Relation of Plasma Levels and Composition of Apolipoprotein B–Containing Lipoproteins to Angiographically Defined Coronary Artery Disease in Young Patients With Myocardial Infarction

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Background. Hypertriglyceridemia is a common metabolic disturbance in men <45 years old with myocardial infarction. To further investigate the relation between triglyceride-rich lipoproteins and severity of coronary atherosclerosis in this subset of postinfarction patients, apolipoprotein B–containing lipoproteins of 64 consecutive patients were subfractionated in connection with coronary angiography.

Methods and Results. Density-gradient ultracentrifugation of plasma and coronary angiography were performed 4 to 6 months after the myocardial infarction. Global coronary atherosclerosis and the number and severity of distinct stenoses were evaluated by semiquantitative analysis of 15 proximal coronary segments. The majority of the patients (60%) were hypertriglyceremic and had higher coronary scores than normotriglyceridemic patients. Of the major plasma lipoproteins, triglycerides and cholesterol in the low-density lipoprotein (LDL) fraction were associated with global coronary atherosclerosis, whereas LDL triglycerides and high-density lipoprotein (HDL) cholesterol correlated directly and inversely, respectively, with the coronary stenosis score. Plasma apolipoprotein B correlated with both coronary scores. The plasma concentrations of lipid and protein in the very-low-density lipoprotein (VLDL) subfractions (VLDL₁ through VLDL₃) and intermediate-density lipoprotein (IDL) did not correlate with either of the coronary scores, whereas the concentration of triglycerides in dense LDL (density >1.040 kg/L) was strongly associated with both coronary scores. Compositional analysis of the smallest VLDL particles (VLDL₀) and IDL revealed a correlation between the number of cholesteryl ester molecules in small VLDL and global coronary atherosclerosis in hypertriglyceridemic patients.

Conclusions. Global coronary atherosclerosis and distinct stenoses in young postinfarction patients are associated with the number of apolipoprotein B–containing particles in plasma and the concentration of LDL triglyceride. Specifically, dense triglyceride-rich LDL particles and, in hypertriglyceridemic patients, small cholesteryl ester–rich VLDL particles relate to coronary artery disease severity. (Circulation. 1993;88[part 1]:2180-2189.)

Key Words • hyperlipoproteinemia • cholesterol • lipoproteins

Prospective epidemiological studies have demonstrated an independent association between the concentrations of low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol and the incidence of coronary heart disease (CHD), particularly in men,1-3 whereas the results regarding very-low-density lipoprotein (VLDL) lipids have been conflicting in this respect.4-6 In cross-sectional studies, the concentration of the structural protein of VLDL and LDL, apolipoprotein B, has been considered a better discriminator between patients with manifest CHD and healthy control subjects or between patients with or without angiographically documented coronary artery disease (CAD) than the levels of lipoprotein lipids.7-9

Recent studies have related the concentrations of lipoprotein lipids and apolipoproteins to severity as well as progression of CAD assessed by angiography. Of the major lipoproteins, high levels of LDL cholesterol and low levels of HDL cholesterol have been consistently linked to more extensive CAD.10 Apolipoprotein B concentrations have in some studies been a better predictor of lesion severity than LDL and HDL cholesterol levels.11-13 Several recent studies have shown that dietary or drug intervention primarily aiming at cholesterol lowering retards progression and in some instances even results in regression of coronary atherosclerosis.14-17 In contrast, the relation of triglyceride-rich lipoproteins to CAD remains unclear, even though some studies have shown positive univariate associations for the concentrations of VLDL and intermediate-density lipoprotein (IDL) lipids.18-25
Both VLDL and LDL are heterogeneous populations of particles varying in origin, structure, and interactions with cellular receptors. Subfractionation of LDL, either by gradient gel electrophoresis or by density-gradient ultracentrifugation, has shown that small and dense LDL particles are more abundant in patients with CHD, whereas the corresponding information on VLDL subclasses is lacking.

Hypertriglyceridemia is a common metabolic disturbance in men with myocardial infarction before the age of 45 years. Furthermore, previous studies in this group of patients have revealed positive associations between the apolipoprotein B level in plasma and the extent of CAD and between the triglyceride content of the dense LDL fraction and global severity and rate of progression of CAD. To further investigate the relation between triglyceride-rich lipoproteins and the severity of coronary atherosclerosis in this subset of postinfarction patients, subfractionation of apolipoprotein B–containing lipoproteins (VLDL, IDL, and LDL) was performed in connection with coronary angiography in consecutive patients admitted to the emergency hospitals in the Stockholm metropolitan area.

