Phenotypic Effects of Apolipoprotein Structural Variation on Lipid Profiles

III. Contribution of Apolipoprotein E Phenotype to Prediction of Total Cholesterol, Apolipoprotein B, and Low Density Lipoprotein Cholesterol in the Healthy Women Study

June E. Eichner, Lewis H. Kuller, Robert E. Ferrell, Elaine N. Meilahn, and Mohammad I. Kamboh

The apolipoprotein (apo) E structural locus has been shown to influence concentrations of total cholesterol, apo B, and low density lipoprotein (LDL) cholesterol in population studies. Apo E has six phenotypes resulting from three common alleles at this locus. In the present study, we have typed 473 healthy white women for apo E. At baseline in 1984, all women were premenopausal. To date, 109 of these women have become postmenopausal and are not on hormone therapy. Statistical analyses were done on both pre- and postmenopausal groups to assess the influence of menopausal status in combination with the apo E locus on lipid profile. Nine lipoprotein lipids and apolipoproteins were categorized by three apo E phenotypes: apo E3-2, apo E3-3, and apo E4-3. These were compared in analysis of variance. At baseline, the apo E3-2 phenotype showed the lowest average concentrations of total cholesterol (170 mg/dl), apo B (80 mg/dl), and LDL cholesterol (91 mg/dl), while the apo E4-3 phenotype demonstrated the highest average concentrations of total cholesterol (192 mg/dl), apo B (104 mg/dl), and LDL cholesterol (116 mg/dl) (p<0.0004). Apo E3-3 homozygotes were intermediate on all three quantitative variables. The postmenopausal subset showed the same trends by phenotype, with overall increases in total cholesterol, apo B, LDL cholesterol, and triglycerides, regardless of phenotype. Women who remained premenopausal generally showed smaller increases in these same measures. Our results suggest that, on average, the lower lipoprotein values for the apo E3-2 phenotype are maintained through early menopause despite a worsening of lipid profiles for all women as they age. There did not appear to be a marked effect of apo E phenotype on change in lipoprotein lipids and apolipoproteins that might reflect an interaction between this locus and hormonal status. In addition, the effects of several environmental determinants of total cholesterol, apo B, and LDL cholesterol, along with apo E, were assessed by multivariate regression analysis. (Arteriosclerosis 10:379-385, May/June 1990)

In an effort to understand the role of genetic variation in determining the risk of coronary artery disease (CAD), we are investigating apolipoprotein polymorphisms and their relationship to quantitative lipids and apolipoproteins in a group of healthy middle-aged women. The study, known as the Healthy Women Study, involves a cohort of 541 women who are being followed up for changes in cardiovascular risk factors through the climacteric. Biochemical, as well as anthropometric, psychosocial, and environmental variables are being collected. Evidence for the role of genes in susceptibility to CAD comes from family studies of single gene defects as well as familial aggregation of CAD of unknown etiologies. As genetic defects that predispose to early atherosclerosis are discovered (e.g., receptor defects and apolipoprotein and enzyme deficiencies), more is learned about the metabolic and regulatory roles of apolipoproteins. However, there are still unanswered questions about the pathogenesis of CAD in individuals without early onset.

To address these questions, particularly as they affect women, we are investigating apolipoprotein structural heterogeneity and its effects on lipid and apolipoprotein profiles. We have previously reported our research findings for apolipoprotein (apo) H and A-IV in this population of women.1,2 Here we assess the influence of the apo E structural locus on lipids and apolipoproteins in the entire group of white premenopausal women and in a subset of women who have become postmenopausal.

Apo E has previously been shown to influence total cholesterol, apo B, and low density lipoprotein (LDL) cholesterol concentrations.3 Apo E acts as a high-affinity ligand for the apo E and LDL (B/E) receptors on the liver.4,5,6 As a part of chylomicron remnants, very low...
density lipoprotein (VLDL) remnants, and a subgroup of high density lipoprotein (HDL) cholesterol, apo E functions as a mediator of endocytosis, and thus modulates the metabolism of atherogenic LDL cholesterol particles and may also function in reverse cholesterol transport. Apo E exhibits structural heterogeneity due to single amino acid substitutions. Three alleles (apo E*2, apo E*3, apo E*4) determine six phenotypes. The E2 [arg158->cys] and E4 [cys122->arg] isoforms probably result from point mutations in the most common isoform, E3. The apo E structural locus is located on chromosome 19 in close proximity to the genes for apo C-I and apo C-II. The locus codes for a polypeptide of 299 amino acids with a calculated molecular weight of 34,145. The complete amino acid sequence of apo E has been published.

