Influence of Dietary Fat, Apolipoprotein E Phenotype, and Sex on Plasma Lipoprotein Levels

Margaret M. Cobb, MD, PhD; Howard Teitlebaum, DO, PhD; Neil Risch, PhD; James Jekel, MD; and Adrian Ostfeld, MD

Background. The "Western" diet, sex, and apolipoprotein (Apo) E polymorphism have been implicated as codeterminants of lipid levels.

Methods and Results. In a retrospective analysis, we evaluated the combined impact of dietary fat, sex, and Apo E phenotype on lipoprotein levels in 67 subjects fed two contrasting, metabolically controlled diets: one a "Western" diet, with a low polyunsaturated to saturated (P:S) fatty acid ratio and the other a "therapeutic" diet, with a high P:S ratio. The high P:S diet compared with the low P:S diet exerted a far stronger predictive influence on lipoprotein concentrations than Apo E phenotype, sex, or the latter two factors combined. Apo E phenotype alone was associated with a stepwise increase in low density lipoprotein cholesterol (LDL-C), such that 3/2 < 3/3 < 4/3 on either the low or the high P:S diets. On the low P:S diet only, sex was shown to be a significant predictor of high density lipoprotein cholesterol (HDL-C) levels, with women greater than men, and the associated LDL/HDL ratio with men greater than women. On the high P:S diet, women displayed a dramatic fall in HDL-C, effectively raising the LDL/HDL ratio to equivalency with men and obliterating the sex influence seen with the low P:S diet. Controlled for dietary fat, Apo E and sex exerted independent, additive effects on lipoprotein levels on the low P:S diet only. Only the Apo E phenotype remained predictive on the high P:S diet.

Conclusions. Women of the Apo E 3/2 phenotype stand to benefit the least from a high P:S diet because of reduction in the more "protective" HDL-C, whereas men of the 4/3 phenotype showed the greatest improvement in the LDL/HDL ratio. (Circulation 1992;86:849–857)

Keywords • apolipoprotein E • fat, dietary • sex

Lipoprotein concentrations are determined by genetic constitution and environmental factors, such as diet. Among constitutional factors, sex remains the most potent predictor of variations in high density lipoprotein cholesterol (HDL-C) and coronary heart disease (CHD) risk. In persons consuming a typical high-fat, "Western" diet, men present with their first onset of myocardial infarction far earlier than age-matched women through about the fifth decade of life. This protective benefit of "femaleness" may be due to high HDL-C, which is influenced by estrogens and a Western diet.

Genetic variation in apolipoproteins has recently emerged as a determinant of variation in low density lipoprotein cholesterol (LDL-C) in men and women. The apolipoprotein (Apo) E gene locus is polymorphic, with three common isoforms, e2, e3, and e4, which are encoded by three different alleles on chromosome 19. The e2 and e4 variants differ from the more common e3 allele by a single amino acid substitution, which affects ligand binding of the triacylglyceride-rich lipoproteins and HDL to the hepatic LDL and Apo E receptor. Individuals carrying the e2 allele show lowered dietary cholesterol absorption, depressed chylomicron and remnant clearance, reduced hepatic cholesterol levels, and enhanced LDL-receptor activity compared with e3 and e4 subjects. Thus, population studies show that normal subjects with the e2 allele have lower and e4 higher LDL-C levels compared with e3.

Both genetic and dietary factors have been implicated in the etiology of type III hyperlipidemia and the high LDL-C levels seen in the Finnish population. Cross-cultural studies show a higher frequency of the e4 allele with enhanced exogenous fat clearance and an exceptionally high CHD risk in Finnish men compared with Japanese men.

Although these types of clinical observations and epidemiological contrasts have set the stage to investigate the role of genetic and nongenetic determinants underlying lipoprotein levels, no study has documented in a metabolically controlled diet setting their collective

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Supported in part by General Clinical Research Center grant RR-00102 at The Rockefeller University and National Institutes of Health grant HG-00348 at Yale University. Dr. Cobb's work was funded by Suzanne and Irving Karpas, Karpas Health Information Center, Beth Israel Medical Center, New York, N.Y., The Margolis Foundation, and donations from Ms. W. Jablonski.

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Received January 25, 1991; revision accepted May 27, 1992.
impact in normocholesterolemic subjects. Over the past 8 years, several clinical studies have been conducted to assess this interrelation.\textsuperscript{10,20–24} However, because of the small sample size and interindividual variation present in any one of these studies, it was not possible to clarify these associations.

The purpose of the present study is to combine these data directly, conducted under metabolically controlled conditions, to define 1) the individual and combined contributions of Apo E phenotype and sex to prediction of lipoprotein levels in subjects given a high-fat Western diet and 2) the relative contributions of these determinants to plasma lipoprotein concentrations after the crossover to a “therapeutic” diet.

