Independent Effects of Apo E Phenotype and Plasma Triglyceride on Lipoprotein Particle Sizes in the Fasting and Postprandial States

Anthony M. Dart, Bridget Cooper

Abstract—LDL particle sizes and Apo E phenotypes were determined in 212 subjects of whom 51 had angina. LDL diameter was significantly less in subjects with an e2 allele (24.76±0.08 vs 24.94±0.02 nm, P=0.02), and this was evident for both E2/E3 (24.77±0.09 nm) and E2/E4 (24.69±0.08 nm) phenotypes. Although there was a negative relation between LDL diameter and plasma triglyceride, the effect of apo E2 was still evident with adjustment for triglyceride. In multiple regression analysis, the significant determinants of LDL diameter were gender (with females having larger particles than males), body mass index, and the presence (or absence) of E2. HDL particle sizes and compositions were determined on fasting samples and, additionally, 5 and 8 hours after a fat-rich meal for 48 coronary heart disease cases and 49 control subjects. Fasting HDL particle sizes were not related to the presence of E2 but were significantly smaller for subjects possessing an e4 allele (8.09±0.08 vs 8.39±0.05 nm, P=0.003) and were negatively related to plasma triglyceride. However, the effect of E4 persisted after adjustment for triglyceride. In a multiple regression analysis, the only significant determinant of fasting HDL diameter was the presence (or absence) of E4 with fasting plasma triglyceride just failing to reach significance (P=0.06). There was a postprandial increase in HDL diameter that was less marked in subjects with coronary heart disease. The postprandial increase in HDL diameter was of sufficient magnitude to result in size reclassification of HDL particles. The influence of E4 was also evident at both postprandial time points. Compositional analysis demonstrated that the increase in HDL diameters postprandially could be attributed to triglyceride enrichment, with an accompanying fall in cholesterol ester content. Phospholipid changes postprandially were biphasic with an initial fall followed by a rise in concentration. The increase in triglyceride content was significantly less in those subjects with angina despite an equivalent rise in plasma triglyceride. The present study demonstrates significant, but different, effects of variation in apo E phenotype on the particle sizes of both HDL and LDL. Such effects were still evident with adjustment for differences in plasma triglyceride and suggests that variation in apo E phenotype exerts effects on lipoprotein particle sizes by mechanisms additional to those dependent on change in plasma triglyceride. (Arterioscler Thromb Vasc Biol. 1999;19:2465-2473.)

Key Words: apo E ■ LDL particle size ■ HDL particle size ■ triglyceride ■ coronary heart disease ■ postprandial

There is considerable evidence that variation in apo E phenotype, dependent on the assortment of e4, e3, and e2 alleles, contributes to the variation in occurrence of coronary heart disease (CHD). Thus the possession of an e4 allele has been associated with the CHD in the European Atherosclerosis Research Study.1 More severe atheroma was found in the coronary vessels of Alzheimer’s patients with an e4 allele than in those without, and elderly Finnish men dying of coronary disease had twice the relative frequency of an e4 allele compared with those not dying from coronary disease.2,3 In addition, an E2/E3 phenotype appears to confer relative protection compared with an E3/E3 phenotype.4–6 The impact of e4 on CHD appears to be similar for men and women.7 The mechanism for the effects of apo E variation are not fully established but may include differences in the receptor mediated clearance of cholesterol and alterations in absorption as well as accompanying differences in a number of other CHD risk factors.6,8–13

In addition to variation in apo E phenotype, there is considerable heterogeneity in the size and density of both LDL and HDL particles. A number of studies have suggested that the presence of small, dense LDL particles is particularly associated with an increased risk of CHD, although negative studies have also appeared as has an association with LDL polydispersity.14–22 LDL particle diameters are associated with a number of other cardiovascular risk factors,14–17,23–26 and a particular dependence on plasma triglyceride concentrations is well established.10–12,16,17 In some studies, the association between LDL particle diameter and CHD is no longer apparent after adjusting for other risk factors.21,22 The presence of an e4 allele is associated with elevated fasting and postprandial triglyceride levels, which could thus lead to an...
influence on LDL particle size. However, such an association was also present even with adjustment for plasma triglyceride levels. On the other hand, a study of apparently healthy 35-year-old men failed to show any relation between apo E phenotype and LDL particle size. HDL particles are known to undergo extensive remodelling during their residence in the plasma by processes involving exchange of triglyceride for cholesterol ester with subsequent lipolysis leading to the formation of smaller particles. Such processes are also dependent on plasma triglyceride levels and changes in HDL diameters have been seen postprandially coincident with the rise in triglyceride levels. However, associations with apo E phenotypes have apparently not been sought... As indicated, apo E polymorphism affects a number of steps in lipoprotein metabolism that may mediate affects on lipoprotein particle size in addition to those related to variation in plasma triglyceride. In addition to affects on cholesterol absorption and synthesis and remnant uptake, there are affects on LDL apo B metabolism. Apo E enhances the lipid exchange between lipoproteins mediated by cholesteryl ester transfer protein (CETP) and apo E polymorphisms influence the response of CETP to dietary cholesterol. Alteration in CETP activity could be expected to contribute to changes in HDL composition and size. The distribution of apo E between apo B containing and noncontaining lipoproteins is influenced by apo E phenotype with individuals carrying E2 having a higher, and those carrying E4 a lower, proportion of apo E in HDL. Recent studies have also demonstrated an effect of apo E containing particles on LDL receptor binding of buoyant LDL, which differs between subjects with predominantly small or large LDL particles.