Methods

Subjects

Sixty-four men with a first myocardial infarction before the age of 45 years were studied consecutively. The patients had initially been admitted to the 10 hospitals in Stockholm County with a coronary care or intensive care unit between April 1989 and October 1990. They were subsequently referred to the Karolinska Hospital for metabolic and cardiological investigation. Metabolic and angiographic studies were performed 4 to 6 months after the acute event, when it was expected that acute-phase reactions resulting from myocardial damage had subsided. A total of 104 men fulfilled the criteria for participation in the study. Of the 40 patients not investigated, 3 died during the acute stage or the early postinfarction period and 10 declined to participate. Nine patients were excluded because of concomitant diseases such as manifest diabetes mellitus (n=2), heterozygous familial hypercholesterolemia (n=1), severely impaired renal function (n=4), or large cerebral infarction (n=2). A further 13 patients were not investigated for other reasons, particularly because of deficient laboratory capacity, and 5 patients were either referred later than 6 months after their infarctions or were unavailable to the research team. None of the patients eligible for the study had hypothyroidism.

Of the patients investigated, none were on lipid-lowering drugs, but all had been informed about a lipid-lowering diet in connection with the first visit to the outpatient clinic 6 weeks after admission to the referring intensive care units. The dietician’s instructions given to the patients aimed at a diet low in fat, rich in complex carbohydrates, and with a limited intake of alcohol. The percentage composition of the different sources of energy in the recommended diet was 10% to 15% protein, 30% fat, and the rest carbohydrates. The ratio of saturated to monounsaturated and polyunsaturated fat was 1:1:1.

Seventy-three healthy men with a similar age distribution (39.6±2.7 years [mean±SD]) were recruited by random selection of individuals born between 1947 and 1956 from a database consisting of all inhabitants in Stockholm County. Of those invited, 68% agreed to participate in the program, which included an interview to exclude individuals with a history of a prior myocardial infarction, angina pectoris, or any other severe illness and blood sampling for determination of plasma and lipoprotein lipids.

Blood Sampling

Blood samples for lipoprotein analyses were taken between 8 and 9 AM after 12 hours of fasting, during which time smokers were asked to refrain from smoking. All subjects were free of symptoms of infectious disease at the time of blood sampling. Venous blood for lipoprotein fractionation was drawn into precooled vacutainer tubes containing Na2EDTA (1.4 mg/mL) and placed in an ice bath. Plasma was then recovered by low-speed centrifugation (1400g, 20 minutes) at +1°C and kept at this temperature throughout the preparation procedures.

Lipoprotein Determinations

The major plasma lipoproteins (VLDL, LDL, and HDL) were determined by a combination of preparative ultracentrifugation and precipitation of apolipoprotein B–containing lipoproteins, followed by lipid analyses as described. The cutoff limits for lipoprotein phenotyping were set to the 90th percentiles of VLDL triglyceride (1.65 mmol/L) and LDL cholesterol values (5.35 mmol/L) in a control population.

VLDL and IDL Fractionation

VLDL subfractions (VLDL1, Svedberg flotation units >100; VLDL2, Sf 100 to 60; and VLDL3, Sf 60 to 20) and an IDL fraction were prepared by cumulative rate ultracentrifugation in a density gradient. The corresponding particle sizes of the lipoproteins measured by electron microscopy were VLDL1, 51.1±3.7 nm; VLDL2, 47.4±4.5 nm; VLDL3, 39.9±3.8 nm; and IDL, 31.5±2.0 nm (mean±SD). In short, plasma was adjusted to density 1.10 kg/L by addition of NaCl. A density gradient consisting of 4 mL of 1.10 kg/L density plasma and 3 mL 1.065, 1.020, and 1.006 kg/L NaCl solutions, respectively, was then formed in cellulose nitrate tubes (Ultraclear tubes, Beckman, Palo Alto, Calif) and centrifuged (Beckman L8-55 ultracentrifuge, 40 000 rpm) for 190 minutes in a Beckman SWT40 swinging bucket rotor at +1°C. The VLDL1 subfraction was then aspirated from the top of the tube, and density 1.006 kg/L NaCl solution was used for refilling before centrifugation was continued. The VLDL2 and VLDL3 subfractions were isolated after 170 minutes and 16 hours of further centrifugation, respectively, by the same procedure. IDL was harvested from the top 3 mL of the tube after VLDL2 had been aspirated and was subsequently concentrated 6.3 times by ultracentrifugation in a fixed-angle rotor for 20 hours (Beckman L8-55 ultracentrifuge, 40 000 rpm).

