Estrogen Replacement Therapy, Serum Lipids, and Polymorphism of the Apolipoprotein E Gene

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Background: Pharmacogenomics, the study of genetic loci that modulate drug responsiveness, may help to explain why estrogen replacement therapy (ERT) has differential effects on serum lipid and lipoprotein concentrations in postmenopausal women who inherit distinct alleles of the apolipoprotein E gene (APOE).

Methods: We compared total-cholesterol, triglyceride, and lipoprotein (LDL and HDL) concentrations in 66 postmenopausal women receiving ERT ([+]ERT) with 174 postmenopausal women not receiving ERT ([−]ERT), controlling for three APOE genotypes divided into three groups: E2 (e2/e3, n = 31), E3 (e3/e3, n = 160), and E4 (e3/e4 + e4/e4, n = 49).

Results: Mean total-cholesterol concentrations were lower in all three [+ERT] groups compared with their −ERT counterparts but were statistically significant only for women in group E4 (P = 0.014). The mean LDL-cholesterol concentrations were significantly lower in all three [+ERT] groups compared with their −ERT counterparts (P ≤ 0.005). Although all three groups of [+ERT] women tended to have higher mean HDL-cholesterol concentrations compared with their −ERT counterparts, the differences were not statistically significant. [+ERT] women in groups E2 and E3 had significantly higher (P < 0.05) triglyceride concentrations than their −ERT counterparts. In [+ERT] women, the ratios of total and LDL-cholesterol to HDL-cholesterol that were comparable to group E2 −ERT women.

Conclusions: Triglyceride concentrations in group E2 [+ERT] women may need to be monitored more closely than those in E3 or E4 [+ERT] women. Group E4 women should probably be targeted for ERT. Results suggest that APOE genotypes have a differential effect on serum lipids and lipoproteins in [+ERT] postmenopausal women.

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Coronary heart disease (CHD)5 accounts for 250,000 deaths per year in American women, with most deaths occurring after menopause (1). A major risk factor for CHD in older women is the lack of ovarian estrogen. Clinical trials and epidemiological studies have shown that estrogen replacement therapy (ERT) reduces the incidence of CHD in postmenopausal women (2–5). One of the beneficial effects of ERT has been attributed to known estrogen effects on lowering LDL-cholesterol (LDL-C) and increasing HDL-cholesterol (HDL-C) concentrations (6).

Recognition of apolipoprotein E (apoE) polymorphism as one determinant of serum lipid and lipoprotein concentrations has led to research on the relationship between apoE phenotypes and CHD risk (7). apoE is a 34-kDa protein that functions in the redistribution of lipids among cells of various organs, based on its ability to bind to two lipoprotein receptors, namely the LDL receptor and the non-LDL receptor, sometimes referred to as the remnant receptor or LDL-related receptor protein (LRP) (8). apoE plays central roles in mammalian cholesterol transport by serving as a ligand for the cell surface

5 Nonstandard abbreviations: CHD, coronary heart disease; ERT, estrogen replacement therapy; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; apo, apolipoprotein; LRP, LDL-related receptor protein; HRT, hormone replacement therapy; BMI, body mass index; NMAPS, New Mexico Aging Process Study; NMMLR, New Mexico Medical Reference Laboratory; CNL, Clinical Nutrition Laboratory; LPL, lipoprotein lipase; HTGL, hepatic lipase; and IDL, intermediate-density lipoprotein.
LDL and LRP receptors that mediate the endocytosis of apoB- and apoE-containing lipoproteins. apoE differentially modulates blood lipid concentrations via three different isoforms, apoE2, E3, and E4 (9). Charge and sulfhydryl differences among the isoforms are thought to be responsible for differences in binding affinity for the LRP and LDL receptor in the liver (9). The three APOE alleles, e2, e3, and e4, determine six genotypes: three homozygous, identified as e2/e2, e3/e3, and e4/e4; and three heterozygous, identified as e2/e3, e3/e4, and e2/e4.