At the population level, the apo E protein polymorphism has been shown to have a consistent impact on total cholesterol, apo B, and LDL cholesterol concentrations. This influence exists in normolipemic persons as well as in patients with angiographic evidence of CAD. While evidence at both the population and molecular levels exists for the impact of this locus on cholesterol and apo B levels, angiographically documented studies in CAD patients have provided conflicting support for a causative role of apo E in myocardial infarction. Based on a study of patients undergoing coronary angiography versus controls, Menzel et al. proposed that, in the absence of hypercholesterolemia, the apo E*2 allele may provide protection against atherosclerosis.

In the present study, we confirm the effects of the apo E structural locus on lipids in a population of healthy premenopausal women, look at changes occurring with menopause, and assess the relative contributions of the apo E phenotype and several environmental predictors on total cholesterol, apo B, and LDL cholesterol.

Methods

Samples

Serum samples, which had been frozen at -20°C for approximately 1 year, were used for apo E phenotyping. Lipid, lipoprotein, and apolipoprotein determinations were done on blood drawn at a baseline clinic visit occurring between November, 1983 and May, 1985. All samples come from the Healthy Women Study, a prospective study of cardiovascular risk factors in 541 premenopausal women (ages 42 to 50) residing in Pittsburgh, Pennsylvania. A total of 4153 women randomly sampled from women with drivers’ licenses in selected zip codes of Allegheny County, Pennsylvania, were recruited by mail. Of these, 2405 completed a telephone recruitment survey. Approximately 58% of this latter group were excluded from the study because of hysterectomy, amnorrhea, hypertension, or other problems with eligibility. Of women contacted and meeting criteria for inclusion, 63.7% (541) agreed to participate in the study, which involved initial home and clinic visits and follow-up over a 7-year period. Initially, each woman had 60 ml of blood drawn for determination of blood biochemistry profiles. A total of 473 Caucasian subjects were typed for apo E. Women not typed were either of other racial origins or did not have serum available. Blood was also drawn at follow-up visits.

Quantitative Lipid and Apolipoprotein Measures

Participants fasted for at least 12 hours before blood drawing for determination of lipoprotein lipids, apolipoproteins, and triglycerides. Blood was centrifuged, and serum was separated. HDL cholesterol (total HDL cholesterol) was measured by a heparin-manganese precipitation method, and the HDL cholesterol subfractions, HDL2 and HDL3, were separated by using dextran sulfate. HDL2 was computed as the difference between total HDL cholesterol and HDL3. Triglycerides were measured enzymatically. LDL cholesterol was calculated by using the Friedewald formula. Electroimmunoassay was used for determination of apo A-I and apo B concentrations. The intraassay coefficient of variation for apo A-I was 6.4% and for apo B, 10.6%. Apo A-II was measured by an enzyme-linked immunoabsorbant assay. The intraassay coefficient of variation for apo A-II was 10.5%.

Apolipoprotein E Typing

Isoelectric focusing (IEF) followed by immunoblotting was used to detect apo E phenotypes. Serum samples were delipidated by using the guanidine-HCl method proposed by Havekes et al. IEF was performed on 5% (wt/vol) flat bed polyacrylamide gels containing 3 M urea. The gels were prepared as follows: 5.4 g of urea was dissolved in 5 ml of monomer solution (29.1% wt/vol), 5 ml of bisacrylamide solution (0.9% wt/vol), 0.45 ml of Pharmalyte ampholines (pH 4.5 to 5.4), and 0.90 ml of Pharmalyte ampholines (pH 5 to 8), and this was adjusted to a total volume of 30 ml with deionized water. An aliquot of 30 ml of a 0.1% riboflavin solution was added directly to this solution to initiate the polymerization reaction. Each gel was photopolymerized under fluorescent light overnight.