LDL Fractionation

LDL subfractions were separated by use of a modification of the density gradient ultracentrifugation procedure described by Chapman et al. LDL (density 1.019 to 1.063 kg/L) was isolated from plasma by two
consecutive runs in a Beckman 50.3 Ti fixed-angle rotor (Beckman L8-55 ultracentrifuge). A discontinuous density gradient was formed in cellulose nitrate tubes (Ultraclear tubes, Beckman) consisting of 4.5 mL of 1.054 kg/L NaBr solution, 3.5 mL of LDL at density 1.040 kg/L, and 2 mL each of 1.024 and 1.019 kg/L NaCl solution. Two gradients were constructed from each LDL preparation. The gradients were centrifuged in a Beckman SW40 Ti swinging bucket rotor for 65 hours at +1°C (Beckman L8-55 ultracentrifuge, 40,000 rpm). After the ultracentrifugation, 15 LDL subfractions of 0.8 mL were recovered with a fraction collector (FRAC-200, Pharmacia, Uppsala, Sweden) coupled to a continuous pump mechanism by puncture of the bottom of the tubes and upward displacement of the gradient with an inert, water-immiscible liquid of density 1.9 kg/L (Maxidens FC-43, Nycopem AS, Oslo, Norway). To control the density profile of the gradient after ultracentrifugation, gradients in which the isolated LDL had been replaced by a salt solution of density 1.040 kg/L were fractionated. The densities of the subfractions recovered were determined with a precision densitometer (Model DMA 60, Anton Paar, Graz, Austria). All salt solutions used to prepare the density gradients were adjusted to pH 7.4 and contained 0.02% sodium azide and 0.01% EDTA. Densities were verified to the fourth decimal place.

Lipid and Apolipoprotein Analyses

Free and esterified cholesterol (14106-14108 Merck Diagnostika, Darmstadt, Germany), triglycerides (877557 Boehringer Mannheim Diagnostica, Germany), and phospholipids (9990-54008 Wako Chemicals, Neuss, Germany) were determined in triplicate in plasma and the lipoprotein subfractions with enzymatic methods. Total and soluble protein were measured with the Lowry et al technique using bovine albumin as protein standard. All samples, including the standards, were extracted with chloroform after color development to remove any turbidity. Soluble protein in the VLDL subfractions and IDL was estimated after extraction with isopropanol. The content of apolipoprotein B was calculated as the difference between total and soluble protein. Apolipoprotein E phenotype was determined by isoelectric focusing on an isolated VLDL subfraction obtained by density gradient ultracentrifugation.

Oral Glucose Tolerance Test

Glucose was ingested in a dose of 1.75 g/kg body weight in 150 to 200 mL water flavored with lemon extract. Venous blood samples were collected before and 15, 30, 45, 60, and 120 minutes after glucose intake. Oral glucose tolerance was assessed according to criteria adopted from Reaven and coworkers.

Coronary Angiography

Percutaneous transfemoral angiography was performed according to a standard protocol and recorded on 35-mm cine film with cesium iodide–activated image intensifiers. All cine angiograms were assessed by one angiographer without knowledge of the patient’s clinical characteristics or biochemical profiles. The presence and severity of coronary atherosclerotic lesions were determined by use of a semiquantitative classification system in 15 proximal coronary arterial segments. Atherosclerotic lesions were defined as sharp-edged, plaque-like, or irregular indentations, often multiple, into the vessel lumen without features suggesting fibromuscular hyperplasia. Accordingly, a single stenosis with smooth contours or a single occlusion in the absence of additional changes in the same or any other coronary artery was not classified as atherosclerosis, whereas multiple lesions always were. Segments located distal to a total occlusion or distal to a significant stenosis in the absence of sufficient poststenotic contrast filling were not evaluated, nor were segments of a hypoplastic coronary artery. Points were assigned for both extension of atherosclerotic lesions and plaque size in each segment. The scores for extension and plaque size were then multiplied to produce a segmental atherosclerosis severity score. A global coronary atherosclerosis score was obtained by dividing the sum of all segmental atherosclerosis scores by the number of segments accessible to evaluation. The estimate of global coronary atherosclerosis obtained by this scoring system included both diffuse lesions and distinct hemodynamically significant stenosis. A global stenosis score was calculated as the global atherosclerosis score but included only lesions that reduced the lumen diameter ≥25%. The method of determining the coronary atherosclerosis and stenosis scores and the statistical analyses of the reliability of this system have been described in detail elsewhere.