**Methods**

**Study Subjects**

Studies providing the data base for this analysis were tabulated in 1988, although publication spanned to 1990. Some data were unpublished but met the inclusive criteria of this study. A total of 70 subjects participated and were admitted to inpatient metabolic wards and studied under strictly controlled conditions. A final cohort of 67 subjects remained after two subjects withdrew upon completion of the first dietary period only and one subject exhibited marked hypertriglyceridemia and was removed from the study.\textsuperscript{22} Two subjects, one man and one woman, smoked less than a pack of cigarettes per day during the entire study. One female subject was on biphasic oral contraceptives during the study.\textsuperscript{20} Neither of these factors was shown to have statistical impact on the lipid parameters studied. Informed consent was obtained from all volunteers after institutional review of the respective study protocols at each of two locations. Before data analysis, predetermined selection criteria were developed. All subjects were older than 18 years, in excellent health, and their lipoprotein values fell between the 10th and 90th percentiles, stratified for age and sex, compared with Lipid Research Clinic standards.\textsuperscript{25} Subjects were volunteers recruited from staffs of the respective universities, were undergraduate work-study students, or were patients who presented to the lipid clinic of each university for routine evaluation. These data were made available for this analysis by the senior investigator on all of these studies (Dr. Jan Breslow, now of Rockefeller University).

**Study Design**

This investigation pooled data from six previous studies,\textsuperscript{10,20–24} some unpublished, into one cohort group. As described above, data were derived from young (less than 35 years of age), healthy, weight-stable, normolipidemic subjects. Investigations were conducted at Harvard Medical School, Boston, and Rockefeller University, New York. Table 1 shows the key characteristics of the six studies used as the basis for this study and the average composition of the low and high polysaturated to saturated (P:S) fatty acid ratio diets.

The individual hypotheses, design features, and two contrasting metabolic diets in each of these studies were

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### Table 1. Study Hypothesis and Design Features of Metabolic Diet Studies

<table>
<thead>
<tr>
<th>Design Features</th>
<th>Men/ Women</th>
<th>Diet type</th>
<th>Dietary P:S ratio</th>
<th>Fat (%kcal/day)</th>
<th>P:S ratio</th>
<th>Cholesterol (mg/1,000 kcal)</th>
<th>Protein (%kcal/day)</th>
<th>Carbohydrate (%kcal/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects (n=67)</td>
<td></td>
<td>Low P:S 37</td>
<td>0.2</td>
<td>196</td>
<td>15</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rockefeller University studies (first author, study hypothesis)</td>
<td></td>
<td>High P:S 28</td>
<td>2.4</td>
<td>67</td>
<td>15</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Britton,\textsuperscript{20} 1990: Dietary fat influences HDL turnover</td>
<td>5/6</td>
<td>Mixed Low P:S 42</td>
<td>0.1</td>
<td>200</td>
<td>15</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Denke,\textsuperscript{21} 1988: An intermittent saturated fat meal influences fasting lipids</td>
<td>7/9</td>
<td>Mixed Low P:S 42</td>
<td>0.1</td>
<td>200</td>
<td>15</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Weintraub,\textsuperscript{22} 1988: Fat saturation affects postprandial lipoprotein metabolism</td>
<td>8/0</td>
<td>Mixed Low P:S 42</td>
<td>1.4</td>
<td>206</td>
<td>15</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Wissel,\textsuperscript{23} 1987: Dietary fat affects lipid levels and drug metabolism</td>
<td>4/0</td>
<td>Mixed Low P:S 42</td>
<td>3.3</td>
<td>226</td>
<td>15</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvard University studies (first author, study hypothesis)</td>
<td></td>
<td>Low P:S 25</td>
<td>3.3</td>
<td>67</td>
<td>15</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Fisher,\textsuperscript{24} 1983: Saturated fat and cholesterol independently affect plasma lipids</td>
<td>10/9</td>
<td>Formula Low P:S 31</td>
<td>0.1</td>
<td>0</td>
<td>15</td>
<td>54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Zanni,\textsuperscript{10} 1987: Dietary fat and egg cholesterol influences fasting lipid levels</td>
<td>0/9</td>
<td>Mixed Low P:S 31</td>
<td>0.6</td>
<td>583</td>
<td>14</td>
<td>55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P:S ratio, polyunsaturated to saturated fatty acid ratio; HDL, high density lipoprotein.

*Study with unpublished data included.
similar and are described in detail in the original references.10,20–24 These studies had several key common features that made pooling possible. Among these were 1) one senior investigator, Dr. Jan Breslow, MD, who conducted all the studies and was responsible for study hypotheses, design, and execution; 2) the research design was the same for all studies (i.e., a matched crossover design on two contrasting diets); 3) all diets were prepared in a clinical research kitchen, weighed out to 0.1 g, and calculated for weight maintenance; 4) in all studies, the averages of at least two lipoprotein determinations at the end of each diet period were used to evaluate the lipoprotein concentrations; and 5) at both study sites, the lipoprotein concentrations were uniformly measured by the same method and monitored by the senior investigator. These facts made the groups equivalent for analysis. The primary data analysis was performed independently at Yale University.