In the present study, we have examined the relationships between LDL and HDL particle sizes, determined after-gradient gel electrophoresis, and apo E phenotypes, in particular the possession of e4 and e2 alleles in control subjects and those with CHD. The role of plasma triglyceride in effecting such modulations has been determined, with particular attention to postprandial changes in HDL particles.

### Methods

#### Study Population

The study population comprised subjects with no known current or past history of coronary artery disease as well as subjects with recent onset of angina. Both groups were recruited from subjects self presenting to health screening clinics either at the Baker Medical Research Institute or one of several locations held by the Anti-Cancer Council of Victoria as part of its Health 2000 study. All attendees completed a questionnaire designed to establish the presence of known CHD (previous angina or myocardial infarction) and the presence of symptoms compatible with recent onset angina. All subjects also had a fasting plasma total cholesterol measurement at the time of their initial screening. Subjects taking lipid-lowering drugs or with a known history of myocardial infarction or angina were not included as cases or controls in the study. We recruited subjects with newly diagnosed, rather than established, CHD so that they could be investigated before possibly adopting dietary and other lifestyle changes or receiving changes to medication that may have modified the parameters under investigation. A similar strategy has been followed elsewhere.

Subjects whose replies to the chest-pain questionnaire were considered to possibly indicate recent onset angina were then invited to attend for a stress ECG test. This was performed on a moving treadmill using a modified Bruce protocol. Subjects with $\pm 1.5$ mV ST segment depression were considered positive and diagnosed as having CHD, which had been previously unsuspected or not diagnosed. No medication, other than short-acting nitrates, was prescribed during the interval between the diagnostic exercise ECG and their study day. Subjects did, however, continue with any preexisting medication.

Three matched asymptomatic control subjects were recruited for each CHD case. Control subjects were recruited from the same source as the respective case and were matched by sex, age (within 2 years), and total plasma cholesterol (within $\pm 1$ mmol/L). Control subjects did not undergo a stress ECG test and were recruited throughout the study in parallel with cases. In addition to fasting samples, each CHD case and the first of the recruited controls were followed elsewhere. Control subjects were recruited from the same source as the respective case and were matched by sex, age (within 2 years), and total plasma cholesterol (within $\pm 1$ mmol/L). No medication, other than short-acting nitrates, was prescribed during the interval between the diagnostic exercise ECG and their study day. Subjects did, however, continue with any preexisting medication.

#### Protocol on Study Day

Subjects reported to the laboratory by 8:30 AM on the day of the study having fasted, except for clear fluids, from the previous midnight. Height and weight were recorded as were details of their medication. Blood pressure was recorded in the seated position from the brachial artery by sphygmomanometry. For subjects participating in the post prandial study, a 20G 3 cm Insyte Catheter was then inserted into the right antecubital vein. After discard of the initial 2 mL, a fasting blood sample was then withdrawn, and a slow infusion of N/saline, without added heparin, was started to maintain catheter patency. The total volume administered was always $<400$ mL. Subjects were then given their test meal, which had to be consumed within 30 minutes of the first (fasting) sample and were then allowed only clear fluids during the time of the postprandial study. Further blood samples were then withdrawn at 5 and 8 hours after the start of the meal. Blood was transported to the laboratory on ice.

#### Meal Composition

The test meal provided approximately 66% of predicted daily energy requirements. It contained 45% fat, 35% (simple) carbohydrate, and 20% protein. All meals comprised 3 slices of bread, 75 g of cheese, 300 mL milk, and 50 mL chocolate topping. Quantities of Promod (D-whey protein concentrate and soy lecithin) and margarine were dependent on the KJ requirement. Cream and sugar (35%) was added for higher KJ requiring meals. The meal was served as 3 slices of cheese in sandwiches made from 3 slices of white bread lightly spread with margarine. The remaining constituents were given as a chocolate milkshake.

### Table 1. Blood Pressure and Fasting Biochemical Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CHD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>51</td>
<td>161</td>
</tr>
<tr>
<td>Male/female</td>
<td>33/18</td>
<td>101/61</td>
</tr>
<tr>
<td>Age (y)</td>
<td>62.1±1.0</td>
<td>62.6±0.5</td>
</tr>
<tr>
<td>Plasma chol (mmol/L)</td>
<td>5.62±0.14</td>
<td>5.77±0.08</td>
</tr>
<tr>
<td>Plasma trig (mmol/L)</td>
<td>1.57±0.09</td>
<td>1.51±0.07</td>
</tr>
<tr>
<td>BP treatment (%)</td>
<td>21 (41)**</td>
<td>10 (6)</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>139.6±3.0</td>
<td>134.4±1.5</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>84.5±1.3</td>
<td>81.4±0.9</td>
</tr>
<tr>
<td>Plasma NEFA (mmol/L)</td>
<td>0.83±0.06</td>
<td>0.99±0.04</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>5.90±0.18</td>
<td>5.64±0.06</td>
</tr>
<tr>
<td>Plasma insulin (IU/L)</td>
<td>8.88±0.55**</td>
<td>7.04±0.31</td>
</tr>
</tbody>
</table>