Calculations and Statistical Methods

The composition of VLDL particles was calculated by use of the model developed by Miller and Small. This computer program was modified to fit a Macintosh computer. The program calculates the number of molecules of lipid constituents (divided into oil and surface phases) and lipoprotein size from the weight percentage of triglyceride, total and free cholesterol, phospholipids, and protein. Calculation of compositional characteristics was possible only for the VLDL subfraction and IDL of all patients, since the plasma levels of lipid and protein in VLDL, and VLDL were occasionally low and obviously out of proportion in some normotriglyceridemic patients.

Statistical methods were applied as recommended by Snedecor and Cochran. Conventional methods were used for calculations of medians, means, and SDs. Coefficients of skewness were calculated to test deviations from a normal distribution. Logarithmic transformations were performed on all skewed lipid and protein variables (plasma concentrations in VLDL and IDL) by ANCOVA using body mass index (BMI) as the covariant. Categorical variables were analyzed with the χ² test. Relations between lipoprotein variables and coronary angiography scores were analyzed by computing simple and partial correlation coefficients. Multiple stepwise linear regression analysis was performed to analyze the independent relations between lipoprotein
variables and coronary angiography scores. The variable with the highest partial correlation coefficient was entered at each step until no variable remained with an F value (F to enter) of 4 or more.

Results

Patient and Control Characteristics

Basic characteristics of the patient group are shown in Table 1. The large majority of the patients were smokers until their myocardial infarction but had quit smoking by the time of the metabolic investigation. About one fourth of the patients had a prior diagnosis of hypertension, and all except three were on \( \beta \)-adrenergic blocker medication. Of the 64 patients investigated, only 20 were normolipidemic, whereas the majority had hypertriglyceridemic phenotypes. Apolipoprotein E isoforms were determined in 61 patients, a majority of whom had a 3/3 phenotype. No patient had a 2/2 phenotype. Of the remaining 3 patients who were not subjected to apolipoprotein E isoform determination, none had hypertriglyceridemia together with a ratio of cholesterol to triglyceride in VLDL >0.77, which would indicate type III hyperlipoproteinemia. Single-vessel disease was the most common CAD category, followed by two-vessel disease. Minor proportions of the patients had either three-vessel or zero-vessel CAD. The coronary angiograms of 5 individuals showed no signs of atherosclerosis.

The patients had considerably higher BMIs than the control subjects (28.2±4.1 versus 24.4±2.8 kg/m\(^2\) [mean±SD], \( P < .001 \)), and a comparable proportion of present smokers was found in the two groups (33% versus 34%). Compared with the controls, marked elevations of VLDL and LDL triglyceride and cholesterol concentrations were found among the patients, whereas the level of HDL cholesterol in the patient group was lower (Table 2). Control for the confounding effect of the group difference in BMI by ANCOVA did not result in elimination of any statistically significant case-control differences for the plasma concentrations of major lipoproteins (data not shown). The potential influence of ongoing \( \beta \)-blocker medication in the vast majority of the patients could not be controlled for, since so few patients were without this medication.

Relations of Plasma Lipoproteins to Coronary Angiography Scores

The global coronary atherosclerosis scores were higher, although not significantly, in all hyperlipoproteinemic groups than in the normolipidemic patients (Table 2). Both coronary angiography scores were significantly higher (\( P < .05 \)) in hypertriglyceridemic (lipoprotein phenotypes IIB and IV) patients than in normotriglyceridemic (normolipidemic and lipoprotein phenotype IIA) patients (2.4±1.7 versus 1.4±1.5 for coronary atherosclerosis and 1.8±1.8 versus 1.0±1.1 for coronary stenosis).

Age and BMI correlated directly with the two global coronary angiography scores (atherosclerosis score, \( r = .27 \) and \( r = .23 \), respectively, and stenosis score, \( r = .28 \) and \( r = .26 \), respectively). As a consequence, all regression analyses were performed with age as a forced variable, whereas computations were made both with and without BMI as a forced variable, since it can be argued that BMI influences plasma lipoproteins, which, in turn, are involved in the atherogenic processes. Tables 3 through 5 show partial correlation coefficients with age as a forced variable. Introducing BMI into the equation weakened the relations between lipoprotein variables and coronary scores without eliminating any significant associations, with a few exceptions that are commented on further.