Electrode strips (6 mm x 25 cm) were saturated with 1 M of NaOH (cathode) and 1 M of H3PO4 (anode) and were placed on opposite sides of the gel. Wicks (5 mm x 3 mm made of 3 MM filter paper) were loaded approximately 0.5 cm from the cathode. The gel was run for 3 hours on an LKB 2217 Ultoraphor electrofocusing unit at 10 W, 2000 V, and 250 mA at 10°C. After 1 hour, the wicks were removed, and the electrofocusing continued. At the conclusion of electrofocusing, the proteins were blotted onto a nitrocellulose filter of 0.2 mm pore size by simple diffusion. This transfer was allowed to proceed for 1 hour. The filter was then removed from the gel, washed in Tris-buffered saline (TBS) solution (0.25 M NaCl, 0.03 M Tris-HCl, pH 8.0), and was incubated overnight with 5% (wt/vol) powdered skim milk to block the remaining protein-binding sites.

A double antibody technique was used to visualize the apo E phenotypes. The filter was rinsed in TBS briefly and then exposed to goat-antihuman apo E polyclonal antisera (Daichii Chemical, Tokyo, Japan) diluted 1:500 in TBS buffer for 3 hours. The filter was washed for 45 minutes with three changes of TBS buffer. The second
incubation was with rabbit-antigoat IgG conjugated with
the enzyme alkaline phosphatase and lasted 1 hour
(1:5000 dilution in TBS). Again, the filter was washed for
45 minutes with three changes of TBS buffer.

The filter was stained histochemically by using 25 mg
of β-naphthyl phosphate, 25 mg of Fast Blue BB salt, and
60 mg of magnesium sulfate in 50 ml of stock buffer
(1.8 g NaOH, 3.7 g boric acid/l).

Collection of Other Variables

Other variables [i., age, race, body mass index (BMI),
cigarette smoking, leisure time energy expenditure, and
alcohol consumption] used in the analyses were col-
lected at the baseline examination. BMI, an index of
obesity, was calculated using weight in kilograms divided
by height in meters squared (kg/m²). Cigarette smoking
was measured as cigarettes per day. The Paffenbarger
questionnaire was used to estimate energy expenditure
in leisure time physical activity and was measured as
kilocalories per week. Alcohol intake, in grams per day,
was based on self-reported frequency, amount, and type
of alcoholic beverages normally consumed.

Statistics

Statistical analyses were done with the computer pack-
age, SPSSx. Analysis of variance (ANOVA) with nine
quantitative lipid measures used individually (total cho-
lesterol, total HDL cholesterol, HDL₂, HDL₃, triglycerides,
apo A-I, apo A-II, apo B, and LDL cholesterol) as depend-
ent variables and three apo E phenotypes (apo E 3-2,
apo E 3-3, and apo E 4-3) used as categorical variables
was performed on residuals. Residuals were saved after
use of stepwise regression analysis to adjust quantitative
measures for age and BMI, since both of these attributes
vary concomitantly with lipid levels. Not every quantitative
lipid measure needed to be adjusted for both age and
BMI; thus, adjustments were only done if age and/or BMI
influenced the outcome variable. One quantitative resid-
ual variable, triglycerides, was transformed using natural
logarithms, because this improved the normality of the
distribution. ANOVA was then done on the transformed
triglyceride residuals. The average allelic effects for apo E
were calculated with the formula of Boerwinkle et al. 25

A multivariate linear model was used to determine the
contribution of the apo E locus along with other determi-
nants of lipid levels in premenopausal women. These
latter variables included cigarette smoking, BMI, age,
alcohol consumption, and leisure time energy expendi-
ture. With the exception of apo E and smoking, all
variables were continuous. Three phenotypes of apo E
(apo E 3-2, apo E 3-3, and apo E 4-3) and cigarettes/day
(1 to 20 and 21 or more) were entered as dummy
variables. The remaining three phenotypes had too few
individuals for modeling. Multiple regression equations
were generated for three dependent variables: total cho-
lesterol, apo B, and LDL cholesterol.

ANOVar were also done on a group of postmenopausal
women not using hormones and on change in quantitative
measures based on the difference between pre- and
postmenopausal status. Postmenopausal is defined as at
least 12 months amenorrheic. Change variables for each
subgroup were computed over a 3- to 3.5-year period.

Results

A total of 473 white women were typed for apo E, and
their phenotypes were used in the statistical analyses.
Allele frequencies in this sample were: apo E*2 = 0.059,
apo E*3 = 0.819, and apo E*4 = 0.122 (Table 1). Alleles at
the apo E locus are in Hardy-Weinberg equilibrium. The
distribution of phenotypes is presented in Table 1. These
frequencies are comparable to those obtained on a white
diabetic population from the same geographical area
(Kamboh, unpublished data).