Several studies have examined the relationship between apoE isoforms and blood lipids in postmenopausal women not receiving ERT and those receiving ERT. In 1994, Schaefer et al. (10) examined the association of APOE genotypes with plasma lipoproteins in pre- and postmenopausal women in the Framingham Offspring Study who were not receiving any type of hormone replacement therapy (HRT). Schaefer et al. (10) found that total-cholesterol and LDL-C concentrations were 12.7% and 19.7% higher, respectively, in postmenopausal women with an e3/e4 genotype compared with postmenopausal women with an e2/e3 genotype. The fact that premenopausal women had similar total-cholesterol and LDL-C values across all three genotypes and that postmenopausal women had variable increases in their total cholesterol and LDL-C would suggest that estrogens modulate total-cholesterol and LDL-C concentrations, depending on APOE genotype. Somekawa and Wakabayashi (11) were the first to examine the association of apoE polymorphism and lipid profiles in three groups of postmenopausal women based on their genotype, E2 (e2/e2 + e2/e3, n = 14), E3 (e3/e3, n = 170), and E4 (e3/e4 + e4/e4, n = 52). One hundred fifty-two women (age range, 42–60 years) completed the study. These women received HRT (0.625 mg/day conjugated equine estrogen and 2.5 mg/day medroxyprogesterone acetate) continuously for 6 months. Serum lipids and lipoproteins were compared before and after HRT. Total cholesterol and LDL-C, but not HDL-C, were significantly reduced by HRT in all three groups. The LDL-C/HDL-C ratio was calculated to determine the risk of atherosclerosis before and after the initiation of HRT in the three groups. The ratio improved from 2.18 before HRT to 1.58 after HRT in group E2, from 2.26 to 1.92 in group E3, and from 2.57 to 2.10 in group E4 women. Of interest was the finding that even before HRT, the LDL-C/HDL-C ratio in group E2 women was similar to those in group E4 after HRT. Serum triglycerides were minimally affected by HRT in these Japanese women; however, unlike unopposed estrogen therapy, combination therapy tends to blunt the increase in triglycerides, as was the case in this study. Somekawa and Wakabayashi (11) concluded from this study that group E4 women had the highest risk of CHD and that group E2 women had the lowest risk. In addition, they suggested that oral HRT (conjugated equine estrogen and medroxyprogesterone) be recommended for those Japanese women with an APOE e4 allele.

The present study, unlike the clinical trial of Somekawa and Wakabayashi (11), reports observed differences in lipid and lipoprotein concentrations in postmenopausal women receiving ERT by APOE genotype after controlling for age and body mass index (BMI). The women’s personal physicians prescribed unopposed estrogen use or combination therapy (estrogen plus progestin) completely independently of the study protocol. Our data suggest that women who inherit distinct alleles of the APOE gene may require individualized ERT or combination therapy.

Materials and Methods

Subjects

Subjects for this study were from the Albuquerque, NM vicinity and enrolled in the New Mexico Aging Process Study (NMAPS). Participation in the NMAPS is entirely voluntary and limited to noninstitutionalized men and women over 60 years of age who are basically healthy for their age. For example, individuals with diagnosed CHD and insulin-dependent diabetes and those with uncontrolled hypertension were not enrolled in this study. In 1996, the median (±SD) age was 76.2 ± 7.1 years, and all participants were Caucasian, with 3% being of Spanish/Hispanic descent. The data in the present report were obtained from all women who were enrolled in the NMAPS and examined in 1996.

Although a more complete description of the NMAPS population can be found in previous publications (12–14), it is important to point out that this population is not a random sample of elderly living in Albuquerque, NM. This is a select group of elderly individuals who are, for the most part, health-conscious, well-educated, financially secure, and highly motivated participants of this longitudinal study. All inferences from these data must be made with this in mind. Women receiving ERT or combination therapy began their therapy after consulting with their personal physicians and were not influenced to do so by investigators in the NMAPS. None of the women were taking any type of cholesterol-lowering drugs. None of the six women with an APOE e2/e3 genotype receiving ERT were taking progestins. Eleven of the 44 women (25%) with an APOE e3/e3 genotype and receiving ERT were taking progestins, and 4 of the 16 women (25%) with an APOE e3/e4 or e4/e4 genotype were also taking progestins. Fifty-six of the 66 women (85%) were taking Premarin™ (Wyeth-Ayerst), 5 were taking Estratab™ (Solvay Pharmaceuticals), 2 were taking Estrace™ (Bristol-Myers Squibb), and 2 were taking other forms of estrogen. Of the 240 women in this study, 5 had had a hysterectomy, 3 of whom were receiving ERT and 2 were not.

All volunteers were seen as outpatients each year in the Clinical Nutrition Program laboratory at the University of New Mexico Health Sciences Center. Outpatient visits were distributed throughout the year. The Human Research Review Committee of the University of New Mexico approved all aspects of the study.
Mexico School of Medicine approved the study. Informed consent was obtained from each participant.