The table shows baseline characteristics and fasting biochemical parameters for subjects with CHD and control subjects. chol indicates total cholesterol; trig, triglyceride; BP, blood pressure medication; SBP, systolic blood pressure; DBP, diastolic blood pressure; NEFA, nonesterified fatty acids. **$P<0.01$ for the difference between CHD and controls.
Biochemical Measurements
Plasma was separated by low-speed centrifugation within 30 minutes of collection. Phenyl methyl sulfonyl fluoride (final concentration 1 mmol/L) was added to separated plasma, which was stored at 4°C until analysis. Biochemical analyses were conducted within 2 days of plasma collection. Plasma lipids were measured enzymatically in a Cobas Bio centrifugal analyser (Roche Products Pty Ltd). Triglyceride levels were measured after removal of free glycerol. Nonesterified fatty acids levels were also measured by enzymatic methods (Wako Pure Chemical Industries, 990-75401). Plasma glucose was determined enzymatically with a Kodak Ektachem DT Micro Glucose kit (1532316). Plasma insulin concentrations were determined in the Endocrine Laboratory, Department of Biochemistry, Royal Melbourne Hospital, by immunoassay with enzyme-linked chemiluminescence.

Lipoprotein Composition Profile
Lipoprotein profiles were determined by equilibrium density gradient ultracentrifugation based on the method by Belcher and colleagues,43 with minor variations.43 VLDL and chylomicrons were removed ultracentrifugation based on the method by Belcher and colleagues,43 with minor variations. VLDL and chylomicrons were removed by ultracentrifugation of 5.0 mL of plasma, overlaid with normal saline, in a Quickseal ultracentrifuge tube (Beckman 344619) for 16 hours at 40 000 rpm and 10°C in a 50.3Ti rotor (Beckman Instruments). The tube was sliced and approximately the top 1.2 mL and bottom 4.5 mL were transferred to separate volumetric tubes. The volumes were increased to 1.5 and 5 mL, respectively, with normal saline. The density gradient ultracentrifugation was performed on the VLDL-free plasma fraction by loading the following solutions sequentially into 11×60 mm wettable Ultracote tubes (Seton Scientific): 0.31 mL of 1.21 g/mL KBr, 1.25 mL of 1.15 g/mL KBr, 0.31 mL of VLDL-free plasma, and 2.13 mL of 1.02 g/mL KBr. Ultracentrifugation was performed in an SW60 rotor (Beckman Instruments) at 58 000 rpm for 21 hours and 20°C with slow acceleration and the brake off.

To fractionate lipoproteins, the centrifuge tubes were pierced from the bottom and Fluorinert FC-40 (Sigma Chemical Co) was pumped in at 0.5 mL/min. Each tube was fractionated into 20 fractions of 0.2 mL each via an optical density monitor reading at 280 nm to monitor the protein profile of each tube. The density of each fraction was determined by refractometry. Each fraction was analyzed for total and free cholesterol, triglyceride, and phospholipid. The composition of HDL, was determined from fractions 11 to 15 inclusive (1.0693 to 1.1141) and HDL, from 16 to 19 inclusive (1.1297 to 1.924).

Apo E Phenotyping
Apo E phenotyping was performed by the method of Menzel and Utermann,44 with minor variations as described previously.6

HDL Particle Profiles
HDL particle profiles were determined on 3% to 30% nonnaturating gradient gels (Gradipore GS330). Total lipoprotein samples were prepared by isolating the density <1.23 g/mL fraction from fresh plasma. Plasma (200 µL) was mixed with 1.9 mL of density 1.25 g/mL KBr solution, placed in a 2.1 mL Quickseal ultracentrifuge tube (Beckman 344625), sealed, and centrifuged at 100 000 rpm for 16 hours. The lipoprotein fraction was removed by slicing the top of the tube and collecting the top 300 µL. Total lipoprotein fraction (20 µL) was loaded onto the gradient gels adjacent to a lane of High Molecular Weight Standards (Pharmacia 17–0445-01). Electrophoresis was carried out in nonnaturating running buffer for 16 hours at 190 V and 10°C; gels were fixed with 10% sulfosalicylic acid for 1 hour, stained with 0.04% PAGE Blue G90 (Electran 44248) for 3 hours, and destained with 5% acetic acid overnight or until the background was clear. Gels were scanned with an LKB Ultrascan XL Laser Densitometer to discern peaks (Figure 1). HDL peak sizes were estimated against a standard curve calibrated with markers of known diameter: thyroglobulin 17 nm, ferritin 12.2 nm, catalase 10.4 nm, lactate dehydrogenase 8.16 nm, and albumin 7.1 nm. HDL particles were classified on the basis of diameter as HDL, (7.2 to 7.8 nm); HDL, (>7.8 to 8.2 nm); HDL, (>8.2 to 8.8 nm); HDL, (>8.8 to 9.7 nm), and HDL, (>9.7 to 12 nm).