Because lipid and apolipoprotein levels are influenced
by age and BMI, these latter two variables were controlled
for before the analysis of variance was performed. Adjust-
ments in the quantitative variables in premenopausal
women were made as follows: age and BMI accounted
for 3% of the variance in total cholesterol, 28% of the
variance in triglycerides, and 12% of the variance in
apo B. BMI accounted for 15% of the variance in HDL
cholesterol, 16% of the variance in HDL₃, 5% of the
variance in HDL₃, 5% of the variance in apo A-I, and 3% of
the variance in LDL cholesterol. Age explained 2% of the
variance in apo A-II. ANOVA was done on residual values
after adjustments. Because of small cell sizes for three
phenotypes (apo E 2-2 = 2; apo E 4-2 = 5, and apo E
4-4 = 6), these were excluded from the analyses. The
mean adjusted values were obtained by adding the
overall mean to the phenotype residual means, thus
restoring recognizable values. Table 2 presents pheno-
typic means and standard errors for the major three
apo E phenotypes. The mean values of relevant quanti-
tative measures for the remaining three phenotypes are
discussed in the text.

A statistically significant effect of the apo E phenotype
on total cholesterol, apo B, LDL cholesterol, and apo A-II
was observed. Apo E 3-2 heterozygotes had lower aver-
age total cholesterol, apo B, and LDL cholesterol than the
apo E 3-3 homozygotes. Apo E 4-3 heterozygotes had
higher average total cholesterol, apo B, and LDL chole-
sterol than the apo E 3-3 homozygotes. Although there
were only five apo E 4-2 heterozygotes at baseline, their

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Table 1. Distribution of Lipoprotein E Phenotypes
and Allele Frequencies in the Healthy Women Study

<table>
<thead>
<tr>
<th>Apolipoprotein E phenotype</th>
<th>No of women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo E*2</td>
<td>2</td>
</tr>
<tr>
<td>Apo E*3</td>
<td>47</td>
</tr>
<tr>
<td>Apo E*4</td>
<td>5</td>
</tr>
<tr>
<td>Apo E 3-2</td>
<td>315</td>
</tr>
<tr>
<td>Apo E 3-3</td>
<td>98</td>
</tr>
<tr>
<td>Apo E 4-3</td>
<td>6</td>
</tr>
</tbody>
</table>

This study includes American white women only.
average total cholesterol (160.3±10.7 mg/dl) and LDL cholesterol (86.8±7.4 mg/dl) were the lowest of all phenotype
classes. The concentration of apo B (95.7±15.6 mg/dl), on the other hand, was close to the overall average. The apo E 4-4 homozygotes, while only six in number, had the highest average cholesterol (202.9±
12.6 mg/dl) and LDL cholesterol (124.7±9.1 mg/dl) levels among all phenotype classes. This was not true for apo B
correlation (90.6±13.1 mg/dl). There were two individuals with the apo E 2-2 phenotype with an average total
levels of 165.1±2.1 mg/dl, an average apo B of 95.7±15.6 mg/dl, and an average LDL of 96.3±5.0 mg/dl.

In this population, the average effect of the apo E*2 allele was to lower total cholesterol by 16 mg/dl, apo B by
11 mg/dl, and LDL cholesterol by 18 mg/dl. The average effect of the apo E*4 allele was to raise cholesterol by
6 mg/dl, apo B by 8 mg/dl, and LDL cholesterol by 6 mg/dl. At baseline in premenopausal women, after adjusting for age and BMI, the apo E structural locus accounted for 3% of the variation in total cholesterol, 8% of the variation in apo B, and 5% of the variation in LDL cholesterol. The apo E phenotype also appeared to influence concentration of apo A-II, with the apo E 4-3 heterozygotes showing a higher average concentration than the apo E 3-3 homozygotes and 3-2 heterozygotes (Table 2). However, this was no longer true in the
postmenopausal subset.