LABORATORY MEASUREMENTS

Blood chemistries. After an overnight fast, −50 mL of blood was obtained from each person between 0800 and 0930 for various biochemical measurements. Blood was drawn at the annual outpatient visit. Analytical measurements were performed in two laboratories: the New Mexico Medical Reference Laboratory (NMMRL), located in Albuquerque, NM, and the Clinical Nutrition Laboratory (CNL) at the University of New Mexico School of Medicine. Tests performed by the NMMRL were serum cholesterol and triglycerides. Cholesterol and triglyceride assays were conducted on a Vitros 950 Analyzer, using dry-slide technology (J & J Orthodiagnostic Products). The NMMRL analyzed all blood samples on the day they were drawn. Quality-control samples were included with each batch of test specimens for monitoring accuracy and precision of biochemical tests. Commercial preassayed controls were obtained from the College of American Pathologists. HDL-C assays were performed in the CNL with an ABA-100 Biochromatic Analyzer (Abbott Laboratories), using a Sigma HDL-C kit. A modified heparin-manganese method was used for the precipitation of LDL- and VLDL-cholesterol (15). We calculated the LDL-C concentration by subtracting the HDL-C and VLDL-cholesterol concentrations from the total serum cholesterol concentration. We estimated the VLDL-cholesterol concentration using the formula of Friedewald et al. (16), which assumes that the concentration of VLDL-cholesterol approximates one-fifth of the plasma triglyceride concentration when triglyceride concentrations are <4 g/L (<400 mg/dL). Control samples provided by the CDC Lipid Laboratory, Atlanta, GA, were included to monitor accuracy and precision of the HDL-C assays. Serum estrone assays were conducted in the CNL using an ICN Pharmaceuticals RIA kit. The minimum detection limit of this kit is 1.2 ng/L (1.2 pg/mL), and the interassay CV, as determined by the manufacturer, is 10.2% at an estrone concentration of 90 ng/L (90 pg/mL). We measured serum estrone because some women, when questioned about their use of exogenous estrogens, equate ERT with occasional use of preparations such as Estrace or Premarin or using transdermal estrogen patches and vaginal creams, which do not have the same effect as oral estrogens in raising serum estrone concentrations (17).

When women in the NMAPS were asked whether they used exogenous estrogens, 31% (n = 74) responded yes to this inquiry. For those women who answered no to this question (n = 166), the mean (± SE) serum estrone concentration was 8.8 ± 0.47 ng/L (8.8 ± 0.47 pg/mL), with a range of 0.90–29.7 ng/L (0.90–29.7 pg/mL). Thus, we chose an estrone value of >30.0 ng/L (>30.0 pg/mL) as the concentrations required to put women in the ERT category. This reduced the number of women from 74 to 66 whom we felt assured were regular ERT users. All of these 66 women had received ERT for at least 1 year.

apoE isotyping. Restriction fragment length polymorphism analysis of PCR products was used to assess APOE genotypes according to the procedure of Hixson and Vernier (18). This assay was conducted in the CNL.

Statistical analyses. The balanced-gene estimates of the APOE allele frequencies were calculated as follows. For example, APOE e4 (frequency) = APOE e4/e4 + ½(APOE e2/e4 + APOE e3/e4). The summary of descriptive statistics was reported. We applied the ANOVA model with age and BMI adjusted to assess the effect of ERT and genotype as well as their interaction on each lipid fraction. To understand the change in magnitude with each lipid fraction that could be attributed to ERT and genotype, we fit a multiple regression model with predictors including age, BMI, and each combination of ERT and genotype, with the reference group being women with the APOE e3/e3 genotype not receiving ERT. Log e transformations were performed on serum triglyceride and estrone concentrations to achieve normality of distributions. All analyses were performed using SAS (release 6.12).

Results

The number of the various APOE genotypes and the allele frequencies for women (n = 248) examined in the NMAPS are shown in Table 1. There were no significant differences in relative allele frequencies between women not taking exogenous estrogens ([–]ERT) and women receiving ERT ([+]ERT), and the relative frequencies were all in Hardy-Weinberg equilibrium. In addition, allele frequencies were similar to those in other healthy Caucasian

