The Effect of Fasting Triacylglyceride Concentration and Apolipoprotein E Polymorphism on Postprandial Lipemia

A.J. Brown and D.C.K. Roberts

To determine if apolipoprotein (apo) E polymorphism influences postprandial lipemia and hence can help explain the wide range of lipemic responses to a standardized fat meal observed previously, blood samples were collected from 25 healthy men whose postprandial responses to a standardized fat meal had been measured. Venous blood samples had been obtained before the fat meal (0.73 g fat/kg containing vitamin A) and hourly thereafter for 8 hours, plasma and chylomicron triacylglyceride (TAG) concentrations had been determined, and retinyl esters (REs) in the chylomicron and nonchylomicron fractions had been measured. The original results were reanalyzed by apo E phenotype (six E2/3; 14 E3/3; four E3/4 and one E4/4, grouped as E4). Contrary to what is known about the e4 allele, the apo E4 group displayed a significantly greater response curve than did either the apo E2/3 or E3/3 groups for both plasma TAG and chylomicron RE concentrations (/;<0.01), as reflected in a later chylomicron RE peak for the apo E4 group (p<0.05). The E4 group tended (p=0.18) to have a 40% higher fasting TAG than did either of the other groups, which may reflect bias in the selection of subjects. As fasting TAG is an important determinant in postprandial lipemia, results were normalized for this variable. After adjustment, the E4 group had the lowest TAG response relative to the E3/3 group (p<0.01). Our findings suggest that controlling for apo E phenotype may help to reduce interindividual variation in the postprandial response to a standard fat meal. Furthermore, our data suggest that in studying the effect of apo E phenotype on postprandial lipemia, it may be necessary to take account of fasting TAG levels. (Arteriosclerosis and Thrombosis 1991;11:1737-1744)

In a recently reported study,1 a large variation in postprandial responses to the standard fat meal was observed between individuals. One possible explanation for this variability is genetic differences in the way the study subjects metabolize an oral fat load.

The observed effect of genetic variation in apolipoprotein (apo) E on plasma lipid and lipoprotein levels in humans (both normolipidemic and dyslipidemic) has led to the hypothesis that apo E polymorphism plays a major role in determining susceptibility to atherosclerosis.2-5 Three common alleles (e2, e3, and e4) code for the three major isoforms (E2, E3, and E4), giving rise to six apo E phenotypes in plasma.6,7 In a general Caucasian population, the approximate frequencies for these phenotypes are 1% for E2/2, 11% for E2/3, 2% for E2/4, 60% for E3/3, 23% for E3/4, and 3% for E4/4.8,9

Apo E located on the surface of remnant particles can be recognized by apo B and chylomicron remnant receptors in the liver. Both apo E2 and E4 are considered to be abnormal isoforms, functionally and metabolically distinct from apo E3.10 The currently accepted hypothesis is that homozygous E2 and E4 are the extremes, whereas the intervening phenotypes (E2/3, E2/4, E3/3, and E3/4) display a spectrum of discrete effects.3,4,11-13 The apo E2 and E4 isoforms are thought to have reciprocal effects on plasma lipid and lipoprotein concentrations.3,11,14 Apo E2 binds poorly to remnant receptors,15,16 delaying remnant clearance but facilitating low density lipoprotein (LDL) catabolism; it is associated with type III (remnant) hyperlipidemia17,18 and lower plasma cholesterol concentrations in normolipidemic persons. Conversely, apo E4 has increased affinity for remnant receptors, enhances remnant clearance,10 and downregulates LDL receptors; it is associated with "polygenic" hypercholesterolemia19-21 and raised apo B22-24 and LDL cholesterol25 levels. Because the structural defects of apo E are identical in