Multivariate regression analyses demonstrate apo E to be an important predictor of total cholesterol, apo B, and
LDL cholesterol levels in premenopausal women given additional information about risk factors for CAD. Determinants of total cholesterol, apo B, and LDL cholesterol are presented in Table 3 with their beta coefficients and standard errors. Each determinant is statistically significant at p<0.05. Beta coefficients give an idea of the impact of each independent variable on the dependent variable. Cumulative adjusted $r^2$ values give the proportion of variance accounted for by the combined variables (i.e., apo E3-2, apo E4-3, BMI, cigarettes/day, age, and alcohol intake). For total cholesterol, $r^2$=8%; for apo B, $r^2$=22%; and for LDL cholesterol, $r^2$=14%.

Because the Healthy Women Study is a longitudinal study, repeat measures of lipids were done approximately 3.5 years after entry and as women became postmenopausal. Of this group, 109 postmenopausal women who were not on hormone replacement therapy showed the same effects of apo E phenotype on total cholesterol, apo B, and LDL cholesterol (Table 4),

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Table 2. Baseline Phenotype Specific Means and Hypothesis Testing Results for White Premenopausal Women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Apo E 3-2</th>
<th>Apo E 3-3</th>
<th>Apo E 4-3</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol**†</td>
<td>170.4 (3.8)</td>
<td>185.7 (1.8)</td>
<td>192.2 (3.1)</td>
<td>8.02</td>
<td>0.0004</td>
</tr>
<tr>
<td>Total HDL cholesterol†</td>
<td>62.4 (1.4)</td>
<td>59.0 (0.7)</td>
<td>58.5 (1.5)</td>
<td>1.69</td>
<td>0.0865</td>
</tr>
<tr>
<td>HDLc†</td>
<td>22.4 (0.5)</td>
<td>20.9 (0.5)</td>
<td>20.5 (1.0)</td>
<td>0.72</td>
<td>0.4863</td>
</tr>
<tr>
<td>HDLc†</td>
<td>40.0 (0.9)</td>
<td>38.1 (0.3)</td>
<td>38.1 (0.7)</td>
<td>1.90</td>
<td>0.1501</td>
</tr>
<tr>
<td>Triglycerides*†</td>
<td>86.4 (5.5)</td>
<td>83.3 (2.3)</td>
<td>90.6 (4.7)</td>
<td>0.91</td>
<td>0.4045†</td>
</tr>
<tr>
<td>LDL cholesterol†</td>
<td>90.9 (3.6)</td>
<td>110.0 (1.6)</td>
<td>115.6 (2.9)</td>
<td>14.70</td>
<td>0.0000†</td>
</tr>
<tr>
<td>Apo A-I†</td>
<td>147.3 (1.9)</td>
<td>141.7 (1.0)</td>
<td>145.1 (2.0)</td>
<td>2.87</td>
<td>0.0579</td>
</tr>
<tr>
<td>Apo A-II*</td>
<td>52.2 (1.2)</td>
<td>51.5 (0.5)</td>
<td>54.5 (0.9)</td>
<td>4.05</td>
<td>0.0181</td>
</tr>
<tr>
<td>Apo B*†</td>
<td>79.7 (3.0)</td>
<td>93.0 (1.3)</td>
<td>104.2 (2.5)</td>
<td>19.24</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

Values for the phenotypes are given in mg/dl and are the means and standard errors.
Quantitative variables are adjusted for age* and body mass index.† Indicates a log, transformation of residuals for calculation of F statistic.

- HDL=high density lipoprotein,
- LDL=low density lipoprotein,
- Apo=apolipoprotein.

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Table 3. Determinants of Cholesterol, Apolipoprotein B, and Low Density Lipoprotein Cholesterol Levels in Premenopausal Women

