Apolipoprotein E4 Homozygosity Predisposes to Serum Cholesterol Elevation during High Fat Diet

Matti J. Tikkanen, J.K. Huttunen, Christian Ehnholm, and P. Pietinen

The hypothesis that apolipoprotein E (apo E)-isoform-related differences in plasma and LDL cholesterol concentrations are due to differential responses to dietary lipids was explored in 110 North Karelian subjects who had previously participated in dietary intervention studies. This was accomplished by collecting fresh blood samples for apo E phenotyping and by re-analysis of the original plasma lipid data according to apo E phenotypes. During high fat, high cholesterol baseline (p = 0.003) and switchback diets (p = 0.002), plasma cholesterol correlated inversely with the sum of subscript numbers (e.g., apo E_{3/4} = 7). Thus, subjects with the apo E_{3/4} phenotype had the highest (7.63±1.32 mmol/l), and subjects with the apo E_{3/3} phenotype had the lowest baseline levels of plasma cholesterol (5.85±1.48 mmol/l). This association became weaker during a low fat, low cholesterol diet intervention (p = 0.069). Greater reductions in plasma cholesterol occurred in subjects homozygous for the apo E_{4/4} allele (−1.84 mmol/l) as compared to subjects with other genotypes (−1.13 mmol/l) (p = 0.0097). Moreover, these subjects responded to the switchback diet by greater increases in plasma cholesterol (1.52 mmol/l) than others (0.92 mmol/l, p = 0.0141). The results suggest that the effect of apo ε genotype on plasma cholesterol is modulated by dietary fat and cholesterol intake.


Apolipoprotein (apo) E is a structural component of plasma chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), and high density lipoproteins (HDL). Apo E is recognized by LDL receptors and chylomicron remnant receptors and has a major regulatory role mediating the cellular uptake of several lipoprotein classes. Genetic variation in the apo E gene results from three common alleles, ε2, ε3, and ε4, which code for the three isoforms apo E_2, apo E_3, and apo E_4, respectively. The early studies of Utermann et al.1,2 demonstrated that individuals carrying the ε2 allele had lower plasma and LDL cholesterol concentrations than did those homozygous for the ε3 allele. Other studies indicated that carriers of the ε4 allele had higher plasma and LDL cholesterol levels than did subjects with the ε3/ε3 genotype.3,4 Further studies have extended these findings and have established that in several populations in industrialized countries, plasma cholesterol, LDL cholesterol, and apo B levels decrease in the following order: 4/4 > 4/3 > 3/3 > 3/2 > 2/2.5–11 According to Utermann,12 the above relationship between cholesterol levels and apo E genotypes is not independent from cultural and ethnic background. Thus, the association of the ε4 allele with high cholesterol is strong in Finns,7 moderate in Germans,13 but less significant or absent in some Asian populations.12 One possibility is that these populations differ significantly from each other in dietary habits such as fat intake and that individuals with the ε4 allele acquire elevated cholesterol levels only while on a high fat diet.12 We set out to test the hypothesis that apo E-isoform-related differences in plasma and LDL cholesterol were due to differential responses to dietary lipids. This was accomplished by determining the apo E phenotypes in North Karelian subjects who had previously participated in diet intervention studies14,15,16 and by re-analysis of the plasma lipid data according to apo E phenotype.

Methods

Subjects

Fresh blood samples were collected from subjects who had participated in the dietary intervention studies in North Karelia, which were reported in 1982 to 1985.14,15,16 The original results were re-analyzed by apo E phenotype. Subjects ages 30 to 50 years were recruited for the three dietary studies through local risk factor screenings14 or from the county-wide hypertension register15,16; they were asked to participate with their spouses. Couples who volunteered were apparently healthy and had no long-term medication or history of specific lipid-lowering therapy. Altogether 250 subjects had participated in the three studies. For the present study, however, only subjects belonging to identical intervention groups (described below) within each study were invited to participate. From those 130 subjects, phenotyping of apo E was possible in 110 individuals (56 men and 54 women).
**Table 1. Total Plasma Cholesterol Concentrations and Changes during Dietary Study**