**Diet Protocol**

Diet studies were conducted under inpatient metabolic ward conditions to ensure consumption of the appropriate diet. Subjects were required to consume all of their food at each meal and, under supervision, were maintained at their usual levels of physical activity throughout all phases of the study. Body weights and activity were monitored at least five times per week. All subjects were placed on two administered metabolic diets in a nonrandomized crossover design with intervening washouts (Table 1). First they were given a low P:S diet, which was an adverse diet designed to aggravate a typical Western diet, rich in saturated fat. Second, they were given a more therapeutic high P:S diet, tailored to simulate a more beneficial diet rich in polyunsaturates, low in saturated fat, and usually lower in dietary cholesterol. Covariate adjustment was used to adjust for group differences in diet composition, as described in “Statistical Analysis.”

Apo E phenotype, sex, and ad libitum lipoprotein profiles were obtained for each subject upon entry and are presented in Table 2. Thirty-four men and 33 women were represented in this pooled analysis, with the women exhibiting higher HDL-C than men ($p<0.05$), a tendency toward lower LDL-C ($p>0.05$), and, consequently, a lower LDL/HDL-C ratio ($p<0.01$). Although Apo E phenotypes were not part of the selection criteria, patients with normolipidemic profiles were sought, and reasonable heterogeneity for Apo E phenotype was obtained. The homozygous phenotypes 3/3 and 4/4 (Table 2) were the most and least prevalent, respectively, with a similar percentage (but less prevalent than the 3/3 phenotype) of the 3/2 and 4/3 phenotypes. Phenotype frequencies were not significantly different between the sexes and study sites ($p>0.05$; data not presented). During the ad libitum period, Apo E phenotype was associated with a stepwise increase in total cholesterol (TC), LDL-C levels, and in the LDL/HDL ratio with 3/2<3/3<4/3.

**Laboratory Analysis**

**Lipoprotein levels.** At both the Rockefeller and Harvard sites, antecubital blood samples were collected after a 12-hour fast, and plasma was quantified for lipoprotein levels as previously described.10,20–24 Across the two study sites, identical laboratory methods were used for these measurements. TC and HDL-C levels were standardized by the Lipid Standardization Program of the Center for Disease Control, Atlanta, Ga.

**Apolipoprotein E phenotyping.** The Apo E phenotyping method (using isoelectric focusing), which was initially used at the lipoprotein laboratory at Harvard, was instituted and adopted at Rockefeller University.26 As DNA sequencing was not used, Apo E phenotype was taken to reflect Apo E genotype.

**Statistical analysis.** Descriptive statistics and analyses were calculated using Biomedical Computer Programs (BMDP) statistical software on a mainframe computer and SAS 6.02 on an IBM AT. Because this investigation pooled subjects from six previous studies, the dependent variables (TC, triglycerides [TG], very low density lipoprotein cholesterol [VLDL-C], LDL-C, and HDL-C) were adjusted for significant ($p<0.05$) group differences in age, dietary saturated fat, and cholesterol content by covariance analysis.

In the study by Fisher et al.,24 a formula diet was used with a very high proportion of polyunsaturated fatty acids

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**Table 2. Characteristics of Subjects by Sex and Apolipoprotein E Genotype at Time of Entry**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (years)</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C/HDL-C</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>67</td>
<td>25±6</td>
<td>168±30</td>
<td>74±38</td>
<td>21±13</td>
<td>97±27</td>
<td>51±11</td>
<td>2.0±0.8</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>34</td>
<td>26±7†</td>
<td>171±27</td>
<td>80±47</td>
<td>22±17</td>
<td>102±25</td>
<td>48±11*</td>
<td>2.3±0.9*</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>33</td>
<td>23±4</td>
<td>165±33</td>
<td>68±25</td>
<td>20±8</td>
<td>92±28</td>
<td>54±11</td>
<td>1.7±0.6</td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein E genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/2</td>
<td>13</td>
<td>24±6</td>
<td>152±28</td>
<td>60±22</td>
<td>19±7</td>
<td>86±23</td>
<td>48±6</td>
<td>1.8±0.5</td>
<td></td>
</tr>
<tr>
<td>3/3</td>
<td>44</td>
<td>25±6</td>
<td>169±29†</td>
<td>81±43</td>
<td>22±15</td>
<td>96±28§</td>
<td>52±12</td>
<td>2.0±0.9</td>
<td></td>
</tr>
<tr>
<td>4/3</td>
<td>8</td>
<td>25±7</td>
<td>183±29</td>
<td></td>
<td></td>
<td>62±20</td>
<td>19±9</td>
<td>117±18§</td>
<td>48±13</td>
</tr>
<tr>
<td>4/4</td>
<td>2</td>
<td>24±5</td>
<td>191±22</td>
<td></td>
<td></td>
<td>58±1</td>
<td>24±1</td>
<td>104±11§</td>
<td>63±10</td>
</tr>
</tbody>
</table>