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Protocol

The protocol, fat tolerance test procedure, and most of the laboratory methods have been described in detail previously.1 After a 12-hour overnight fast, subjects were given a standard test meal to be eaten in 10 minutes. It was presented as a cream/yogurt/peanut oil blend and incorporated an aqueous solution of vitamin A in the form of retinyl palmitate (429 retinol equivalents/kg body wt; Bioglan, Marrickville, Sydney, Australia) to label the chylomicrons. The test meal contained 0.73 g fat/kg body wt (polyunsaturated-saturated fatty acid ratio=0.4; 1.3 mg cholesterol/kg body wt), consisting of 89% of energy as fat and 5.5% each as protein and carbohydrate. Blood samples (20 ml) were taken from the fasting subjects (before treatment) for determination of fasting plasma triacylglyceride (TAG), cholesterol, lipoprotein lipids, and, several months after treatment, for apo E phenotyping. Lipoproteins were separated from an aliquot (4.0 ml) of the initial fasting sample by density gradient ultracentrifugation (modified from that reported in Reference 31) into four fractions (very low density lipoprotein [VLDL]<1.006, 1.006<LDL<1.063, 1.063<high density lipoprotein2 [HDL2]<1.125, and 1.125<high density lipoprotein3 [HDL3]<1.21 g/ml). The ultracentrifugal isolation of chylomicrons used in this experiment (1.6×10^6 g·min^-1) is thought to separate a predominantly chylomicron population (SF>1,000) from a predominantly remnant population (nonchylomicron fraction, SF<1,000).27,32,33 Retinyl esters (REs) were determined in the chylomicron and nonchylomicron fractions by isocratic normal-phase liquid chromatography (adapted from the methods of Paanakker and Groenendijk34 and Bankson et al35).

Subjects

Blood samples were collected from 25 healthy men whose postprandial responses to a standard fat meal had been measured after conditioning with moderate olive oil (OO) or fish oil (FO) supplementation. The original results1 were reanalyzed by apo E phenotype.

Methods

The postprandial response to the test meal was characterized by a number of different methods for total plasma TAG, chylomicron TAG, chylomicron REs, and nonchylomicron REs: 1) Two-way analysis of covariance (ANCOVA) was performed on the group data using hour (0–8) and apo E phenotype (E2/3, E3/3, and E4) as the independent variables and oil supplement (OO or FO) as a covariate. This analysis calculated the mean value at each time point for each phenotype, adjusted (by regression) to remove the effect of the oil supplements. 2) The average of the peak (y_{max}) and second highest (y_{2nd}) concentration above the baseline concentration (y_0) was calculated by the equation

{\text{Postprandial increase} = \left(\frac{(y_{\text{max}} + y_{\text{2nd}})}{2}\right) - y_0}

3) The average of the time to peak concentration (x_{max}) and the time to the second highest concentration (x_{2nd}) was calculated by the equation

{\text{Time to peak} = \left(\frac{x_{\text{max}} + x_{\text{2nd}}}{2}\right)}
TABLE 1. Anthropometric Characteristics and Fasting Total Lipid and Lipoprotein Levels of Healthy Men by Apolipoprotein E Phenotype

<table>
<thead>
<tr>
<th>Variable</th>
<th>E2/3 (n=6)</th>
<th>E3/3 (n=14)</th>
<th>E4 (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>25±5</td>
<td>30±11</td>
<td>26±3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.6±4.9</td>
<td>77.4±8.4</td>
<td>73.6±5.2</td>
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<tr>
<td>Height (cm)</td>
<td>183±6</td>
<td>184±7</td>
<td>187±8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.9±1.7</td>
<td>23.0±2.8</td>
<td>21.1±2.3</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.83±0.03</td>
<td>0.85±0.04</td>
<td>0.87±0.06</td>
</tr>
<tr>
<td>Lipids (mM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAG</td>
<td>1.02±0.41</td>
<td>1.00±0.43</td>
<td>1.43±0.52</td>
</tr>
<tr>
<td>VLDL-T</td>
<td>0.61±0.35</td>
<td>0.54±0.31</td>
<td>0.75±0.53</td>
</tr>
<tr>
<td>Chol</td>
<td>4.39±0.54</td>
<td>5.07±1.02</td>
<td>5.11±1.10</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>0.52±0.26</td>
<td>0.65±0.35</td>
<td>0.81±0.51</td>
</tr>
<tr>
<td>LDL-C</td>
<td>2.09±0.50</td>
<td>2.44±0.72</td>
<td>2.27±0.64</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.66±0.22</td>
<td>0.74±0.21</td>
<td>0.71±0.29</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.49±0.08</td>
<td>0.55±0.10</td>
<td>0.63±0.13</td>
</tr>
</tbody>
</table>

Values are mean±SD.

BMI, body mass index; TAG, triacylglyceride; Chol, cholesterol; VLDL-T/C, very low density lipoprotein triacylglyceride/cholesterol, respectively; HDL₂/₃-C, high density lipoprotein cholesterol subfractions 2 and 3, respectively.