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of subjects</th>
<th>% of total</th>
<th>Allele</th>
<th>All women (n = 248)</th>
<th>[–]ERT women (n = 180)</th>
<th>[+]ERT women (n = 68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>e2/e2</td>
<td>0</td>
<td>0</td>
<td>e2</td>
<td>7.86%</td>
<td>8.61%</td>
<td>5.88%</td>
</tr>
<tr>
<td>e3/e3</td>
<td>160</td>
<td>64.52</td>
<td>e3</td>
<td>79.84%</td>
<td>80.00%</td>
<td>80.15%</td>
</tr>
<tr>
<td>e4/e4</td>
<td>4</td>
<td>1.61</td>
<td>e4</td>
<td>12.30%</td>
<td>11.39%</td>
<td>13.97%</td>
</tr>
<tr>
<td>e2/e3</td>
<td>31</td>
<td>12.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e2/e4</td>
<td>8</td>
<td>3.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e3/e4</td>
<td>45</td>
<td>18.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. APOE genotypes and allele frequencies of NMAPS women.
women (19). In subsequent analyses, the women were divided into three groups. Group E2 included 31 women with an APOE ε2/ε3 genotype, group E3 included 160 women with an APOE ε3/ε3 genotype, and group E4 included 36 women with an APOE ε3/ε4 genotype and 4 women with an APOE ε4/ε4 genotype. Eight women with an APOE ε2/ε4 genotype were not included in subsequent analyses because we chose not to assign these women to either group E2 or E4. Three women with fasting triglyceride concentrations >4.92 mmol/L (400 mg/dL; one in group E4 and two in group E3) were excluded from the LDL-C calculation. Table 2 shows the mean age, BMI, serum lipid concentrations, and estrone type, showed a significant negative association with triglyceride concentrations (β-coefficients = −0.025 ± 0.005, P = 0.0001; and 0.025 ± 0.007, P = 0.0004, respectively). Women receiving ERT, regardless of genotype, showed a significant negative association with LDL-C, P < 0.02. The negative association between ERT

Table 2. Summary of ages, BMI, serum estrone and lipids, and ratios of total cholesterol and LDL-C to HDL-C of women in the NMAPS, according to ERT status: receiving ([+]ERT) or not receiving ([−]ERT) ERT.

<table>
<thead>
<tr>
<th></th>
<th>−[ERT]</th>
<th></th>
<th>[+]ERT</th>
<th></th>
<th>P *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean (SE)</td>
<td>n</td>
<td>Mean (SE)</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>174</td>
<td>77.4 (0.55)</td>
<td>66</td>
<td>73.2 (0.82)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>174</td>
<td>25.1 (0.32)</td>
<td>66</td>
<td>25.8 (0.53)</td>
<td>0.759</td>
</tr>
<tr>
<td>Estrone, ng/L</td>
<td>174</td>
<td>8.8 (0.47)</td>
<td>66</td>
<td>12.4 (11.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>174</td>
<td>5.72 (0.07)</td>
<td>66</td>
<td>5.39 (0.11)</td>
<td>0.0138c</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>171</td>
<td>3.55 (0.06)</td>
<td>66</td>
<td>2.95 (0.10)</td>
<td>0.0001c</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>174</td>
<td>1.46 (0.03)</td>
<td>66</td>
<td>1.59 (0.04)</td>
<td>0.0173c</td>
</tr>
<tr>
<td>Loge triglycerides, mmol/L</td>
<td>174</td>
<td>0.351 (0.035)</td>
<td>66</td>
<td>0.501 (0.057)</td>
<td>0.0283c</td>
</tr>
<tr>
<td>Triglycerides, mmol/Ld</td>
<td>174</td>
<td>1.42 (1.03)</td>
<td>66</td>
<td>1.65 (1.06)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol/HDL-C ratio</td>
<td>174</td>
<td>4.13 (0.08)</td>
<td>66</td>
<td>3.54 (0.14)</td>
<td>0.0003c</td>
</tr>
<tr>
<td>LDL-C/HDL-C ratio</td>
<td>171</td>
<td>2.55 (0.07)</td>
<td>66</td>
<td>1.96 (0.10)</td>
<td>0.0001c</td>
</tr>
</tbody>
</table>

* Represents differences between those receiving and those not receiving ERT.

** To convert mmol/L values for cholesterol to mg/dL divide by 0.02586.

* Adjusted for age and BMI.

* To convert mmol/L values for triglycerides to mg/dL divide by 0.01129.

Table 3. Multiple regression of total cholesterol, LDL-C, and HDL-C and loge triglycerides for each separate APOE genotype, controlling independently for ERT, age, and BMI.*