**Notes:**
- TC, total cholesterol; TG, triglycerides; VLDL-C, very low density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C/HDL-C ratio, LDL-C/HDL-C. Values are mean±SD.
- *Significantly different from women, $p<0.05$.
- †Significantly different from women, $p<0.01$.
- ‡Significantly different from 3/2, $p<0.05$.
- §Significantly different from 3/2, $p<0.01$.
- ||Significantly different from 3/3, $p<0.05$. 
and essentially no dietary cholesterol. Because this study contrasted with the other natural food studies, 20-23, 25 the data were analyzed with and without inclusion of this study. No statistical differences were found between these analyses.

In the studies by Denke and Breslow, 21 Brinton et al., 20 and Wissel et al., 23 diets with both a reduction in total fat and increase in P:S ratio were used, as compared with an increase in P:S ratio alone in the remaining investigations. 10, 22, 24 Others 27 have shown that a reduction of total fat and change in the P:S ratio may exert separate additive influences; therefore, separate analysis by diet and sex by diet were performed. No statistical differences were found between these analyses; therefore, data from these studies were included in the pooled analysis.

Because of the highly intercorrelated nature of the dependent variables, a multivariate analysis of covariance (MANCOVA) with repeated measures was used. MANCOVA for repeated measures was used at the 5% significance level (two-tailed) to test for effects of these independent variables [diet (low P:S versus high P:S diet), sex (male versus female), and Apo E phenotype (3/2, 3/3, 4/3)]. The analytic strategy for assessing the effect of the independent variables (diet, Apo E, and sex) on lipid profiles was as follows: this MANCOVA testing procedure was initially used to detect overall differences due to the independent variables on the low and then the high P:S diet treatments using age and group differences in diet composition as a covariate. If this statistical test was significant at the 0.05 level, then MANCOVA univariate tests were performed on each dependent variable. When statistical significance was found in these analyses, post hoc testing procedures (Tukey post hoc comparisons test) were used to isolate individual group differences. Because the distribution of the TG and VLDL-C concentrations were skewed, a natural logarithmic transformation was used in the analyses. For clarity of presentation, unadjusted tabular data on plasma lipid levels are presented before the logarithmic transformation.

**Results**

**Diet Influence**

Table 3 presents the unadjusted lipoprotein profiles for subjects on the low and high P:S diets. (The statistics regarding differences are presented as footnote.) The diet crossover was the most statistically dominant predictor of changes in lipoprotein levels for all lipids and subfractions (p<0.0001).

The effect of the high P:S diets was striking for all lipids except TG and VLDL-C, as there were decreases of 39 mg/dl for TC, 27 mg/dl for LDL-C, and 8 mg/dl for HDL-C subfractions, respectively, after the crossover from the low to high PS diet. The LDL/HDL ratio was favorably decreased by 0.3 because of a greater reduction of LDL-C than HDL-C. These absolute changes corresponded to reductions in TC, LDL-C, HDL-C, and the LDL/HDL ratio of 22%, 26%, 15%, and 14%, respectively.

**Apo E Phenotype Influence**

Table 4 shows the lipoprotein profiles of study subjects while they were on the low and high P:S diets. The two Apo E 4/4 phenotype subjects were excluded from the formal analysis because of the small numbers. As shown, Apo E phenotype is seen to be a significant predictor of the lipoprotein profile (p<0.015, Table 4). Univariate tests revealed that Apo E phenotype was predictive of TC and LDL-C on the low and high P:S diets, with the effect of Apo E phenotype 3/3 and 4/3 differing statistically from the 3/2 phenotype (p<0.01).

On the low P:S diet, Apo E phenotype was associated with a stepwise increase in TC and LDL-C levels, with Apo E 3/2<3/3<4/3. The LDL/HDL ratio displayed the same relation for the three Apo E phenotypes. No significant or clinically important differences were noted among the Apo E phenotypes for the other lipid fractions.

The change to a high P:S diet decreased the TC and LDL levels for all three Apo E phenotypes, but the phenotypic relation was maintained, with the association of 3/2<3/3<4/3. TC was lowered by 32 mg/dl (−20%), 41 mg/dl (−22%), and 38 mg/dl (−20%) in the
Apo E 3/2, 3/3, and 4/3 phenotypes, respectively. These decreases were mainly in the LDL-C subfraction, with declines of 21 mg/dl (−24%), 29 mg/dl (−27%), and 25 mg/dl (−21%) in the respective profiles when stratified by Apo E phenotype. Apo E phenotype did not predict HDL-C levels on either diet, but the high P:S diet reduced the LDL/HDL ratio by 0.2, 0.3, and 0.2 for the respective Apo E 3/2, 3/3, and 4/3 phenotypes, which corresponded to reductions of 11%, 14%, and 8%, respectively.