LDL Particle Sizes
LDL was prepared from plasma frozen at −80°C. The density of the plasma was increased to 1.25g/mL by addition of KBr, 0.8 mL of which was overlaid with saline (density 1.006), sealed in a 2.0-mL ultracentrifuge tube, and centrifuged for 60 minutes at 100 000 rpm at 20°C. The LDL band was removed by aspiration with a small gauge needle and syringe and analyzed for LDL particle size within 3 days. LDL particle diameters were determined on 3% to 13% nonnaturating gradient gels (Gradipore GS313) as described previously.45 LDL peak sizes was estimated against a standard curve created from the markers of known diameter: thyroglobulin 17 nm, ferritin 12.2 nm, catalase 10.4 nm, lactate dehydrogenase 8.16 nm, and albumin 7.1 nm. LDL particle profiles were determined on 3% to 30% nonnaturating gradient gels (Gradipore GS330). Total lipoprotein samples were prepared by isolating the density <1.23 g/mL fraction from fresh plasma. Plasma (200 µL) was mixed with 1.9 mL of density 1.25 g/mL KBr solution, placed in a 2.1 mL Quickseal ultracentrifuge tube (Beckman 344625), sealed, and centrifuged at 100 000 rpm for 16 hours. The lipoprotein fraction was removed by slicing the top of the tube and collecting the top 300 µL. Total lipoprotein fraction (20 µL) was loaded onto the gradient gels adjacent to a lane of High Molecular Weight Standards (Pharmacia 17–0445-01). Electrophoresis was carried out in nonnaturating running buffer for 16 hours at 190 V and 10°C; gels were fixed with 10% sulfosalicylic acid for 1 hour, stained with 0.04% PAGE Blue G90 (Electran 44248) for 3 hours, and destained with 5% acetic acid overnight or until the background was clear. Gels were scanned with an LKB Ultrascan XL Laser Densitometer to discern peaks (Figure 1). HDL peak sizes were estimated against a standard curve calibrated with markers of known diameter: thyroglobulin 17 nm, ferritin 12.2 nm, catalase 10.4 nm, lactate dehydrogenase 8.16 nm, and albumin 7.1 nm. HDL particles were classified on the basis of diameter as HDL, (7.2 to 7.8 nm); HDL, (>7.8 to 8.2 nm); HDL, (>8.2 to 8.8 nm); HDL, (>8.8 to 9.7 nm), and HDL, (>9.7 to 12 nm).

Data and Statistical Analysis
Data were tabulated and analyzed using spsspc+/v2. Values are shown as mean±SEM unless stated otherwise. Plasma triglyceride concentrations were log, transformed before statistical analysis to achieve a normal distribution. Transformation was not required for other baseline plasma levels. Between-group comparisons for cardinal measurements were analyzed by unpaired t test, whereas proportions were analyzed by χ² test. ANOVA and repeated measures MANOVA were used as indicated in the text. Multiple regression analysis was undertaken with a stepped entry and removal method...
with the probability of F to enter variables being set at 0.05 and the probability of F to remove variables being set at 0.1. Statistical significance was assumed for P < 0.05.

**Ethics**

The study was approved by the local ethics committee and all participants signed an informed consent form at the time of their enrolment into the study.

**Results**

Data on LDL particle sizes, determined by gradient gel electrophoresis, and apo E phenotypes were available for 51 of the CHD cases and 161 asymptomatic controls. CHD cases and controls were well matched in age, gender, and total plasma cholesterol, but more CHD cases than controls were receiving blood pressure lowering medication (Table 1). The effect of apo E phenotype on LDL particle diameters and other parameters is shown in Table 2. Both E2/E3 (24.77 ± 0.09 nm, n = 30) and E2/E4 (24.69 ± 0.08 nm, n = 6) had smaller mean LDL diameters than those with E3/E3 (24.95 ± 0.03 nm, n = 161). Subjects with an e2 allele had significantly smaller LDL particles than subjects without this allele (Table 2). The effect of possession of an e2 allele persisted with inclusion of plasma triglyceride as a covariate in an ANOVA in which both triglyceride (P = 0.014) and e2 status (P = 0.017) were significant. Similar trends were seen when males and females were analyzed separately (Table 2), although differences were not statistically significant (P = 0.06 and 0.08, respectively). To further establish an independent effect of e2, multiple regression analyses were performed without and with inclusion of e2 status in the list of independent variables (Table 3). The inclusion of e2 status resulted in a significant increase in multiple r (Tables 1 and 2), with 45% of these receiving beta adrenoceptor antagonists and 22.5% a diuretic; other medication included calcium channel blockers, ACE inhibitors, alpha adrenoceptor antagonists, and centrally active agents. The use of blood pressure lowering medication was almost identical between those with and