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Beta coefficient (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td></td>
</tr>
<tr>
<td>Apo E 3-2</td>
<td>−15.4 (4.8)</td>
</tr>
<tr>
<td>Apo E 4-3</td>
<td>6.7 (3.6)</td>
</tr>
<tr>
<td>Age</td>
<td>2.4 (0.9)</td>
</tr>
<tr>
<td>BMI</td>
<td>0.9 (0.3)</td>
</tr>
<tr>
<td>1–20 cigarettes/day</td>
<td>3.4 (3.8)</td>
</tr>
<tr>
<td>21+ cigarettes/day</td>
<td>17.0 (4.7)</td>
</tr>
<tr>
<td>Apo B</td>
<td></td>
</tr>
<tr>
<td>Apo E 3-2</td>
<td>−13.9 (3.5)</td>
</tr>
<tr>
<td>Apo E 4-3</td>
<td>11.3 (2.6)</td>
</tr>
<tr>
<td>Age</td>
<td>3.2 (0.6)</td>
</tr>
<tr>
<td>BMI</td>
<td>1.5 (0.2)</td>
</tr>
<tr>
<td>1–20 cigarettes/day</td>
<td>7.9 (2.8)</td>
</tr>
<tr>
<td>21+ cigarettes/day</td>
<td>13.3 (3.5)</td>
</tr>
<tr>
<td>Alcohol/day</td>
<td>−0.3 (0.1)</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td></td>
</tr>
<tr>
<td>Apo E 3-2</td>
<td>−20.0 (4.3)</td>
</tr>
<tr>
<td>Apo E 4-3</td>
<td>6.1 (3.2)</td>
</tr>
<tr>
<td>Age</td>
<td>1.6 (0.8)</td>
</tr>
<tr>
<td>BMI</td>
<td>0.9 (0.3)</td>
</tr>
<tr>
<td>1–20 cigarettes/day</td>
<td>7.7 (3.4)</td>
</tr>
<tr>
<td>21+ cigarettes/day</td>
<td>21.4 (4.2)</td>
</tr>
<tr>
<td>Alcohol/day</td>
<td>−0.5 (0.1)</td>
</tr>
</tbody>
</table>

Apo=apolipoprotein, BMI=body mass index, LDL=low density lipoprotein.
Table 4. Phenotype Specific Means and Hypothesis Testing Results in White Postmenopausal Women Not Taking Hormones

<table>
<thead>
<tr>
<th>Variable</th>
<th>Apo E 3-2 (n=12)</th>
<th>Apo E 3-3 (n=79)</th>
<th>Apo E 4-3 (n=18)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol*</td>
<td>189.4 (10.9)</td>
<td>206.3 (3.6)</td>
<td>213.3 (10.2)</td>
<td>1.78</td>
<td>0.1734</td>
</tr>
<tr>
<td>Total HDL cholesterol†</td>
<td>56.2 (2.8)</td>
<td>57.7 (1.6)</td>
<td>56.3 (3.3)</td>
<td>0.12</td>
<td>0.8880</td>
</tr>
<tr>
<td>HDL3*</td>
<td>17.5 (2.7)</td>
<td>18.3 (1.1)</td>
<td>15.4 (2.1)</td>
<td>0.62</td>
<td>0.5380</td>
</tr>
<tr>
<td>HDL4*</td>
<td>39.4 (1.7)</td>
<td>39.3 (0.9)</td>
<td>41.0 (2.2)</td>
<td>0.32</td>
<td>0.7255</td>
</tr>
<tr>
<td>Triglycerides†</td>
<td>100.5 (13.2)</td>
<td>105.7 (7.5)</td>
<td>119.6 (15.5)</td>
<td>0.35</td>
<td>0.70471</td>
</tr>
<tr>
<td>LDL cholesterol†</td>
<td>113.3 (10.1)</td>
<td>127.3 (3.3)</td>
<td>132.6 (9.3)</td>
<td>1.37</td>
<td>0.2579</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>151.8 (5.0)</td>
<td>144.3 (2.5)</td>
<td>148.5 (5.7)</td>
<td>0.77</td>
<td>0.4869</td>
</tr>
<tr>
<td>Apo A-II</td>
<td>49.1 (3.9)</td>
<td>50.8 (1.1)</td>
<td>53.3 (2.8)</td>
<td>0.58</td>
<td>0.5592</td>
</tr>
<tr>
<td>Apo B†</td>
<td>95.5 (7.9)</td>
<td>108.4 (2.7)</td>
<td>113.3 (7.4)</td>
<td>1.81</td>
<td>0.1683</td>
</tr>
</tbody>
</table>

The values for the phenotypes are given in mg/dl and are the means and standard errors. Quantitative variables were adjusted for age* and body mass index.† Indicates a log transformation of residuals for calculation of F statistic.

HDL = high density lipoprotein, LDL = low density lipoprotein, Apo = apolipoprotein.