4) The area under the curve above the fasting concentration (0 hour) was calculated by the trapezoidal rule.38

The latter three measures were tested for statistical significance among the apo E phenotypic groups by one-way ANCOVA, again with oil supplement as the covariate. The first measure was also examined after controlling for fasting TAG. This involved dividing each individual value by the fasting plasma TAG concentration (test day sample), so that all three phenotypic groups began the test day with identical fasting plasma TAG concentrations. Data are expressed as mean±SD in the tables. No error bars are given in Figures 2 and 3 because these are related to the individual variations and may have given a false impression of the significance of the results.39 Statistical comparisons were performed by use of a computer package (MACSS, Statsoft Inc., Tulsa, Okla.). A probability value of less than 0.05 (two-tailed) was considered significant.

Results

Subject Characteristics

Of the 25 subjects, 14 (56%) had the phenotype E3/3, six (24%) had the phenotype E2/3, four (16%) had the phenotype E3/4, and one subject (4%) was an E4 homozygote. The five subjects displaying an e4 allele were grouped together and designated as the E4 group.

Anthropometric characteristics (Table 1), vigorous exercise, and alcohol consumption (as assessed by questionnaire) were similar for the three apo E phenotypic groups. Fasting plasma TAG tended to be higher (40%) in the E4 group, but this did not reach statistical significance (p=0.18). Plasma cholesterol and lipoprotein lipid concentrations were not significantly different (Table 1).

Effects of Fish Oil on Dietary Fat Clearance in Apolipoprotein E3 Homozygotes

When only the 14 apo E3 homozygotes were considered (Figure 1), the chylomicron RE response curve was statistically lower (p=0.025) for the FO-fed group (n=7) when compared with that of the OO-fed group (n=7). This difference was twice as significant as that for the corresponding response curves in our previously reported data,1 when each oil supplementation group contained a mixture of apo E phenotypes. None of the other measures of postprandial response (data not shown) reached statistical significance when only the apo E3 homozygotes were included in the analysis.
Effects of Apolipoprotein E Phenotype on Dietary Fat Clearance

Although the apo E phenotype distribution between the oil supplementation groups (E2/3: three in the OO group and three in the FO group; E3/3: seven in the OO group and seven in the FO group; E4: four in the OO group and one in the FO group) was homogeneous ($\chi^2=1.46, df=2, p>0.25$), comparisons were performed after removing the effect of the oil supplementation to increase the sample size (see "Statistics" above).

Chylomicron TAG responses were not statistically different ($p=0.105$) among the three apo E phenotypic groups, tending to mirror those of total plasma TAG.

For both plasma TAG and chylomicron REs, the apo E4 group displayed a significantly greater response curve than did either the apo E2/3 or E3/3 group ($p<0.01$) (Figures 2A and 2B). This greater response was reflected in a later chylomicron RE peak for the apo E4 group ($p=0.036$; Table 2), although other measures of postprandial response were not significantly different (Table 2). Although nonchylomicron RE responses were not significantly altered by apo E phenotype (Figure 2C), the time to peak again tended to be delayed in the apo E4 group ($p=0.093$; Table 2). Postprandial responses to the standard fat meal were not significantly different between the apo E2/3 and E3/3 groups.

Adjustment for the higher fasting plasma TAG concentration in the E4 group reversed the observed effect in lipemic response (Figure 3 and Figure 2A), with the E4 group now having the lowest TAG response relative to the E3/3 group ($p=0.004$) and the E2/3 group being intermediate between the two.

Discussion

The oral fat tolerance test reflects overall chylomicron metabolism and cannot be used to distinguish between chylomicron clearance and decreased entry into the plasma pool. But because the effect of apo E polymorphism on postprandial lipemia has been largely attributed to the apo E isoforms having different affinities for the remnant receptor, any observed differences in lipemic response between the apo E phenotypic groups in this article are ascribed to differences in clearance rather than absorption.