**TABLE 2. LDL Diameters by Apo E Phenotype**

<table>
<thead>
<tr>
<th>Apo E</th>
<th>LDL Dia (nm)</th>
<th>Age (y)</th>
<th>BMI (kg/m²)</th>
<th>Total chol (mmol/l)</th>
<th>Triglyceride (mmol/l)</th>
<th>Glucose (mmol/l)</th>
<th>NEFA (mmol/l)</th>
<th>Insulin (UI/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2/E3</td>
<td>24.86 ± 0.10 (15)</td>
<td>62.5 ± 1.4</td>
<td>26.5 ± 0.3</td>
<td>1.40 ± 0.30</td>
<td>6.10 ± 0.10</td>
<td>0.92 ± 0.04</td>
<td>6.94 ± 0.57</td>
<td>7.32 ± 0.34</td>
</tr>
<tr>
<td>E2/E4</td>
<td>24.89 ± 0.12 (6)</td>
<td>62.0 ± 1.6</td>
<td>26.0 ± 0.3</td>
<td>1.45 ± 0.30</td>
<td>6.10 ± 0.10</td>
<td>0.92 ± 0.04</td>
<td>6.94 ± 0.57</td>
<td>7.32 ± 0.34</td>
</tr>
<tr>
<td>E3/E3</td>
<td>24.94 ± 0.15 (2)</td>
<td>62.6 ± 1.4</td>
<td>26.0 ± 0.3</td>
<td>1.40 ± 0.30</td>
<td>6.09 ± 0.10</td>
<td>0.92 ± 0.04</td>
<td>6.95 ± 0.57</td>
<td>7.32 ± 0.34</td>
</tr>
<tr>
<td>E3/E4</td>
<td>25.07 ± 0.17 (6)</td>
<td>62.1 ± 1.4</td>
<td>26.1 ± 0.3</td>
<td>1.45 ± 0.30</td>
<td>6.10 ± 0.10</td>
<td>0.92 ± 0.04</td>
<td>6.94 ± 0.57</td>
<td>7.32 ± 0.34</td>
</tr>
<tr>
<td>E4/E4</td>
<td>25.56 ± 0.10 (5)</td>
<td>62.5 ± 1.4</td>
<td>26.0 ± 0.3</td>
<td>1.40 ± 0.30</td>
<td>6.09 ± 0.10</td>
<td>0.92 ± 0.04</td>
<td>6.95 ± 0.57</td>
<td>7.32 ± 0.34</td>
</tr>
</tbody>
</table>

**TABLE 3. Multiple Regression Analysis**

<table>
<thead>
<tr>
<th>Gender</th>
<th>BMI</th>
<th>Fasting Triglyceride</th>
<th>e2 Allele</th>
<th>e4 Allele</th>
<th>Multiple r</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL Diameter</td>
<td>0.187</td>
<td>-0.181</td>
<td>-0.10</td>
<td>xxxxx</td>
<td>xxxxx</td>
</tr>
<tr>
<td>Model 1</td>
<td>(0.006)</td>
<td>(0.007)</td>
<td>(&gt;0.1)</td>
<td>(&lt;0.000)</td>
<td>(&lt;0.000)</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.019</td>
<td>-0.159</td>
<td>-0.119</td>
<td>-0.143</td>
<td>xxxxx</td>
</tr>
<tr>
<td></td>
<td>(0.003)</td>
<td>(0.020)</td>
<td>(0.086)</td>
<td>(0.033)</td>
<td>(&lt;0.000)</td>
</tr>
<tr>
<td>HDL Diameter</td>
<td>0.047</td>
<td>0.031</td>
<td>-0.269</td>
<td>xxxxx</td>
<td>xxxxx</td>
</tr>
<tr>
<td>Model 1</td>
<td>(0.67)</td>
<td>(0.79)</td>
<td>(0.016)</td>
<td>(&lt;0.016)</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>0.003</td>
<td>-0.32</td>
<td>-0.020</td>
<td>xxxxx</td>
<td>-0.399</td>
</tr>
<tr>
<td></td>
<td>(0.97)</td>
<td>(0.76)</td>
<td>(0.064)</td>
<td>(0.002)</td>
<td>(0.002)</td>
</tr>
</tbody>
</table>

The Table shows the results of multiple regression analyses with fasting LDL or HDL particle diameters as the dependent variables. Analyses were conducted with or without (xxxxx) the inclusion of e2 (for LDL particles) or e4 (for HDL particles) status. Results are shown as beta coefficients with corresponding P values in brackets. In LDL Model 2, the significance (P) of the other terms was plasma NEFA (0.09), insulin (0.20), cholesterol (0.26), glucose (0.82), CHD status (0.27), and use of blood pressure medication (0.78). In HDL Model 2, the significance of the other terms was plasma NEFA (0.22), cholesterol (0.76), insulin (0.81), glucose (0.82), CHD status (0.29), and use of blood pressure lowering medication (0.97).
TABLE 4. HDL Diameters by Apo E Phenotype