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Total cholesterol, mmol/L</th>
<th>LDL-C, mmol/L</th>
<th>HDL-C, mmol/L</th>
<th>Loge triglycerides, mmol/L</th>
<th>P *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-Coefficientb</td>
<td>P</td>
<td>β-Coefficient</td>
<td>P</td>
<td>β-Coefficient</td>
</tr>
<tr>
<td>Intercept</td>
<td>7.17 ± 0.81</td>
<td></td>
<td>4.63 ± 0.72</td>
<td></td>
<td>2.16 ± 0.31</td>
</tr>
<tr>
<td>Group E2, [+ERT]</td>
<td>−0.67 ± 0.24</td>
<td>0.075</td>
<td>−1.51 ± 0.33</td>
<td>0.0001</td>
<td>0.37 ± 0.14</td>
</tr>
<tr>
<td>Group E3, [+ERT]</td>
<td>−0.22 ± 0.16</td>
<td>0.172</td>
<td>−0.51 ± 0.14</td>
<td>0.0004</td>
<td>0.10 ± 0.06</td>
</tr>
<tr>
<td>Group E4, [+ERT]</td>
<td>−0.48 ± 0.24</td>
<td>0.048</td>
<td>−0.65 ± 0.22</td>
<td>0.0032</td>
<td>0.11 ± 0.09</td>
</tr>
<tr>
<td>Group E2, [−ERT]</td>
<td>−0.23 ± 0.20</td>
<td>0.247</td>
<td>−0.43 ± 0.17</td>
<td>0.0134</td>
<td>0.15 ± 0.07</td>
</tr>
<tr>
<td>Group E3, [−ERT]</td>
<td>0.20 ± 0.18</td>
<td>0.260</td>
<td>0.13 ± 0.16</td>
<td>0.4134</td>
<td>−0.06 ± 0.07</td>
</tr>
<tr>
<td>Age, years</td>
<td>−0.017 ± 0.008</td>
<td>0.045</td>
<td>−0.031 ± 0.007</td>
<td>0.0796</td>
<td>−0.001 ± 0.003</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>−0.006 ± 0.014</td>
<td>0.663</td>
<td>−0.002 ± 0.012</td>
<td>0.8875</td>
<td>−0.025 ± 0.005</td>
</tr>
</tbody>
</table>

* The reference group included women in group E3 (ε3/ε3) not receiving ERT.

* Estimate ± stranded error of the estimate.
and LDL-C in each of the genotypes paralleled the changes noted for total cholesterol. ERT had variable associations with HDL-C in groups E2, E3, and E4. Of interest was the finding that ERT had significant positive associations with triglyceride concentrations in group E2 (P = 0.0082) and E3 women (P = 0.0067), but not in group E4 women (P = 0.8068), that were independent of age and BMI. We next examined for interactive effects between APOE and ERT on serum lipids and lipoproteins by fitting an ANOVA model, controlling for age and BMI. The only significant interaction was for triglycerides, P = 0.026. This is demonstrated in Fig. 4.

The observed differences between [-]ERT and [+]ERT women for mean total-cholesterol, LDL-C, HDL-C, and triglyceride concentrations within each apoE group, adjusted for age and BMI, are shown in Fig. 1. Women receiving ERT tended to have lower total cholesterol (Fig. 1A) and higher HDL-C (Fig. 1C) than [-]ERT women, but the only statistically significant finding was that group E4 [+]ERT women had significantly lower total cholesterol than group E4 [-]ERT women (P = 0.014). [-]ERT women in groups E3 and E4 had significantly higher LDL-C than [-]ERT women in group E2, P = 0.0079 and 0.0031, respectively (see Fig. 1B). All three groups of [+]ERT women had significantly lower LDL-C than their [-]ERT counterparts (P =0.005). HDL-C concentrations were higher, but not significantly higher, in all three groups of [+]ERT women compared with their [-]ERT counterparts. We also observed that group E2 [-]ERT women had significantly higher mean HDL-C than either group E3 or E4 [-]ERT women, P = 0.0533 and 0.0219, respectively. The observed effect of ERT on triglyceride concentrations is shown in Fig. 1D. Women in groups E2 and E3 receiving ERT had significantly higher triglycerides than group E4 [+]ERT women, P = 0.0489 and 0.0067, respectively. There were no differences in triglyceride concentrations between E4 [-]ERT and E4 [+]ERT women.

A summary of the percentage of difference (+ or −) in the serum lipid fractions in group E2, E3, and E4 women receiving ERT compared with their counterparts not receiving ERT is provided in Fig. 2. Women in group E2 who were receiving ERT showed the greatest decrease in LDL-C (~34%) and also the greatest increase in triglyceride concentrations (~50%).

Ratios of total cholesterol or LDL-C to HDL-C have been proposed as important determinants of CHD relative risk (20, 21). We observed that each group of women receiving ERT had significantly reduced ratios with the exception of total/HDL-cholesterol in group E2 women (Fig. 3).

