93 mg Ca\(^{2+}\)/d. Study design and formation of the present study population are depicted by the flow diagram (Figure 1).

Written informed consent was obtained from the participants, and the study design was approved by the ethics committee of Kuopio University Hospital. The daily calcium intake was calculated as the intake in grams. Each subject visited the outpatient clinic once a year. Fasting blood samples were taken in the morning for determi-

nations in total cholesterol, HDL cholesterol, and triglyceride analyses within the series have been 1.5%, 4%, and 2%, respectively, at the levels in question using fully automated methods for total cholesterol and triglycerides and semi-

automated measurement of HDL cholesterol. The long-term varia-

tions in total cholesterol, HDL cholesterol, and triglyceride analyses were followed by assay of quality control fresh samples from Labquality Ltd, and the between-series variations have been below 3%, 7%, and 4.5%, respectively. No drift in the analyte levels was found during the present study.

The apoE genotype was determined from blood leukocytes. DNA was extracted by standard phenol/chloroform extraction. \(^{19}\) ApoE genotypes were analyzed by using the polymerase chain reaction

### Table 1. Distribution of ApoE Genotypes and Gene Frequencies in 2 Different Treatment Groups

<table>
<thead>
<tr>
<th>Allele/Genotype</th>
<th>HRT (n=61)</th>
<th>Placebo (n=98)</th>
<th>All (n=159)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene frequency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e2</td>
<td>0.041</td>
<td>0.046</td>
<td>0.044</td>
</tr>
<tr>
<td>e3</td>
<td>0.779</td>
<td>0.781</td>
<td>0.780</td>
</tr>
<tr>
<td>e4</td>
<td>0.181</td>
<td>0.173</td>
<td>0.179</td>
</tr>
<tr>
<td>Genotype, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2/3</td>
<td>1 (1.6)</td>
<td>8 (8.2)</td>
<td>9 (5.7)</td>
</tr>
<tr>
<td>E2/4</td>
<td>4 (6.6)</td>
<td>1 (1.0)</td>
<td>5 (3.1)</td>
</tr>
<tr>
<td>E3/3</td>
<td>40 (65.6)</td>
<td>57 (58.2)</td>
<td>97 (61.0)</td>
</tr>
<tr>
<td>E3/4</td>
<td>14 (23.0)</td>
<td>31 (31.6)</td>
<td>46 (28.9)</td>
</tr>
<tr>
<td>E4/4</td>
<td>2 (3.3)</td>
<td>1 (1.0)</td>
<td>3 (1.9)</td>
</tr>
<tr>
<td>E2/3 or E3/3</td>
<td>41 (67.2)</td>
<td>65 (66.4)</td>
<td>107 (67.3)</td>
</tr>
<tr>
<td>E3/4 or E4/4</td>
<td>16 (26.2)</td>
<td>32 (32.6)</td>
<td>48 (30.2)</td>
</tr>
</tbody>
</table>
TABLE 2. Baseline Characteristics on 154 Postmenopausal Women According to Treatment Groups and ApoE Genotypes

<table>
<thead>
<tr>
<th>HRT, ApoE</th>
<th>HRT, ApoE</th>
<th>Placebo, ApoE</th>
<th>Placebo, ApoE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/3, 3/3 (n=41)</td>
<td>3/4, 4/4 (n=16)</td>
<td>2/3, 3/3 (n=65)</td>
<td>3/4, 4/4 (n=32)</td>
</tr>
<tr>
<td>Age, y</td>
<td>52.5±0.3</td>
<td>52.1±0.6</td>
<td>52.4±0.3</td>
</tr>
<tr>
<td>Time since menopause, y</td>
<td>1.1±0.08</td>
<td>1.00±0.16</td>
<td>1.12±0.06</td>
</tr>
<tr>
<td>Previous HRT use, y</td>
<td>0.67±0.30</td>
<td>0.28±0.14</td>
<td>0.32±0.09</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.2±0.6</td>
<td>27.2±1.0</td>
<td>26.0±0.5</td>
</tr>
<tr>
<td>Dietary Ca-intake, mg/d</td>
<td>726±48</td>
<td>855±140</td>
<td>848±55</td>
</tr>
<tr>
<td>Smoking, pack-years†</td>
<td>8.38±1.39</td>
<td>13.1±1.87</td>
<td>8.01±1.92</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>17.1</td>
<td>12.5</td>
<td>21.5</td>
</tr>
<tr>
<td>Physically active persons, %‡</td>
<td>34.1</td>
<td>18.7</td>
<td>27.7</td>
</tr>
<tr>
<td>Alcohol, absolute ethanol g/week</td>
<td>18.3±4.9</td>
<td>26.2±14.6</td>
<td>20.6±4.5</td>
</tr>
<tr>
<td>FSH, IU/L</td>
<td>65.7±4.5</td>
<td>47.8±6.3</td>
<td>62.8±3.3</td>
</tr>
<tr>
<td>E₂, nmol/L</td>
<td>0.14±0.02</td>
<td>0.18±0.04</td>
<td>0.13±0.02</td>
</tr>
</tbody>
</table>

*Values are the mean±SEM by Kruskal-Wallis test, (in number of smokers and physically active persons, χ² test); no statistically significant differences between the groups.
†Smoking is the life-time number of cigarettes/20×365.
‡Physically active person is 3 or more hours of physical activity/week.