<table>
<thead>
<tr>
<th>Apo E Phenotype</th>
<th>Total n (CHD)</th>
<th>E2/E3</th>
<th>E2/E4</th>
<th>E3/E3</th>
<th>E3/E4</th>
<th>E4/E4</th>
<th>No e4</th>
<th>e4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL Diameter (nm)</td>
<td>11 (3)</td>
<td>3 (2)</td>
<td>61 (30)</td>
<td>21 (12)</td>
<td>1 (1)</td>
<td>72</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>8.33 ± 0.07</td>
<td>7.57 ± 0.34</td>
<td>8.40 ± 0.05</td>
<td>8.18 ± 0.06</td>
<td>8.11</td>
<td>8.39 ± 0.05*</td>
<td>8.09 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>5 hours Postprandial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL Diameter (nm)</td>
<td>8.43 ± 0.05</td>
<td>7.81 ± 0.19</td>
<td>8.43 ± 0.03</td>
<td>8.28 ± 0.07</td>
<td>8.11</td>
<td>8.43 ± 0.03*</td>
<td>8.22 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>2.57 ± 0.35</td>
<td>2.68 ± 0.45</td>
<td>2.78 ± 0.14</td>
<td>3.86 ± 0.42</td>
<td>4.45</td>
<td>2.75 ± 0.13*</td>
<td>3.73 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>8 hours Postprandial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL Diameter (nm)</td>
<td>8.54 ± 0.07</td>
<td>7.90 ± 0.39</td>
<td>8.59 ± 0.07</td>
<td>8.26 ± 0.08</td>
<td>8.52</td>
<td>8.58 ± 0.08*</td>
<td>8.21 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>2.22 ± 0.37</td>
<td>2.22 ± 0.38</td>
<td>1.90 ± 0.12</td>
<td>2.57 ± 0.27</td>
<td>2.57</td>
<td>1.94 ± 0.12*</td>
<td>2.53 ± 0.23</td>
<td></td>
</tr>
</tbody>
</table>

The table shows HDL particle diameters and plasma triglyceride concentrations for fasting and 5 and 8 hours postprandial samples. *P<0.05 for the difference between subjects with or without an e4 allele.

Discussion

The principal new findings of this study were that the diameter of the most prevalent LDL species was smaller in subjects with an e2 allele (Table 2). Furthermore, use of blood pressure medication did not make a significant contribution in the multiple regression analyses (Table 3). There was no influence of the presence of an e4 allele on LDL particle diameters with mean particle sizes of 24.87 ± 0.05 and 24.92 ± 0.03 nm for those with and without an e4 allele, respectively.

HDL particle diameters and compositions were available for 48 CHD cases and 49 controls both fasting and 5 and 8 hours after the fat-rich meal. There was a significant inverse correlation between the diameter of the most prevalent HDL species and plasma triglyceride for fasting (r = −0.30, P < 0.01) and postprandial samples. The influence of apo E phenotype on HDL diameters and plasma triglyceride concentrations is shown in Table 4. E2/E4, E3/E4, and E4/E4 phenotypes had smaller mean HDL diameters than E3/E3. Subjects with an e4 allele had significantly smaller HDL particles both fasting and postprandially than those without this allele (Table 4). There was no significant difference in diameter between subjects with or without an e2 allele (data not shown). In a repeated measures MANOVA including postprandial time, triglyceride concentration, and e4 status, both e4 status (P < 0.01) and triglyceride (P < 0.05) were significant. To further establish an independent effect of e4 status, multiple regression analyses were performed without and with inclusion of e4 status as an independent variable (Table 3). Although plasma triglyceride in the absence of e4 was significant (P = 0.016), there was a further increase in multiple r with inclusion of e4, but only e4 status then remained a significant term. In contrast to LDL diameters, neither gender or BMI was significant in these analyses. The mean size of the second largest HDL peak was also greater in those without an e4 with values in the fasting state of 8.36 ± 0.12 and 8.03 ± 0.14 nm, respectively (P = 0.09).

Under fasting conditions there was no difference in the diameter of the most prevalent species between CHD cases and controls (8.28 ± 0.04 vs 8.28 ± 0.03 nm). A postprandial increase in the diameter of the most prevalent species was evident for both CHD cases and controls but with a tendency (P = 0.09) for a smaller increase in cases than controls (Figure 2). The postprandial increase was also significant when subjects were subdivided on the basis of their fasting samples into those whose most numerous HDL species lay in the range of HDL3a or HDL3b (Table 5). However, for those with HDL3a at baseline, the postprandial increase was seen for control subjects but not those with CHD (Table 5).

In addition to particle size determination by gradient gel electrophoresis, compositional analysis after density gradient ultracentrifugation was also available for the fasting and postprandial samples. Compositional analysis (Figure 3) is presented for fractions 11 to 19 (total HDL) as well as fractions 11 to 15 (HDL2) and 16 to 19 (HDL3). The triglyceride content rose significantly in HDL2 by 5 hours and in both total HDL and HDL3 by 8 hours. Similar trends were evident in HDL3 but failed to reach statistical significance. Phospholipid content rose significantly in total, HDL2, and HDL3 by 8 hours but showed a significant temporary fall at 5 hours in both total and HDL3. Cholesterol ester fell significantly at both time points in both HDL subfractions. Subjects with CHD showed a less marked postprandial increase in triglyceride content in HDL (Figure 4), particularly at 5 hours, than asymptomatic controls with the difference in triglyceride profiles being significant (P < 0.05) when analyzed by repeated measures MANOVA.
subjects with an ε2 allele and the diameter of the most prevalent HDL species was smaller in the presence of an ε4 allele. Although both LDL and HDL particle diameters were shown to be related to the plasma triglyceride concentration, the associations with ε alleles were still present with inclusion of triglyceride as a covariate. In addition, in multiple regression analysis LDL particle size was dependent on gender, BMI, and the presence (or absence) of an ε2 allele, with triglyceride no longer being significant. The diameter of HDL particles was significantly dependent solely on the presence of an ε4 allele. HDL particle diameters increased postprandially due to triglyceride enrichment, particularly in those subjects without CHD. However, an additional influence of ε4 allele status was also evident postprandially.