The correlations between the natural log (log_e) of serum estrone and log_e serum triglyceride concentrations for each of the apoE groups are shown in Fig. 4. One woman in group E2 [triglyceride concentration, 3.61 mmol/L; estrone concentration, 1.1 ng/L (1.1 pg/mL)] and one in group E4 [triglyceride concentration, 7.86 mmol/L; estrone concentration, 2.2 ng/L (2.2 pg/mL)] were determined to be outliers and were not included in the regression analyses. In group E2 women, 31.1% of the variance in triglyceride concentrations could be explained by serum estrone concentrations. When we eliminated 11 group E3 and 4 group E4 women who were receiving combination therapy (estrogen plus progesterone) from the regression analyses shown in Fig. 4, the slope relationships between the three apoE groups remained the same, but slightly lower triglyceride values were measured at a given estrone concentration. For example, at an estrone concentration of 100 ng/L (100 pg/mL; log_e 4.6), triglyceride concentrations were lower in group E3 and E4 women receiving combination therapy compared with those receiving only ERT [0.12 mmol/L (10 mg/dL) and 0.07 mmol/L (6 mg/dL), respectively]. No E2 women were receiving combination therapy. Serum estrone also showed a significant negative correlation with LDL-C in group E2 women (r = −0.50, P <0.05), but not in group E3 women (r = −0.161) or E4 women (r = −0.308; data not shown).

**Discussion**

This study supports the concept that genes or loci can influence response to a given drug. In the present study, the “drug” of interest was ERT in postmenopausal women, primarily conjugated estrogen, Premarin (Wyeth-Ayerst) or estradiol tablets, e.g., Estrace (Mead Johnson). The majority of women (85%) were taking Premarin, and the remainder took nonconjugated estrogen; ERT was verified by measuring serum estrone concentrations. The genetic polymorphism of interest involved alleles of the APOE gene. The response variables were serum lipid fractions, i.e., total, LDL-, and HDL-cholesterol and triglycerides.

Of the three groups of women studied not receiving ERT, group E2 women have the lowest risk for CHD because they have the lowest total cholesterol and LDL-C and the highest HDL-C compared with either group E3 or E4 women (see Fig. 1, B and C). Because ERT produced a significant increase in triglyceride concentrations in group E2 women compared with E2 women not receiving ERT, their triglycerides may need to be monitored more closely than either group E3 or E4 women. As noted in Fig. 2, mean increases in triglyceride concentrations ranged from ~50% for group E2 women to ~25% in group E3 women, with a decrease of ~17% in group E4 women receiving ERT compared with their counterparts not receiving ERT. One limitation of the present study was that only six women in group E2 were receiving ERT; however, four of these six women had hypertriglyceridemia [triglyceride concentrations ≥2.46 mmol/L (200 mg/dL)].

Group E4 women should probably be targeted for ERT because they have the highest total cholesterol and LDL-C and the lowest HDL-C when compared with group E2 or E3 women not receiving ERT. We also observed that group E4 women receiving ERT had lower total choles-
terol, LDL-C, and triglycerides and higher HDL-C than group E4 women not receiving ERT.

Observational data suggest that the total-cholesterol/HDL-C and the LDL-C/HDL-C ratios are better predictors of subsequent CHD than are individual lipid concentrations, especially in the presence of increased triglycerides (20, 21). We examined the ratio of total-cholesterol/HDL-C and LDL-C/HDL-C by APOE genotype and the effects of ERT. The effects noted for the LDL-C/HDL-C ratio between those receiving and not
receiving ERT were greater than noted for the total/HDL-cholesterol ratio (see Fig. 3). These results show that ERT may help group E4 women lower their ratios, either total cholesterol or LDL-C to HDL-C, to concentrations equivalent to those in group E2 women not receiving ERT.

Wakatsuki and Sagara (22) reported that plasma LDL-C concentrations showed a significant negative correlation with plasma estrone concentrations ($r = -0.64$, $P < 0.001$) in 20 premenopausal, 10 postmenopausal, and 10 bilaterally oophorectomized women, but did not examine for any APOE genotypic differences. Our results depended on the various genotypes, e.g., log$_e$ serum estrone concentrations showed a significant negative correlation with LDL-C in group E2 women but not in group E4 women. This information may be useful for group E2 women in that a lower dose of exogenous estrogen intake could minimize any increase in their triglycerides, and at the same time be beneficial in lowering their LDL-C.

This observational study suggests that individualized ERT or combination HRT, based on APOE genotype and dose of estrogen used, may be more beneficial than standard ERT or combination therapy, e.g., 0.625 or 1.25 mg/day conjugated estrogen with or without progestogens. Whether individualized HRT will have an increased cardioprotective effect over standard therapy in women with and without established CHD awaits the outcome of well-designed case-control and cohort studies. It should be noted that Hulley et al. (23) conducted a randomized, blinded, placebo-controlled secondary prevention trial with daily oral conjugated equine estrogen (0.625 mg) plus medroxyprogesterone acetate (2.5 mg) or a placebo in >2700 women with established coronary disease. During a mean follow-up of 4.1 years, HRT did not reduce the overall rate of CHD events in postmenopausal women with established coronary disease.