**Apo E Allele Influence**

The phenotypic variations (milligrams per deciliter) of the three common Apo E phenotypes on the major lipoprotein profiles after the low P:S and high P:S diets are presented in Table 5. The phenotypic variation is defined as the observed deviation (milligrams per deciliter) of the group mean of the Apo E phenotypes 3/2 or 4/3 from the more common Apo E 3/3 phenotype.

In the present study, with a normolipidemic study population and no (or low) representation of the rare Apo E phenotypes (2/2, 4/2, and 4/4), the Apo E allele impact cannot be fully assessed quantitatively. A qualitative description of the direction and magnitude of the allele effect in these subjects is discussed below. By inspection, the greatest impact of the Apo E locus was on the TC level, principally the LDL-C subfraction. The direction of cholesterol divergence produced by the Apo E alleles was remarkably constant on both metabolic diets: the TC and LDL-C levels were lowered, at median, or raised in subjects with the e2, e3, or e4 alleles, respectively, with e2<e3<e4. The magnitude of TC deviation produced by the e2 allele was nearly two to three times as great as the influence of the e4 allele on both diets.

On the low P:S diet, in absolute amounts, the TC and LDL-C deviations produced by the e2 allele appeared slightly augmented compared with the high P:S diet.

### Table 4. Lipoprotein Levels and Ratio by Apolipoprotein E Genotype and Type of Diet

<table>
<thead>
<tr>
<th>Group</th>
<th>Apo E</th>
<th>n</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL/HDL Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low P:S diet</td>
<td>Apo E</td>
<td>3/2</td>
<td>13</td>
<td>158±25</td>
<td>73±16</td>
<td>19±4</td>
<td>88±21</td>
<td>51±9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3/3</td>
<td>44</td>
<td>183±30a</td>
<td>76±31</td>
<td>19±8</td>
<td>109±29a</td>
<td>55±15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4/3</td>
<td>8</td>
<td>189±22a</td>
<td>52±18</td>
<td>16±8</td>
<td>120±18a</td>
<td>64±19</td>
</tr>
<tr>
<td>High P:S diet*</td>
<td>Apo E</td>
<td>3/2</td>
<td>13</td>
<td>126±23</td>
<td>66±26</td>
<td>18±9a</td>
<td>67±21</td>
<td>43±9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3/3</td>
<td>44</td>
<td>142±28a</td>
<td>71±27</td>
<td>17±8</td>
<td>80±25ab</td>
<td>45±12</td>
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<td></td>
<td></td>
<td>4/3</td>
<td>8</td>
<td>151±26a</td>
<td>63±22</td>
<td>16±7</td>
<td>95±17abc</td>
<td>41±11</td>
</tr>
</tbody>
</table>

TC, total cholesterol; TG, triglycerides; VLDL-C, very low density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; LDL/HDL ratio, LDL-C/HDL-C. Values are mean±SD. P:S, polyunsaturated to saturated fatty acid ratio; Apo, apolipoprotein.

*Main effect of Apo E genotype (p<0.01): TC (p<0.01); LDL-C (p<0.01). Interactions, NS.
*Significantly different from 3/2 genotype, same diet groups, p<0.05.
*Significantly different from 3/2 genotype, same diet groups, p<0.01.
*Significantly different from 3/3 genotype, same diet groups, p<0.01.

### Table 5. Change in Lipoprotein Levels Between Apolipoprotein E 3/3 and Other Apolipoprotein E Genotypes on Low and High P:S Diet

<table>
<thead>
<tr>
<th>Group/Apo E genotype</th>
<th>Total lipids</th>
<th>Cholesterol subfractions</th>
<th>LDL-C/HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TC mg/dl %Δ</td>
<td>TG mg/dl %Δ</td>
<td>VLDL-C mg/dl %Δ</td>
</tr>
<tr>
<td>Low P:S diet</td>
<td>Δ3/2</td>
<td>−25</td>
<td>−14</td>
</tr>
<tr>
<td></td>
<td>3/3</td>
<td>+6</td>
<td>+3</td>
</tr>
<tr>
<td>High P:S diet</td>
<td>Δ3/2</td>
<td>+16</td>
<td>−11</td>
</tr>
<tr>
<td></td>
<td>3/3</td>
<td>+9</td>
<td>+6</td>
</tr>
</tbody>
</table>

TC, total cholesterol; TG, triglycerides; VLDL-C, very low density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; LDL/HDL ratio, LDL-C/HDL-C. Values are mean±SD. P:S, polyunsaturated to saturated fatty acid ratio; Apo, apolipoprotein.