CHD subjects in the current study were identified on the basis of a recent history of exertional angina, identified from a chest pain questionnaire together with a positive exercise ECG test. Subjects were then investigated within days of the ECG test. This approach was adopted to minimize interference from changes in diet and lifestyle that may have altered lipoprotein particle sizes and plasma triglyceride. A positive stress ECG in the presence of a history of exertional chest pain has a very high (>.95%) specificity and sensitivity for the detection of angiographically positive coronary disease in men but may yield some false-positive results in women, although some such women may indeed have myocardial ischemia on the basis of other disease, such as coronary artery spasm or microvascular disease. Control subjects had not experienced exertional chest pain (or suffered a previous myocardial infarction) but did not undergo an exercise ECG test. However, because the estimated percentage of males and

### Table 5. Test Meal Effects on HDL Subfractions

<table>
<thead>
<tr>
<th>HDL Subfraction</th>
<th>Fasting</th>
<th>5 hours Postprandial</th>
<th>8 hours Postprandial</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL3α**##</td>
<td>All (64)</td>
<td>8.40±0.01</td>
<td>8.44±0.03</td>
</tr>
<tr>
<td></td>
<td>CHD (32)</td>
<td>8.41±0.01</td>
<td>8.40±0.05</td>
</tr>
<tr>
<td></td>
<td>Control (32)</td>
<td>8.38±0.02</td>
<td>8.47±0.04</td>
</tr>
<tr>
<td>HDL3β***</td>
<td>All (27)</td>
<td>8.10±0.01</td>
<td>8.26±0.03</td>
</tr>
<tr>
<td></td>
<td>CHD (13)</td>
<td>8.11±0.02</td>
<td>8.32±0.06</td>
</tr>
<tr>
<td></td>
<td>Control (14)</td>
<td>8.08±0.01</td>
<td>8.21±0.03</td>
</tr>
</tbody>
</table>

Mean±SEM for the most prominent HDL species (nm) before and 5 and 8 hours after the test meal. Subjects were classified with respect to their fasting HDL diameters. Results are shown for all subjects and for CHD cases and controls separately with the number of respective subjects in parentheses. Statistical analysis was by repeated measures MANOVA with CHD status as a categorical variable.

**P<0.01 and ***P<0.001 for the effect of time. ##P<0.01 for a significant interaction between CHD status and time.

#### Figure 3. Changes in lipid composition of HDL after fat ingestion. Concentration of phospholipid (circles), triglyceride (squares), free cholesterol (upright triangles), and cholesteryl ester (upside down triangles) in HDL (A) and the subclasses, HDL2 (B), and HDL3 (C) over the postprandial period (n=97). *, **, and # indicate P<0.05 for fasting versus 5 hours, fasting versus 8 hours, and 5 hours versus 8 hours, respectively, by repeated measures MANOVA.

#### Figure 4. Distribution of triglyceride between lipoproteins determined after density gradient ultracentrifugation. Profiles are shown for coronary heart disease cases (circles, n=48) and controls (squares, n=49) over the postprandial period: A, fasting; B, 5 hours; and C, 8 hours. Density (g/mL) was determined by refractometry of the individual fractions. Triglyceride profiles of CHD cases and controls were significantly different (P<0.05) when analyzed by repeated measures MANOVA.
females aged 55 to 64 with any form of heart disease is approximately 9% for men and 4.5% for women, and because most of these with CHD would be expected to be symptomatic, it is unlikely that this would have introduced a significant error.

Approximately 14% of the total cohort was receiving medication to lower blood pressure. There was an increased use of antihypertensive medication among those diagnosed with CHD, consistent with the role of hypertension as a risk factor for CHD. However, the use of blood pressure lowering medication was not significantly different among different apo E phenotypes, and the use of such medication was not a significant factor in any of the multiple regression analyses of lipoprotein particle size, achieving probability value of only 0.78 (LDL diameter) and 0.97 (HDL diameter). It is therefore unlikely that the continued use of such medication contributed to the differences observed in the present study. We elected not to discontinue medication before the study because we wished to investigate CHD subjects under the conditions pertaining during the development of their disease.