Our current knowledge of possible mechanisms that help explain the various lipid responses to ERT or combination therapy in women with different APOE genotypes comes from human and animal studies examining the effects of estrogens, progestogens, lipoprotein lipase (LPL), hepatic lipase (HTGL), APOE genotypes, and known interactions between these variables on lipoprotein metabolisms. For example, Walsh et al. (17) conducted a double-blind crossover study in which healthy postmenopausal women were given only conjugated estrogen (Premarin) at two different doses, either 0.625 or
1.25 mg/day. The results, after 3 months of treatment, were given in terms of percentage of change (+ or −) plus 95% confidence intervals. Walsh et al. (17) found that the higher dose of Premarin, 1.25 mg/day, did not have a substantially greater effect than the lower dose of Premarin on lowering total cholesterol or LDL-C or increasing HDL-C. However, Walsh et al. found that Premarin doses of 1.25 mg/day produced a mean increase in the total-triglyceride concentration of 38% (range, 29–47%), which was significantly higher (P < 0.05) than in women taking 0.625 mg/day, who had a mean increase of 24% (range, 15–33%). Our results would suggest that the variation in triglycerides at two levels of Premarin intake noted by Walsh et al. could be explained in part by differences in APOE genotype. We found a strong positive correlation between loge serum estrone and loge triglyceride concentrations in group E2 women (r = 0.56, P < 0.01), whereas there was no association between estrone and triglyceride concentrations in group E4 women (Fig. 4).

The significant increase in triglyceride and decrease in LDL-C concentrations attributable to ERT in group E2 women mimic those in transgenic mice expressing human apoE2 (24). A study by Huang et al. (24) showed that human-E2-induced hypertriglyceridemia in mice could be caused by a pathway unrelated to the current hypothesis that ERT causes increased triglycerides and low LDL-C concentrations because of differences in binding affinity between the apoE isoforms for the hepatic lipoprotein receptor. Huang et al. found that increasing concentrations of apoE2 significantly lowered LDL-C (r = −0.92, P < 0.001) in transgenic female mice lacking LDL receptors by impairing the lipolysis of triglyceride-rich lipoproteins by LPL. Huang et al. showed that apoE2-containing VLDLs were poor substrates for lipolysis and hypothesized that apoE2 could possibly displace or mask apoC-II, which is an absolute cofactor for LPL activity. Huang et al. also noted that all three apoE isoforms inhibit LPL activity to a similar extent; thus, there needs to be an explanation for the increased triglyceride concentrations in women receiving ERT with an APOE e2 allele and not in women with an e4 allele. Several factors could explain this difference. Somekawa and Wakabayashi (25) showed that ERT in postmenopausal women produced significantly higher (P < 0.05) serum apoE concentrations in women with an e2 allele than in women with an e4 allele. This could be the result of reduced clearance of the apoE2 isoform brought about by its lower binding affinity for the hepatic lipoprotein receptors. Although we did not have any homozygous APOE e2 women, our results show that women with an APOE e2 allele receiving ERT have significantly higher triglycerides and lower LDL-C concentrations than women with an APOE e4 allele. These results indicate that, as postulated by Huang et al. (24), the increased lowering of LDL-C in women expressing an APOE e2 vs an APOE e4 allele could involve two different mechanisms: (a) The VLDL → IDL → LDL lipolytic cascade in women with an APOE e2 allele is slowed by increased circulating apoE concentrations. The partial inhibition of LPL activity attributable to ERT may be further enhanced and produces higher triglyceride and lower LDL-C concentrations. (b) The lower binding capacity of apoE2 for the hepatic lipoprotein receptors lowers the transport of cholesterol-rich remnant particles into the liver, causing an up-regulation of hepatic LDL receptors and thereby accelerating the clearance of any LDL-C produced in the VLDL → IDL → LDL lipolytic cascade. In women with an APOE e4 allele, the opposing conditions would prevail, i.e., less inhibition of LPL activity as a result of normal catabolism of apoE and down-regulation of the LDL receptor as a result of increased hepatic uptake of cholesterol remnants containing apoE4 by hepatic lipoprotein receptors. Thus, women receiving ERT who have an APOE e4 allele would tend to have an increased VLDL → IDL → LDL lipolytic cascade, with lower triglyceride and higher LDL-C concentrations than women with an e2 allele.