<table>
<thead>
<tr>
<th>Diet</th>
<th>E4/E4 (n=6)</th>
<th>E4/E3 (n=42)</th>
<th>E3/E3 (n=48)</th>
<th>E3/E2 (n=12)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Baseline</td>
<td>7.63±1.32</td>
<td>6.31±1.15</td>
<td>6.07±1.00</td>
<td>5.86±1.48</td>
<td>0.003</td>
</tr>
<tr>
<td>B. Intervention</td>
<td>5.79±0.86</td>
<td>5.12±0.85</td>
<td>4.99±0.94</td>
<td>4.73±1.10</td>
<td>0.069</td>
</tr>
<tr>
<td>C. Switchback</td>
<td>7.31±1.03</td>
<td>6.0±0.99</td>
<td>5.99±0.97</td>
<td>5.53±1.23</td>
<td>0.002</td>
</tr>
<tr>
<td>Change (B-A)</td>
<td>-1.84±0.81</td>
<td>-1.19±0.88</td>
<td>-1.07±0.62</td>
<td>-1.17±0.59</td>
<td>0.064</td>
</tr>
<tr>
<td>Change (C-B)</td>
<td>+1.52±0.79</td>
<td>+0.88±0.70</td>
<td>+0.99±0.64</td>
<td>+0.79±0.45</td>
<td>0.069</td>
</tr>
</tbody>
</table>

Values are in mmol/l and are the means±SD.

**Diet**

The three studies consisted of a 2-week baseline period, a 6- or 12-week intervention period, and a 5- to 6-week switchback period. During the baseline and switchback periods, the participants were on their normal free-choice diets. During the intervention period, the diet was modified to provide a lower fat content and a higher ratio of polyunsaturated to saturated fatty acids (P/S ratio). The energy content of the diet was kept as constant as possible.

Intervention groups differed within each study: In the first study, all subjects followed a low fat, low cholesterol diet with a P/S ratio of about 1.14 In the second study, Group 1 followed the same low fat diet; Group 2, a low salt diet; and Group 3 constituted the control group.15 In the third study, Group 1 again followed the low fat, P/S ratio 1 diet, and Group 2 followed a low fat, P/S ratio 0.4 diet.16 Only individuals who had been on the identical low fat, low cholesterol, P/S ratio 1 diet were invited to participate in the present study. To match the comparisions, only serum lipid changes during the first 6-week period were used for the subjects from the third study, in which the total duration of intervention was 12 weeks.

**Measurements**

Cholesterol and triglyceride concentrations in serum were determined by using an AutoAnalyzer II apparatus (Technicon Instruments, Tarrytown, NY) and enzymatic assays (Boehringer Mannheim, Mannheim, FRG). HDL cholesterol was measured after precipitation of VLDL and LDL with dextran sulfate-magnesium chloride.17 The LDL cholesterol concentration was calculated by using the Friedewald approximation.18 The concentration of apo B was determined by using the radial immunodiffusion kit M-Partigen-Apolipoprotein B (Behring-Werke AG, Marburg, FRG), and apo A-I, with another immunodiffusion method.19 Apo E phenotyping was carried out directly in fresh serum by using a modification20 of the method of Havekes et al.,21 which is based on isoelectric focusing of delipidated plasma followed by immunoblotting with antiapo E antisera. Cysteamine treatment of plasma before isoelectric focusing was performed as described by Weisgraber et al.22

Statistical analyses were carried out with the BMDP statistical software package (University of California, Los Angeles, CA, 1985). Analysis of variance and covariance was used for comparison of means. When appropriate, baseline adjustment was performed. For pairwise comparison of means, either the t test or Wilcoxon's test was applied, depending on the normality of distributions.

**Results**

Distribution of apo E phenotypes resembled that previously reported in Finns7: The total number of subjects was 110 (56 men and 54 women); eight subjects (six men, two women) had E4/E4; 42 subjects (19 men, 23 women) had E4/E3; 48 subjects (26 men, 22 women) had E3/E3; and 12 subjects (four men, eight women) had E3/E2. A single subject with the apo E2 phenotype was not included in the study population. The concentrations and changes in plasma cholesterol, triglycerides, and HDL cholesterol in subjects with different apo E phenotypes during the dietary study are shown in Tables 1 to 3. The total cholesterol concentrations were significantly influenced by the apo E phenotype during the baseline (p=0.003) and switchback diets (p=0.002), with decreasing levels in the following order: E4/E4>E4/E3>E3/E3. During intervention, the correlation became nonsignificant (p=0.069). Analogous correlations between apo E phenotype and LDL cholesterol concentration, which were approximated by the Friedewald equation, also persisted during intervention (p=0.043, data not shown). The decreases and increases in total cholesterol during intervention and switchback, respectively, appeared to be greater in subjects with the apo E4 phenotype as compared to the changes in other phenotypes (Table 1). Total and LDL cholesterol values in E4/E4 sub-
The mechanisms by which different isoforms of apo E regulate plasma cholesterol concentrations have been partly elucidated. Briefly, triglyceride-rich particles containing apo E2 are removed more slowly by the liver, and apo E-containing particles are removed more efficiently than apo E-containing particles. Thus, the presence of triglyceride-rich particles with apo E2 results in impaired cholesterol uptake by the liver, with a consequent up-regulation of hepatic LDL receptors and a lowering of plasma cholesterol levels. Conversely, efficient uptake of apo E-containing triglyceride-rich particles causes hepatic cholesterol loading with down-regulation of LDL receptors and increased plasma cholesterol levels. This model implies that the effects of apo E isoforms on plasma cholesterol could be modified by changes in dietary fat and cholesterol intake. This theory receives support from a recent study indicating that hepatic clearance of dietary fat was more rapid in carriers of the e4 allele than in e3/e3 subjects. On the other hand, the fat clearance was much slower in e3/e2 subjects compared to those with other genotypes. In addition, the intestinal cholesterol absorption efficiency was shown to be greater in carriers of the e4 allele as compared to others. This enhanced cholesterol absorption may explain the increased serum cholesterol response to dietary cholesterol observed in e4 carriers as compared to e2 carriers.