Previous studies of the relationship between LDL particle size and apo E phenotype have given conflicting results and differ in a number of ways from the present study. The subjects in the study of Zhao et al were all asymptomatic men and 35 years old, and as found in other studies, there was a negative correlation with plasma triglyceride that persisted in a multiple regression analysis, although in that study BMI was not included as a variable. However, the LDL particle diameter was not different between subjects with an E3/E3 phenotype compared with those with either an e2 or an e4 allele. In the study by Haffner et al, the mean LDL diameter was less in those with an E3/E4 phenotype than those with an E3/E2 or E3/E3. This difference was significant for both men and women after adjustment for age, ethnicity, BMI, waist hip ratio, triglyceride, HDL cholesterol, and fasting insulin. In the study of Schaefer et al, an effect of apo E phenotype was evident for male but not female participants in the Framingham Offspring Study. In that study, an LDL particle score was used that took account of the presence of up to 3 LDL peaks. The authors of the study comment that there was a trend for smaller LDL particles from E2 to E4 subjects, although this was only significant for men. Inspection of the data in Table 1 of Schaefer et al suggests that men with an E2/E2 phenotype had markedly larger LDL particles than those with other phenotypes and this may have contributed to the graded effect of phenotype observed. A similar finding was present for women although only 4 of the 1805 women were of this phenotype in contrast to 10 of 1764 men that may have contributed to the lack of significance in elation to this group. As in the present study, the particle diameters data were adjusted for plasma triglyceride levels and, in addition, for age and BMI. There were no subjects in the present study with an E2/E2 phenotype so that direct comparison on this point is not possible. LDL diameters in the current study were somewhat smaller than in some other reported studies. As discussed previously, the use of 3% to 13% polyacrylamide gradient gels, rather than the 2% to 12% used in many other studies, may have accounted for this difference. The relationships between LDL diameters and a number of other parameters reported here and previously are consistent with the literature and suggest that although absolute diameters may have been slightly different the rank ordering of diameters was maintained.

Examination of fasting and postprandial HDL particle sizes according to apo E phenotype indicated that the presence of an e4 allele was associated with smaller HDL particles. As expected there was an inverse relation between HDL particle size and plasma triglyceride levels, and as already discussed, the presence of an e4 allele was itself associated with an increased plasma triglyceride level. However, the influence of apo E phenotype was still present when adjustment was made for plasma triglyceride level. The relationship between triglyceride level and HDL particle size can be explained in terms of initial triglyceride enrichment of HDL in exchange for cholesterol ester with subsequent lipolysis leading to the production of smaller HDL particles. Although the presence of elevated triglyceride levels in subjects with an e4 allele would be expected to facilitate the operation of this mechanism, it appears that other mechanisms must also be operating. It is also of interest to note that the postprandial change in HDL particle size is large enough, despite only a modest degree of lipemia, to result in reclassification of HDL particle subclasses.

Postprandial changes to HDL and the mechanisms involved in its modification are well documented. After the consumption of a fat-rich meal, HDL becomes enriched with triglyceride in exchange for cholesteryl ester. With the increase in HDL triglyceride content, susceptibility to hydrolysis, particularly by hepatic lipase, increases. In the current study, HDL triglyceride concentrations increased and cholesteryl ester concentrations fell throughout the postprandial period. In addition, HDL phospholipid concentration fell at 5 hours but then increased, particularly in HDL2, by 8 hours. This is consistent with a number of other studies showing changes in the postprandial composition of HDL2 and HDL3 directly related with the extent of postprandial lipemia. In another study, HDL exhibited postprandial enrichment in triglyceride in exchange for cholesteryl ester 4 to 8 hours after a fat load, and in addition, the phospholipid content of HDL, specifically HDL3, increased after 8 hours. Due to the complex nature of the changes in lipoprotein metabolism induced by the consumption of an oral fat load, as indicated by studies that have investigated lipoprotein size and composition, CETP and lecithin:cholesterol acyltransferase activities and the concentrations of the lipases, it is evident that compositional findings may be different depending on the particular time points chosen for analysis. In the current study, we chose a time point corresponding to the peak plasma triglyceride level, but it is possible that earlier time points may have yielded different findings.

We also found that the postprandial increase in HDL triglyceride concentration was smaller in subjects with newly-diagnosed coronary disease than in control subjects and that this was reflected in the differing postprandial HDL size increases between the 2 groups. Although only tending toward a significant difference between the groups when all subjects were combined, when classified according to the predominant HDL species in the fasting state, CHD subjects with a majority of HDL3 particles at baseline showed significantly less postprandial increase in particle diameter than asymptomatic controls. Such a difference cannot be due
to a difference in postprandial triglyceride load because we have previously shown higher plasma triglyceride levels postprandially in these subjects.6 Karpe et al found a 1% to 2% increase in HDL particle size 6 hours after intake of an oral fat load, although this was not different between men with previous myocardial infarction and controls.52 Lewis and Cabana did not find any such shift in the particle size of either HDL$_2$ or HDL$_3$ but rather that the ratio of HDL$_3$ to HDL$_2$ increased postprandially.29 Analysis of the transfer of cholesterol ester from HDL to VLDL and LDL showed greater evidence of transfer in subjects with angiographic evidence of coronary disease than controls, although no examination of changes in HDL size were noted.53 Other studies confirm the increase in HDL$_2$ and HDL$_3$ size after the consumption of a fat load.47,54

The present study has demonstrated significant associations between apo E phenotypes, specifically the possession of e2 and e4 alleles and LDL and HDL particle sizes. However, it is important to note that this was not a representative population-based sample, and the effects observed may not be subject to generalization. The possible contribution of such associations to the observed relationship between apo E polymorphism and CHD events also remains to be clarified by prospective studies. Although plasma triglyceride is correlated with both LDL and HDL diameters, the present study also indicates that apo E polymorphisms influence LDL and HDL particle sizes by mechanisms independent of this association.

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References


Independent Effects of Apo E Phenotype and Plasma Triglyceride on Lipoprotein Particle Sizes in the Fasting and Postprandial States
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