Results

Frequencies of apoE alleles and genotypes are outlined according to the treatment group in Table 1. The distribution of alleles was similar to that reported earlier in Finnish and Swedish populations but with a higher frequency of apoE4 compared with other white populations. There were no statistically significant differences between the study groups in the baseline characteristics or the laboratory parameters (Table 2).

The relative body weight (difference between follow-up and baseline, divided by baseline level, as a percentage) increased similarly in all 4 treatment–genotype groups after 5 years (HRT–apoE4-negative group, 4.7±0.8%; HRT–apoE4-positive group, 3.3±2.0%; placebo–apoE4-negative group, 5.5±1.0%; and placebo–apoE4-positive group, 4.3±1.6% (P=0.708). Concentrations of serum E₂ increased in the HRT group but did not change significantly in the placebo group. The 5-year concentrations in serum E₂ were identical between apoE4-negative and apoE4-positive subjects (HRT group, 0.19±0.02 and 0.23±0.06 nmol/L, respectively, P=0.507; placebo group, 0.04±0.02 and 0.04±0.02 nmol/L, respectively, P=0.861).

The concentrations of serum total cholesterol decreased in the HRT group by 5.1% after 2 years (P=0.014) and by 8.1% after 5 years (P<0.001) in apoE4-negative subjects, but they did not decrease significantly in apoE4-positive subjects. In the placebo group, the concentrations of serum total cholesterol did not change in apoE4-negative subjects, whereas they had increased by 3.9% after 2 years in apoE4-positive subjects (P=0.015), but they had returned to the baseline level after 5 years. The changes between the HRT and the placebo groups were statistically significant (MANOVA, P<0.001) (Table 3, Figure 2).

LDL cholesterol concentrations had decreased in the HRT group by 10.3% after 2 years (P<0.001) and by 17.1% after 5 years in apoE4-negative subjects (P<0.001). There was a slight but nonsignificant tendency for decreases in LDL cholesterol concentrations in the HRT group in apoE4-positive subjects and in the placebo group in apoE4-negative subjects, whereas LDL cholesterol concentrations had increased by 5.7% (P=0.010) in the placebo group in apoE4-positive subjects after 2 years but had returned to the baseline level after 5 years. The time-related changes between the treatment groups were statistically significant (MANOVA, P<0.001) (Table 3, Figure 2).

Furthermore, in the multivariate model (analysis of covariance), the LDL cholesterol levels at 5-year examination were associated with apoE genotype, taking into account the effects of age, body mass index, smoking, and interaction between HRT and apoE4 allele (Table 4).
The concentrations of serum HDL cholesterol had increased by 4.3% \((P=0.042)\) and 3.1% \((P=\text{ns})\) after 2 and 5 years, respectively, in the HRT group in apoE4-negative subjects, but no significant changes in apoE4-positive subjects were observed. In the placebo group, serum HDL cholesterol concentrations had decreased after 5 years in apoE4-negative subjects by 3.9% \((P=0.015)\) and in apoE4-positive subjects by 8.1% \((P=0.004)\). However, no significant changes between the treatment groups or genotypes were observed (Table 3, Figure 2).

Serum triglyceride levels increased in all subjects irrespective of the genotype during the follow-up period. After 5 years, the increase was 36.2% \((P<0.001)\) in the apoE4-negative HRT group, 26.7% \((P=0.030)\) in the apoE4-positive HRT group, 15.1% \((P<0.001)\) in the apoE4-negative placebo group, and 17.2% \((P=0.003)\) in the apoE4-positive placebo group. No statistically significant differences between the treatment groups or genotypes were observed (Table 3, Figure 2).

In this study we observed a reduction in LDL cholesterol of 0.4 mmol/L between those with apoE4 allele and those without it in subjects receiving HRT. When we set the \(\alpha\) (probability excluding type 1 error) to 5% and \(\beta\) (probability of type 2 error) to 20% with SD of 0.4 mmol/L, the required sample size will be 16.7 subjects per group. In the HRT group there were 16 subjects with apoE4 allele and 41 without it; therefore, the sample size here is adequate to show these changes, given the changes are so prominent.