ERT has also been shown to increase VLDL production (26) and lower LPL and HTGL activity (22). The suppression of HTGL activity by ERT may decrease conversion of intermediate-density lipoprotein (IDL) to LDL and thus lower LDL-C (27). In addition, the decrease in HTGL activity, attributable to ERT, has been shown to increase HDL2-C concentrations in postmenopausal women (28); this could account for the increased HDL-C concentration noted in the present study. Progestogens have been shown to inhibit HTGL activity; however, progestogens

Fig. 4. Linear regression relationship between loge serum triglyceride concentrations and loge serum estrone concentrations for women in groups E2 (e2/e3), E3 (e3/e3), and E4 (e3/e4 + e4/e4).

Line 1, E2/E3 phenotype \( n = 29, r^2 = 0.311 \); line 2, E3/E3 phenotype \( n = 160, r^2 = 0.071 \); line 3, E3/E4 phenotype \( n = 48, r^2 = 0.011 \).
can partially restore LPL activity (29). Thus, combination therapy (estrogen + progestogen) should reduce triglyceride concentrations compared with estrogen-only treated women as noted in the present study.

In studies of populations not selected for health, the presence of an APOE ε2 allele is usually associated with higher triglyceride concentrations compared with the APOE ε4 allele (9). Our findings, and those of Xhignesse et al. (19), show an opposite trend for women not receiving ERT. For example, women in the present study with an APOE ε2 allele had a geometric mean triglyceride concentration of 1.62 mmol/L compared with 1.77 mmol/L in those women with an ε4 allele (Fig. 1D). In contrast to what might be observed in the general population, both the women in the present study and those in the Xhignesse et al. (19) study were selected on the basis of their being healthy and had few cardiovascular risk factors such as obesity and diabetes. Thus, caution should be taken when comparing APOE results between a random sampling and a select group of elderly. For example, the APOE ε4 allele, which promotes premature atherosclerosis, is associated with decreased longevity (30). Thus, the frequency of the APOE ε4 allele would be expected to be different in a random vs an elderly population selected on the basis of good health.

The significant interaction between APOE and ERT in determining triglyceride concentration is demonstrated in Fig. 4. Although there was a trend for interactions between APOE and ERT in affecting total-cholesterol and LDL-C concentrations, the results were not statistically significant. A larger sample would be required to show significant interactions. Further studies are needed to uncover the exact mechanisms responsible for the interactions between APOE polymorphism and ERT or combination therapy in modifying triglyceride and other lipid concentrations. There are other potential regulatory mechanisms that could explain the differences in mean triglyceride and lipoprotein concentrations noted in the women in the present study receiving ERT with different APOE genotypes.

There are several issues that need to be considered in assessing these results. First, the present study was not a randomized trial of ERT in elderly women, and this should be kept in mind when assessing the results of this study. Second is the limited sample size. As noted previously, there were few women with an APOE ε2 allele (group E2, n = 6) who were receiving ERT. Third, there is no way to determine what the lipid concentrations would be in the [+]ERT women if they were not taking exogenous estrogens. However, the serum lipid concentrations that we observed between women receiving ERT and those not receiving ERT were similar to those reported by Walsh et al. (17) in their carefully controlled study. Fourth, there is evidence that progestogens, in combination with oral estrogens, may prevent the increase in triglyceride concentrations (31). None of the women in the NMAPS with an APOE ε2/ε3 genotype (group E2) used progestogens, whereas 11 of 44 (25%) of APOE ε3/ε3 (group E3) and 4 of 16 (25%) of APOE ε3/ε4 + ε4/ε4 (group E4) women used a combination of oral estrogens and progestogens. Our data indicate that the use of estrogens plus progestogens blunts the rise in triglycerides because women using progestogens had significantly lower mean log e serum triglyceride values than women not using progestogens: 0.351 ± 0.497 (n = 15) and 0.688 ± 0.508 mmol/L (n = 51), respectively (P = 0.027). These log e values, when transformed, equate to geometric mean triglyceride concentrations of 1.42 mmol/L (116 mg/dL) and 1.99 mmol/L (162 mg/dL), respectively. Lastly, population admixture, unknown differences in CHD risk factors, and other environmental factors could undoubtedly explain some of the between-subject lipid variances noted within each APOE genotype.

In summary, we have presented data that support considering APOE genotypes in treating postmenopausal women with ERT to potentially reduce their risk of CHD. Our data indicate that of the three groups not receiving ERT, women with an APOE ε2/ε3 genotype have the lowest risk of CHD. If these women were placed on oral estrogens, then a combination therapy would seem to be warranted to avoid hypertriglyceridermia. The same recommendation would hold for women with an APOE ε3/ε3 genotype. Women with an APOE ε3/ε4 or ε4/ε4 genotype could possibly benefit the most from ERT, and the use of progestogens in these women would not seem to be warranted unless it is deemed necessary to reduce rates of endometrial hyperplasia.

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