Table 5. Mean Change in Quantitative Measure by Apolipoprotein E Phenotype in White Women Who Experienced Natural Menopause

<table>
<thead>
<tr>
<th>Variable</th>
<th>Apo E 3-2 (n=12)</th>
<th>Apo E 3-3 (n=79)</th>
<th>Apo E 4-3 (n=18)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>14.2 (4.6)</td>
<td>15.7 (2.6)</td>
<td>15.7 (7.1)</td>
<td>0.02</td>
<td>0.9778</td>
</tr>
<tr>
<td>Total HDL cholesterol</td>
<td>-6.0 (2.8)</td>
<td>-0.7 (0.9)</td>
<td>-3.2 (2.7)</td>
<td>2.17</td>
<td>0.1194</td>
</tr>
<tr>
<td>HDL3†</td>
<td>-6.0 (2.0)</td>
<td>-2.3 (0.8)</td>
<td>-7.2 (2.0)</td>
<td>3.86</td>
<td>0.0242</td>
</tr>
<tr>
<td>HDL4†</td>
<td>0.0 (1.9)</td>
<td>1.7 (1.0)</td>
<td>4.0 (2.0)</td>
<td>0.94</td>
<td>0.3940</td>
</tr>
<tr>
<td>Triglycerides†</td>
<td>15.8 (13.1)</td>
<td>13.3 (5.3)</td>
<td>7.7 (11.5)</td>
<td>0.14</td>
<td>0.8738</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>17.0 (4.6)</td>
<td>13.7 (2.3)</td>
<td>17.4 (6.0)</td>
<td>0.31</td>
<td>0.7366</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>6.0 (4.7)</td>
<td>1.1 (2.7)</td>
<td>-1.1 (7.0)</td>
<td>0.31</td>
<td>0.7343</td>
</tr>
<tr>
<td>Apo A-II</td>
<td>-1.3 (3.8)</td>
<td>-1.5 (1.2)</td>
<td>-1.8 (2.3)</td>
<td>0.01</td>
<td>0.9898</td>
</tr>
<tr>
<td>Apo B</td>
<td>15.9 (6.6)</td>
<td>10.4 (2.8)</td>
<td>-0.7 (3.9)</td>
<td>2.13</td>
<td>0.1244</td>
</tr>
</tbody>
</table>

Values for the phenotypes are given as mg/dl and are means and standard errors. HDL = high density lipoprotein, LDL = low density lipoprotein, Apo = apolipoprotein.

although the results did not reach statistical significance. It is possible that hormonal status may temporarily obscure the more consistent differences seen by apo E phenotype in premenopausal women. As the postmenopausal group increases and more data are available, these estimates will become more stable.

The change variable, which is based on the difference between pre- and post-lipoprotein lipid and apolipoprotein levels in this small group of women who became postmenopausal demonstrates, in general, an increase in total cholesterol, LDL cholesterol, apo B, and triglycerides (Table 5). There is, however, no clear evidence that apo E phenotype is related to the degree of change over time. Change in HDL3 in postmenopausal women, which had a p value of 0.0242 in ANOVA, did not show this difference in women who remained premenopausal (Tables 5 and 6). More data are needed to determine if this is a true association related to menopause and not due to small cell sizes.

Table 6 presents change variables in 250 premenopausal women typed for the most common apo E phenotypes. These women who remained premenopausal after approximately 3.5 years of follow-up also demonstrated changes in lipoprotein lipids and apolipoproteins but not as dramatically as those experiencing a natural menopause. Apo E phenotype did not show a statistically significant effect on change in women who remained premenopausal.

Discussion

Many variables were collected on each subject in the Healthy Women Study to help characterize the complex nature of CAD risk. Because of this, it was possible to assess the impact of the apo E locus, given other determinants of lipid and apolipoprotein levels. The multiple linear regression analyses demonstrate the contribution of both genetic and environmental factors in predicting quantitative lipid and apolipoprotein levels. It is interesting to note that the greatest amount of variance accounted for, 22% for apo B, required four environmental variables, BMI, age, cigarettes/day, and alcohol/day, and one genetic variable, apo E phenotype. Each of the attributes made a small independent contribution to the
Table 6. Mean Change in Quantitative Measure by Apolipoprotein E Phenotype in White Women Who Remained Premenopausal