Major plasma lipoproteins. Among determinations of the major plasma lipoproteins, both cholesterol and triglyceride concentrations in the LDL fraction related significantly to the coronary atherosclerosis score, whereas only the LDL triglyceride level correlated
TABLE 2. Major Plasma Lipoproteins and Coronary Angiography Scores

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n=73)</th>
<th>IV (n=30)</th>
<th>II B (n=7)</th>
<th>IIA (n=7)</th>
<th>NLP (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triglycerides</strong></td>
<td>1.8±0.7</td>
<td>2.3±1.5</td>
<td>2.2±1.7</td>
<td>1.7±0.9</td>
<td>1.4±0.8</td>
</tr>
<tr>
<td><strong>LDL, mmol/L</strong></td>
<td>3.6±1.2</td>
<td>4.1±1.5</td>
<td>3.6±1.2</td>
<td>3.8±1.4</td>
<td>3.4±1.2</td>
</tr>
<tr>
<td><strong>HDL, mmol/L</strong></td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td><strong>Plasma apolipoprotein B, mg/L</strong></td>
<td>1.2±0.4</td>
<td>1.2±0.4</td>
<td>1.2±0.4</td>
<td>1.2±0.4</td>
<td>1.2±0.4</td>
</tr>
</tbody>
</table>

Values are mean±SD or median (ranges). NLP indicates normolipoproteinemic; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ND, not determined. Normal: VLDL triglycerides <1.65 mmol/L, LDL cholesterol <5.35 mmol/L; II A, VLDL triglycerides <1.65 mmol/L, LDL cholesterol >5.35 mmol/L; II B, VLDL triglycerides >1.65 mmol/L, LDL cholesterol >5.35 mmol/L; IV, VLDL triglycerides >1.65 mmol/L, LDL cholesterol <5.35 mmol/L.

*P<.05, †P<.001 between all patients and control subjects (Student's t test).

significantly with the stenosis score (Table 3). The only significant relation to coronary scores for the VLDL and HDL fractions was found between the HDL cholesterol concentration and the stenosis score. This inverse correlation became insignificant when BMI was included as a forced variable. Plasma apolipoprotein B concentration, an index of VLDL and LDL particle number, correlated with both severity of coronary atherosclerosis and stenoses (Table 3). In multiple stepwise regression analysis, only the plasma apolipoprotein B level was independently related to the coronary atherosclerosis score (multiple \( R^2 = .24 \)), and the HDL cholesterol concentration was independently associated with the coronary stenosis score (multiple \( R^2 = .18 \)). Addition of BMI to the multiple stepwise regression analysis added a further 10% to the variation in the stenosis score.

Subfractions of apolipoprotein B–containing lipoproteins. No significant relations were found between the concentrations of lipid and protein in VLDL\(_1\) through VLDL\(_3\) and IDL and the coronary scores (data not shown).

Except for a positive association between triglycerides and the severity of coronary atherosclerosis, no significant associations were found for lipid and protein concentrations in light LDL (density <1.040 kg/L) (Table 4). In contrast, all constituents of dense LDL (density >1.040 kg/L) correlated significantly with both coronary angiography scores. The simple correlations for two of these relations are illustrated in Figs 1A and 1B. No significant relations to the coronary angiography scores were found for the ratio of triglyceride to protein in dense LDL, namely, the triglyceride content per dense LDL particle \( r = .14 \) for atherosclerosis and \( r = .18 \) for stenosis scores, respectively.

When the particle composition of lipoproteins in VLDL\(_1\) and IDL was related to the coronary scores, associations were found between the number of molecules of cholesterol and cholesteryl ester in VLDL\(_3\),...
TABLE 4. Partial Correlation Coefficients Between Plasma Concentrations of Lipid and Protein in Light and Dense LDL and Coronary Angiography Scores (n=60)

<table>
<thead>
<tr>
<th></th>
<th>Atherosclerosis Score</th>
<th>Stenosis Score</th>
<th>Atherosclerosis Score</th>
<th>Stenosis Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light LDL (density &lt;1.040 kg/L)</td>
<td></td>
<td>Dense LDL (density &gt;1.040 kg/L)</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>.369†</td>
<td>.188</td>
<td>.443†</td>
<td>.411†</td>
</tr>
<tr>
<td>Cholesteryl ester</td>
<td>-.006</td>
<td>-.002</td>
<td>.344†</td>
<td>.323†</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>.019</td>
<td>-.027</td>
<td>.341†</td>
<td>.353†</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>.049</td>
<td>-.029</td>
<td>.325*</td>
<td>.330†</td>
</tr>
<tr>
<td>Protein</td>
<td>.026</td>
<td>-.044</td>
<td>.324*</td>
<td>.331†</td>
</tr>
</tbody>
</table>

*P<.05, †P<.01, ‡P<.001.