The lipid and lipoprotein concentrations were analyzed yearly. The results of 1-, 3-, and 4-year examinations were in line with the reported 2- and 5-year results (data not shown).

### Discussion

The new finding in the present study was that the beneficial response of total and, especially, LDL cholesterol to HRT in

### Table 3: Concentrations of Serum Lipids and Lipoproteins on 154 Postmenopausal Women According to Treatment Groups and ApoE Genotypes*

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>0.415</td>
<td>0.524</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>2.985</td>
<td>0.094</td>
</tr>
<tr>
<td>ApoE4 allele</td>
<td>1.468</td>
<td>0.047</td>
</tr>
<tr>
<td>Current smoking</td>
<td>0.235</td>
<td>0.631</td>
</tr>
<tr>
<td>Interaction for HRT and apoE4</td>
<td>4.298</td>
<td>0.290</td>
</tr>
</tbody>
</table>
postmenopausal women was related to the apoE genotype. Additionally, our results confirm the persistence of truly long-term effects of sequential E2 (2 mg) and CPA (1 mg) treatment on serum total and LDL cholesterol. This study was placebo-controlled, population-based, successfully randomized, and well matched regarding baseline characteristics and weight changes during the follow-up period, which all strengthen our findings.

It is well established that postmenopausal estrogen therapy has favorable effects on serum lipoprotein concentrations. However, a wide variation in the lipid responses has been observed in previous reports, which have been attributed either to the effect of the added progestin, and by larger responses in hypercholesterolemic subjects. It has also been suggested that positive lipid effects of HRT may diminish with the duration of treatment.

However, there are no studies in which the impact of genetic factors on lipid responses to HRT have been investigated. It is fairly well established that apoE allele 2 is associated with lower and allele 4 with higher serum total and LDL cholesterol concentrations compared with those associated with allele 3. Furthermore, there is evidence that hormonal status modulates the lipoprotein variation related to apoE genotype. In postmenopausal women, the association between apoE phenotype and LDL cholesterol levels has been stronger than in premenopausal women or in men. The relatively high serum total cholesterol levels in subjects with apoE4 have been suggested to respond more favorably to a cholesterol-reducing diet, as these subjects show enhanced absorption of dietary cholesterol, especially in populations consuming diets rich in saturated fat, although this is not confirmed in all studies. Our findings are unlikely to be caused by different dietary habits between the HRT and placebo groups. During the 5-year follow-up period, LDL cholesterol levels changed relatively little in the placebo group, although menopause should result in an increase of about 10%. This most likely reflects the decreased intake of cholesterol and saturated fat during these years in the Finnish population.

The finding that apoE polymorphism determines the LDL cholesterol response to HRT has not been previously recognized. In the cross-sectional analysis of the Framingham Offspring Study, no association between apoE genotype and serum LDL cholesterol was found, but the number of postmenopausal women using HRT was small. Although our finding of this genetic determinant of LDL cholesterol to HRT is a new one, there is evidence that apoE polymorphism may influence the responses to statins in familial and nonfamilial hypercholesterolemia; apoE4-positive subjects have more sluggish response than apoE4-negative ones. Taken together, apoE polymorphism seems to be a clinically important determinant of the various lipid-lowering interventions: subjects with apoE4 allele have enhanced response to dietary therapy, whereas the response to treatment with statins and postmenopausal HRT is impaired.

ApoE plays a role in liver lipoprotein metabolism and clearance, and LDL receptor affinity varies according to the apoE isoform. ApoE4 downregulates hepatic LDL receptors, enhances liver lipoprotein uptake, and is associated with increased serum LDL cholesterol concentrations. On the other hand, oral estrogen therapy mediates an upregulation of liver LDL receptors and therefore has a LDL-lowering effect. Hence it is plausible, on the basis of our findings, to suggest that the upregulation of LDL receptors induced by estrogen is impaired in menopausal subjects with the apoE4 allele. The exact mechanisms remain to be demonstrated.

This study was not originally aimed at examining the interaction between genotype and HRT. There were 42 (36.2%) dropouts in the HRT group. Although this number is very low compared with long-term compliance of HRT, the number of apoE4-positive subjects was relatively low in the HRT group in the final analysis. The magnitude of the effect of apoE4 was rather strong, and therefore the sample sizes are adequate considering the homogeneity of the study population and the large number of apoE4-negative subjects in the present study. However, our findings are limited to the Finnish population and need to be confirmed in other studies. To conclude, the apoE genotype modulates the response of serum LDL cholesterol to HRT in postmenopausal Finnish women. This finding may be of potential importance, especially in the treatment of hypercholesterolemic postmenopausal subjects, if confirmed in other studies.

Acknowledgments

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References


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