Values represent deviations of lipoprotein profiles from the more common Apo E 3/3 genotype on the respective diet (calculated from Table 4).
However, when calculated as the percentage change from the common e3 allele, the e2 allele produced a comparable degree of change in TC and LDL-C on both diets (Table 5). In contrast, the effect of the e4 allele on elevating TC and LDL-C levels, in milligrams per deciliter or percentage change (Δ%), was remarkably consistent on both the low and high P:S diets.

One striking finding of the present study was that the magnitude of the LDL/HDL-C ratio deviations produced by the e2 and e4 alleles were independent of diet. On the low P:S diet, the e2 allele depressed the LDL/HDL-C ratio by 22% and the e4 allele elevated the ratio by 18%. On the high P:S diet, the e2 allele depressed the LDL/HDL-C ratio by 16%, whereas the e4 allele produced an elevation of 26%. The effects of the Apo E locus on TG, VLDL-C, and HDL-C levels were inconsistent.

Apo E polymorphism explained an average of 10–12% of the variance in LDL-C on both metabolic diets. The Apo E phenotype–diet interaction was not statistically significant (i.e., the inherent differences among the phenotypes were independent of the effects of the dietary P:S ratio). Thus, Apo E phenotype and diet produced independent but additive effects on lipoprotein levels.

**Sex Influence**

When the subjects entered the study, the HDL-C concentration was lower and the corresponding LDL/HDL ratio was higher in men compared with women (Table 2).

**Low P:S diet.** The low P:S diets, compared with ad libitum diets (Table 3 versus Table 2), increased HDL-C concentrations in both men and women, but the relative relation remained the same (i.e., the HDL-C level was higher by 16% in women; Table 3), whereas male subjects alone exhibited statistically higher TG concentrations by 16% (p<0.02). Both men and women on the low P:S diet showed an increased LDL/HDL-C ratio compared with ad libitum levels, but men continued to maintain higher ratios than women by 24%.

**High P:S diet.** The LDL-C levels were comparably reduced on the high P:S diet by 27% and 23% for men and women, respectively, compared with the low P:S diet. Sex difference in this effect, however, was seen in response to the high P:S diet (diet by sex interaction, p<0.01). Whereas the high P:S diet reduced the LDL-C appreciably in both sexes, male subjects showed reductions in TG, VLDL-C, and HDL-C levels of 16%, 25%, and 12%, respectively, thus decreasing TC by 23% compared with the low P:S diet (Table 3). Female subjects, however, responded differently to the high P:S diet, showing a concomitant increase in TG and VLDL-C levels by 5% and 6%, respectively, surpassing the TG level attained in their male counterparts on the high P:S diet.

On the high P:S diet, women displayed a dramatic fall in HDL by 18% compared with the low P:S diet, effectively reducing their levels to equivalency with men. In women, the LDL/HDL ratio was reduced by only 5% after the high P:S diet, remaining comparable to ad libitum levels (1.7±0.2). In men, however, the LDL/HDL ratio showed a 20% reduction on the high P:S diet. This apparent equalizing effect on sex differences in HDL-C and LDL/HDL (diet by sex interaction, p<0.05) produced by the high P:S diet is potentially of clinical significance and is addressed below.

**Composite Diet, Apo E, and Sex Influence**

Dietary fat, Apo E phenotype, and sex produced additive but not interactive effects on lipoprotein profiles (p>0.05). Further inspection of these data is presented pictorially in Figure 1. A stepwise effect of Apo E phenotype on LDL-C levels was evident for both sexes on the ad libitum, low, and high P:S diets, with 3/2<3/3<4/3. Conversely, in men on the two administered low and high P:S metabolic diets, Apo E phenotype was inversely correlated with TG levels, with 3/2>3/3>4/3. No parallel consistent effect was evident for TG in women. Apo E phenotype did not consistently predict the HDL-C levels, but men with Apo E 3/2 showed the lowest HDL-C. Thus, Apo E 3/2 phenotype and “maleness” predicted the highest TG level and lowest LDL-C and HDL-C subfractions on both metabolically controlled diets.

Figure 1 shows the effects of sex on HDL-C levels. For all three Apo E phenotypes, women showed higher LDL-C levels than men on the ad libitum and low P:S diets, with no strong Apo E phenotypic pattern evident.
After the crossover to the high P:S diet, sex no longer predicted HDL-C because the differences between the sexes became statistically nonsignificant for all three phenotypes. Among all women, the greatest decline in HDL-C levels was evident in the Apo E 4/3 and 3/3 phenotypes. Apo E 3/3 and Apo E 4/3 phenotypes showed decreases of nearly 20% in the more protective HDL-C fraction. However, LDL-C levels fell by over 25% after the crossover, suggesting dietary benefits for these Apo E phenotypes.