The distribution of apo E phenotypes found in this study is comparable with that found in a general Caucasian population. The homozygous E3 phenotype occurs most frequently in the population and is considered "normal." Because the apo E3 homozygotes were evenly divided between the OO- and FO-fed groups, it was possible to evaluate if the effects of the oil supplement were better resolved when apo E phenotype was controlled. In our previously reported data when apo E phenotype was not considered, the FO-fed group ($n=11$) only displayed a significantly lower postprandial chylomicron RE response than did the OO-fed group ($n=14$) after the first 2 hours ($p=0.048$). A clearer difference between the OO- and FO-fed groups emerged when only the E3 homozygotes were considered (Figure 1), a significant difference ($p=0.025$) being observed for the entirety of the fat tolerance test (8 hours).
TABLE 3. Mean Fasting Plasma Triacylglyceride and Cholesterol Concentrations for Six Apolipoprotein E Phenotypes in Six Studies

<table>
<thead>
<tr>
<th>Variable</th>
<th>Postprandial increase (mg/l)</th>
<th>Time to peak (hr)</th>
<th>Area under curve (hr • mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicron RE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2/3</td>
<td>6</td>
<td>2.19±0.49</td>
<td>3.51±0.32 8.71±1.66</td>
</tr>
<tr>
<td>E3/3</td>
<td>14</td>
<td>1.91±1.06</td>
<td>3.27±0.91 7.53±5.09</td>
</tr>
<tr>
<td>E4</td>
<td>5</td>
<td>2.74±1.74</td>
<td>4.67±1.35* 12.2±7.41</td>
</tr>
<tr>
<td>Nonchylomicron RE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2/3</td>
<td>6</td>
<td>0.35±0.16</td>
<td>4.77±0.88 1.72±0.73</td>
</tr>
<tr>
<td>E3/3</td>
<td>14</td>
<td>0.40±0.10</td>
<td>4.55±1.23 1.90±0.46</td>
</tr>
<tr>
<td>E4</td>
<td>5</td>
<td>0.41±0.12</td>
<td>5.88±0.54 1.93±0.82</td>
</tr>
</tbody>
</table>

Values are mean±SD. The statistics paragraph at the end of the "Methods" section describes how these characteristics of the postprandial response were derived.

It is possible that the apo E4 group's higher fasting plasma TAG concentration was either the result of an unrepresentative sample or is a general characteristic of individuals possessing the e4 allele. One might expect that apo E4, by having opposing effects on plasma lipids and lipoprotein concentrations relative to E2, should be associated with lower TAG levels, and there is some evidence to support this view.11,14 However, apo E4 is also linked with type V hyperlipidemia46 and hypertriglyceridermia in obesity.49,50 In the present study, the three phenotypic groups were similar in terms of mean body mass index (BMI) (Table 1), and none of the subjects were clinically obese (BMI range, 18.7-28.1 kg/m²). With sufficient population studies of apo E polymorphism,8,9,25,51-53 it was possible to use a meta-analysis to study trends in the effect of the various isoforms on plasma lipids. The results from six studies, each presenting fasting TAG and cholesterol data for more than 200 healthy individuals (Table 3), were pooled. Relative to the normal homozygous E3 phenotype, plasma cholesterol increased progressively from the homozygous E2 phenotype to the other extreme of the homozygous-
fasting TAG levels. After adjustment, the present data showed (Figure 3) that E4 displayed a lower lipemic response relative to the homozygous E3 group, which is more in keeping with literature expectations.

The previous studies examining the effects of the common apo E phenotypes did not note the influence of fasting TAG levels, as these were similar for all groups. However, it is noteworthy that Rubin- 
szeit et al concluded that normolipidemic apo E2 homozygotes had grossly delayed clearance of chylo-
micron remnants compared with that of a normolip-
idemic control group not possessing an e2 allele.

Although all subjects were apparently normotriacyl-
glyceridemic, the homozygous apo E2 group began the fat tolerance test with an average fasting TAG concentration that was more than twice that of the non-E2 group. It is unclear whether the authors' conclusions would need to have been modified if both groups had been matched according to fasting plasma TAG concentration.

In conclusion, use of a single apo E phenotype improved the "precision" of the fat tolerance test by reducing the interindividual variation in response to a standardized test meal. In addition, this work has indicated that in studying the effect of apo E phenotype on postprandial lipemia, it may be necessary to take account of fasting TAG levels. Any future implementation of the fat tolerance test for clinical use should perhaps be done in parallel with apo E phenotyping. If used after different dietary treat-
ments, thought should be given to controlling for fasting TAG as well. Further studies with larger numbers of subjects and with each phenotypic group matched according to fasting plasma TAG concentration will be needed to confirm the preliminary relations reported here.

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**KEY WORDS** • apolipoprotein E phenotype • fat tolerance test • postprandial • chylomicrons • triacylglycerides • plasma lipids • retinyl esters • fish oils
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