The design of the dietary studies reported here allowed us to evaluate the effects of a low fat, low cholesterol diet in subjects with common apo E phenotypes. In addition, the switchback provided an opportunity to analyze the effects of switching to an atherogenic, free-choice North Karelian diet. As expected, the cholesterol levels were strongly related to apo E phenotype during the free-choice diets (baseline and switchback), decreasing with decreasing sum of subscript numbers (e.g., E4/E4). This correlation became weaker during intervention, suggesting that the regulatory effect of the apo E phenotype may depend on the fat and cholesterol intake. Individuals homozygous for the e4 allele exhibited the greatest reduction in serum cholesterol and LDL cholesterol during intervention and, conversely, they exhibited the greatest increase in these levels during switchback. There is no ready explanation for the lack of a gene dosage effect, i.e., that the E4/e3 subjects' serum cholesterol responses were not significantly greater than those of e3/e3 homozygotes, but this could be related to the relatively small size of the study population. Our results give support to the contention that the effect of the apo E phenotype on plasma cholesterol is modulated by dietary fat and cholesterol intake.

Table 3: Plasma High Density Lipoprotein Cholesterol Concentrations and Changes during Dietary Study

<table>
<thead>
<tr>
<th>Diet</th>
<th>E4/E4 (n=8)</th>
<th>E4/E3 (n=42)</th>
<th>E3/E3 (n=40)</th>
<th>E3/E2 (n=12)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Baseline</td>
<td>1.39±0.37</td>
<td>1.47±0.40</td>
<td>1.38±0.25</td>
<td>1.48±0.30</td>
<td>0.54</td>
</tr>
<tr>
<td>B. Intervention</td>
<td>1.14±0.19</td>
<td>1.24±0.34</td>
<td>1.18±0.23</td>
<td>1.22±0.28</td>
<td>0.69</td>
</tr>
<tr>
<td>C. Switchback</td>
<td>1.47±0.37</td>
<td>1.47±0.37</td>
<td>1.43±0.26</td>
<td>1.47±0.23</td>
<td>0.92</td>
</tr>
<tr>
<td>Change (B-A)</td>
<td>-0.28±0.21</td>
<td>-0.23±0.17</td>
<td>-0.20±0.14</td>
<td>-0.25±0.13</td>
<td>0.39</td>
</tr>
<tr>
<td>Change (C-B)</td>
<td>+0.33±0.29</td>
<td>+0.23±0.16</td>
<td>+0.25±0.14</td>
<td>+0.25±0.13</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Values are given in mmol/l and are the means±SD.

Discussion

The mechanisms by which different isoforms of apo E regulate plasma cholesterol concentrations have been partly elucidated. Briefly, triglyceride-rich particles containing apo E2 are removed more slowly by the liver, and apo E-containing particles are removed more efficiently than apo E-containing particles. Thus, the presence of triglyceride-rich particles with apo E2 results in impaired cholesterol uptake by the liver, with a consequent up-regulation of hepatic LDL receptors and a lowering of plasma cholesterol levels. Conversely, efficient uptake of apo E-containing triglyceride-rich particles causes hepatic cholesterol loading with down-regulation of LDL receptors and increased plasma cholesterol levels. This model implies that the effects of apo E isoforms on plasma cholesterol could be modified by changes in dietary fat and cholesterol intake. This theory receives support from a recent study indicating that hepatic clearance of dietary fat was more rapid in carriers of the e4 allele than in e3/e3 subjects. On the other hand, the fat clearance was much slower in e3/e2 subjects compared to those with other genotypes. In addition, the intestinal cholesterol absorption efficiency was shown to be greater in carriers of the e4 allele as compared to others. This enhanced cholesterol absorption may explain the increased serum cholesterol response to dietary cholesterol observed in e4 carriers as compared to e2 carriers.

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Acknowledgment

Statistical analysis of the data by Terhi Hakala is greatly appreciated.

References


Index Terms: apolipoprotein E4 • high-fat diet • cholesterol
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doi: 10.1161/01.ATV.10.2.285
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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