**Discussion**

The purpose of this composite retrospective analysis was to examine the individual impact of Apo E and sex on lipoprotein profiles in normolipidemic subjects fed an adverse Western diet (low P:S) and a more beneficial therapeutic (high P:S) diet. Data were collected and analyzed by pooling results across six different dietary studies conducted in a similar manner under a single senior investigator at two study sites. We found that on a high-fat Western diet, Apo E phenotype predicted LDL-C, whereas sex predicted TG and HDL-C levels. Women of the Apo E 3/2 phenotype stand to benefit the least from a diet crossover because of their initially low LDL-C level and the diet-induced reduction in the more “protective” HDL-C, whereas men of the 4/3 phenotype showed the greatest improvement in the LDL/HDL ratio.

Western societies characteristically consume diets rich in saturated fat and dietary cholesterol and maintain high blood cholesterol levels.5-8 The magnitude and direction of the effect of dietary fat on plasma lipoprotein levels in the present study support the results of large trials that dietary change to high P:S diets can reduce plasma TC and LDL-C concentrations in Western populations.26-29 Because of the wide interindividual differences in lipoprotein response to dietary change, we used two contrasting dietary regimens at the opposite ends of the usual clinical spectrum. In our study, even in normolipidemic subjects, dietary change exerted a major influence on both components of the LDL/HDL ratio (Table 3).

In the present study, subjects with the 4/3 Apo E phenotype maintained the highest LDL-C level across all dietary treatments, with levels being low P:S diet greater than ad libitum greater than high P:S diet, in decreasing order of plasma LDL-C levels. In fact, several of the 4/3 subjects (and one of the two Apo E 4/4 individuals), when fed the low P:S diet, exceeded the adult treatment panel guidelines after this study below this point. This panel recommends that LDL-C be maintained below 130 mg/dl.32 Thus, subjects carrying the e4 allele appear to be at especially high risk of developing elevated cholesterol levels compared with those of other phenotypes when fed a Western diet. Indeed, ad libitum TC levels and average allelic effects were increased on the low P:S diet in the e4 subjects (Tables 2, 4, and 5), contributing to the stepwise elevation in TC with 3/2<3/3<4/3. It is therefore likely that subjects of the 4/3 phenotype will require more vigorous treatment with a high P:S diet than other phenotypes because of the underlying genetic variation at the Apo E locus. In the present study, atherosclerotic susceptibility could not be accurately assessed. Using the LDL/HDL ratio (Table 4) as a potential predictor of CHD propensity,5 the order of increasing vulnerability to atherosclerosis as proposed by Davignon et al12 and Sing and Davignon,15 3/2<3/3<4/3, held in the present study.

The precise molecular basis underlying genetic variation in LDL-C levels for the general population is currently undefined. The Apo E locus was the first polymorphic gene implicated in regulation of LDL-C levels, accounting for 8–16% of the variance in resultant LDL-C levels.12,15 In the present work, 10–12% of the LDL-C variance was explained by this single gene locus, which is in agreement with these reported findings. However, the balance of the LDL-C variance remains unexplained. Clearly, the Apo E locus is but one site contributing to the total genetic variation in LDL-C levels. Research suggests that, using restriction fragment length polymorphisms of the Apo B gene (XbaI, MspI, and BseRI) and the Apo Al-CIII-AIV gene cluster, other apolipoprotein genes explain much of the variation in LDL levels.33

Genetic epidemiological studies suggest that Apo E phenotype may interactively magnify the effects of a high-fat and high-cholesterol diet on LDL-C levels.12 Boerwinkle and Utermann34 and Sing et al35 have speculated that the TC response to dietary cholesterol is greater in subjects with the e4 allele. The present work supports an additive rather than interactive effect of diet and Apo E phenotype on the low and high P:S diets.

In our study, calculated as the average deviation from Apo E 3/3 levels, the lowering effect of the e2 allele and elevating influence of the e4 allele were remarkably similar in magnitude on LDL-C levels but opposed in direction on both the low and high P:S diets (Table 5). These findings corroborate, in part, those of Xu et al33 who recently published an association between Apo E genotype and LDL-C levels in a basal high-fat diet. This Apo E influence disappeared, however, after a crossover to a low-fat, high P:S diet, a result that is at variance with ours. This difference may be attributed to the counseled outpatient diet in the Xu et al33 group, which could have had a confounding effect on the Apo E locus influence on the high P:S diet. Manttari et al39 also reported some predictive power of the Apo E4 allele on TC and LDL-C change or response after crossover to a low-fat, high P:S diet. However, again, the therapeutic diet was outpatient and counseled rather than under strict metabolic diet control. In summary, whereas diet remained the overwhelming factor in the improvement or deterioration of one’s lipoprotein profile, the Apo E allele exerts an additional negative or positive effect on these levels relative to the predominant alleles.

The independent effects of sex on atherosclerotic susceptibility have been well established.5,9 Women generally exhibit lower LDL/HDL ratios than men at baseline, before any type of controlled dietary intervention.5,7 This was found in the present study during ad libitum and after the low P:S diet phase (Tables 2 and 3). In the present work, the LDL-C concentrations on ad libitum, low P:S, and high P:S diets tended to be higher but did not reach statistical significance.