<table>
<thead>
<tr>
<th>Variable</th>
<th>Apo E 3-2 (n=30)</th>
<th>Apo E 3-3 (n=164)</th>
<th>Apo E 4-3 (n=56)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>8.9 (3.0)</td>
<td>9.4 (1.5)</td>
<td>6.3 (2.9)</td>
<td>0.5191</td>
<td>0.5957</td>
</tr>
<tr>
<td>Total HDL cholesterol</td>
<td>0.6 (1.1)</td>
<td>-0.6 (0.6)</td>
<td>-1.6 (1.3)</td>
<td>0.8149</td>
<td>0.4439</td>
</tr>
<tr>
<td>HDL₄</td>
<td>-1.7 (0.9)</td>
<td>-2.4 (0.5)</td>
<td>-1.5 (1.1)</td>
<td>0.4646</td>
<td>0.6289</td>
</tr>
<tr>
<td>HDL₂</td>
<td>2.4 (0.9)</td>
<td>1.6 (0.4)</td>
<td>-0.1 (0.8)</td>
<td>2.5343</td>
<td>0.0814</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>2.1 (5.2)</td>
<td>2.4 (2.5)</td>
<td>10.5 (4.6)</td>
<td>1.4072</td>
<td>0.2468</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>7.9 (2.8)</td>
<td>9.5 (1.4)</td>
<td>5.8 (3.1)</td>
<td>0.8387</td>
<td>0.4335</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>4.3 (2.4)</td>
<td>3.9 (1.6)</td>
<td>-1.0 (3.0)</td>
<td>1.3180</td>
<td>0.2696</td>
</tr>
<tr>
<td>Apo A-II</td>
<td>-1.9 (1.4)</td>
<td>-2.3 (0.7)</td>
<td>-5.2 (1.5)</td>
<td>2.1568</td>
<td>0.1179</td>
</tr>
<tr>
<td>Apo B</td>
<td>10.3 (3.3)</td>
<td>8.3 (1.8)</td>
<td>6.4 (2.9)</td>
<td>0.3577</td>
<td>0.6997</td>
</tr>
</tbody>
</table>

Values for phenotypes are given in mg/dl and are the means and standard errors.

HDL=high density lipoprotein, LDL=low density lipoprotein, Apo=apolipoprotein.

concentration of apo B. Still, 78% of the variation was not associated with these known risk factors. The apo E locus provided only modest additional information in determining cholesterol, apo B, and LDL cholesterol concentrations.

Estrogen, which offers protection from CAD risk, does not mask the allelic effects of the apo E locus. At baseline, all women were premenopausal. Subsequent analyses in a group of postmenopausal women not on estrogen replacement therapy demonstrated similar, albeit not statistically significant, phenotypic effects on total cholesterol, apo B, and LDL cholesterol (Table 4). Lack of statistical significance is most likely due to the smaller sample size, but might also be due to the decline in estrogen output. In this group of women who became postmenopausal, the average age at baseline was 47.9 years and the average BMI, 25.3. For their postmenopausal visit, the mean age was 51.1 with a mean BMI of 26.3.

Results from the Healthy Women Study, as well as from other longitudinal studies, have shown a greater increase in total cholesterol over time among women who became postmenopausal than among those who remained premenopausal. In the Healthy Women Study, along with increases in age and BMI, there were concomitant increases in total cholesterol, triglycerides, apo B, and LDL cholesterol. Total HDL cholesterol was reduced, largely due to HDL₂. In between 3 to 4 years, there has been an overall worsening of the lipid profiles of the study subjects. This was not entirely due to cessation of menses since a control group of women, who remained premenopausal at follow-up, showed similar trends (Table 6). A smaller group of women who began hormone replacement therapy also demonstrated increases in total cholesterol and apo B; however, LDL cholesterol was not as dramatically increased. Contrary to the pre- and postmenopausal groups, women on hormone therapy showed increases in HDL cholesterol and apo A-I. Triglycerides rose in all groups, especially in those using hormones.

Apo E phenotype clearly influenced total cholesterol, apo B, and LDL cholesterol in cross-sectional analyses. However, in preliminary analyses, phenotype did not markedly affect changes in lipid parameters either in women who remained premenopausal or in those who experienced a natural menopause. Knowledge of the combined effects of hormones on lipids and genes that function in regulatory roles may aid in understanding the complex influences on lipid profiles in women as they age.

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References

Index Terms: apolipoprotein E • lipoproteins • Healthy Women Study • apolipoprotein polymorphisms
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