Meanwhile, the HDL-C level was higher in women ad libitum and on the low P:S diet. However, this level was then roughly equalized between sexes on the high P:S diet (Table 3). Women showed no improvement in the
LDL/HDL ratio on the more therapeutic high P:S diet compared with ad libitum, whereas men significantly improved their LDL/HDL ratios (diet by sex interaction, p<0.05). These findings are in apparent conflict with two prior studies by Kuusi et al.\textsuperscript{27} and Ehnholm et al.,\textsuperscript{28} who found no significant differences between the sexes in response to a diet crossover; however, these diets lacked metabolic ward control, and participants showed an elevated risk for CHD, whereas ours were low-risk subjects and normocholesterolemic by Lipid Research Clinic standards.\textsuperscript{25}

Previous work from our laboratory, cited as a component of this pooled analysis,\textsuperscript{20} has demonstrated distinct metabolic mechanisms between a low LDL-C on a given diet and a diet-induced decrease in HDL-C. Although the desirability of a high P:S diet for relatively normolipidemic women may come into question, the lack of mortality data in women with diet-induced HDL-C reduction leaves our data inconclusive.

For the purposes of applying the three influences of diet, sex, and Apo E phenotype to patient management, the clinical aspects of these findings are reinforced by our statistical findings. We define risk as an increase and benefit as a decrease in the LDL/HDL ratio on an administered, metabolic diet and use this ratio to devise a risk/benefit scheme. We may speculate which groups, stratified for sex and Apo E phenotype, are most at risk on a low P:S (Western) diet and which can most benefit from a high P:S (therapeutic) diet.

The population with the best overall disposition, in terms of atherosclerotic susceptibility as represented by the LDL/HDL ratio, would appear to be 3/2 women. Again, Figure 1 can be used to consider relative contributions to protection conferred by “femaleness” and the 3/2 phenotype. No significant difference was seen in LDL-C levels between 3/2 women and 3/2 men (Figure 1). The HDL-C concentration is appreciably higher in women of this phenotype, suggesting a major contribution of femaleness to the low atherosclerotic susceptibility of 3/2 women, even on a low P:S diet.

At the other end of the Apo E spectrum (i.e., Apo E 4/3 subjects), HDL-C levels are higher in women than in men (as in Apo E 3/2) only on the low P:S diet. After the crossover to the high P:S diet, the 4/3 men showed a dramatic reduction in the LDL/HDL ratio, whereas 4/3 women failed to show a proportionate ratio decline comparable to men. Thus, the high P:S diet in men nearly equalized the apparent risk (as predicted by the LDL/HDL ratio) in this group to that of the 4/3 women. This is illustrated in Figure 1, where femaleness is associated with a higher HDL-C than for men despite the presence in either sex of the e4 allele. This effect was evident only with the low P:S diet.

Men with the e2 allele exhibited the lowest TC (mainly in the LDL-C and HDL-C subfractions) and the highest TG concentrations (Figure 1). These subjects were relatively unresponsive to the diet crossover (i.e., maintained a constant LDL/HDL ratio). These findings suggest that Apo E phenotype and sex may exert additive influences to the amounts of LDL relative to HDL-C independent of dietary fat.

The clinician, then, would consider the male patient with an e4 allele on a low P:S Western diet most at risk. A diet very low in total fat with a high P:S ratio would be recommended. Men of the 3/2 phenotype are at less risk of developing high cholesterol levels on a low P:S diet, but inspection of TG and HDL-C levels would be indicated, with diet and lifestyle changes in 3/2 men aimed at reduction of TG and boosting of HDL-C levels.

On the other hand, a crossover from a typical Western diet to a more therapeutic low-fat, high P:S diet may not improve the atherosclerotic risk of women who have the more protective Apo E phenotypes (3/2). Based on this study, women who show elevated plasma cholesterol concentrations would best be placed on a diet of only mildly reduced saturated fat with replacement by monounsaturated fatty acids.

Conclusions

The work presented here shows an additive effect of dietary fat, Apo E polymorphism, and sex on lipoprotein profiles. Knowledge of diet composition, genetics, and sex influences may, in the future, lead to more effective gender-specific intervention strategies to lower lipoprotein levels and CHD susceptibility.

Acknowledgments

The authors acknowledge Dr. Jan Breslow for providing a portion of the original data and appreciate his suggestions on preparation of the manuscript. We thank Cynthia Seidman and Donna Tesi of the Rockefeller University Dietetics Department for formulation and preparation of the metabolic diets. Special appreciation is extended to Marie Phillips and the nursing service on the Rockefeller University Metabolic Unit for their continued assistance. Gratitude is offered to Michael Friedmann for his assistance in preparation of the manuscript.

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Circulation. 1992;86:849-857
doi: 10.1161/01.CIR.86.3.849

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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