LDL indicates low-density lipoprotein. Patients' ages had first been entered in the regression model.

Furthermore, a significant relation was also found between the number of molecules of cholesteryl ester in VLDL3 and the coronary atherosclerosis score in the hypertriglyceridemic patient group. The simple correlations for these relations are presented in Figs 2A and 2B. No significant associations were found between the composition of IDL and the coronary angiography scores.

When plasma concentrations of lipid and protein in dense LDL were included in multiple stepwise regression analysis along with HDL cholesterol and apolipoprotein B in plasma, 29% of the variability in global coronary atherosclerosis score could be accounted for by age and dense LDL triglycerides. In comparison, age and dense LDL triglycerides explained 32% of the variation in the coronary stenosis score. Addition of other lipoprotein variables, although statistically correlated with the stenosis score when age had been taken into account, did not increase the value of multiple $R^2$, whereas addition of BMI added another 12% to the variation in the coronary stenosis score (Table 6).

In the hypertriglyceridemic patients, 49% of the variability in the coronary atherosclerosis score could be explained by age and the number of cholesteryl ester molecules in VLDL3. Addition of other lipoprotein variables, plasma apolipoprotein B, or dense LDL triglycerides did not result in a significant increase in the multiple $R^2$.

**Interrelations Between Lipoprotein Fractions and Subfractions**

The concentrations of triglyceride and protein in dense LDL were strongly associated with the apolipoprotein B concentration in plasma ($r=.51$ and $r=.52$, respectively; $P<.001$). Dense LDL triglyceride but not protein correlated with the VLDL cholesterol ($r=.37$, $P<.01$) and the VLDL triglyceride ($r=.34$, $P<.01$) concentrations. Similar relations were found between the triglyceride but not the protein concentration in dense LDL and the number of molecules of cholesteryl ester in VLDL3 particles ($r=.33$, $P<.05$) and the HDL cholesterol concentration ($r=.40$, $P<.01$), respectively (Figs 3A and 3B). In all, 43% of the variability in dense LDL triglycerides could be explained by plasma apolipoprotein B, number of cholesteryl ester molecules in VLDL3, and HDL cholesterol (Table 6). No associations were found between the number of cholesteryl ester molecules in VLDL3 and lipoprotein lipids or apolipoprotein B in plasma.

**Nonlipid Risk Factors in Patients Grouped by the Degree of CAD**

The proportions of patients who were smokers or hypertensive or had decreased oral glucose tolerance in each tertile of the coronary angiography scores were tested by $\chi^2$ test (data not shown). Only hypertension related significantly to both coronary atherosclerosis and stenosis ($P<.05$ and $P<.01$), whereas smoking was more common among patients with a high stenosis score ($P<.01$).

**Discussion**

The majority of the patients in this angiographic study, which focused on the relations of apolipoprotein B–con-
taining lipoproteins to global coronary atherosclerosis and stenoses, had hyperlipoproteinemia, predominantly type IV hypertriglyceridemia. The hypertriglyceridemic patients had higher coronary angiography scores than the normolipidemic patients. Among the major plasma lipoproteins and plasma apolipoprotein B, the concentration of apolipoprotein B was the strongest predictor of both the global coronary atherosclerosis and stenosis scores. There was no consistent association between the total VLDL cholesterol concentration and the coronary scores, whereas positive relations existed between the number of molecules of cholesteryl esters in small VLDL particles and the coronary atherosclerosis score in hypertriglyceridemic patients and with the stenosis score in the entire patient group. Increased amounts of lipid, especially triglycerides, and protein in dense LDL (density > 1.040 kg/L) correlated strongly with the severity of coronary atherosclerosis as well as with the number and severity of distinct stenoses. Of note, the results of the multivariate analysis should be regarded with due caution, since the $R^2$ statistic is influenced by sample size, and the values of multiple $R^2$ not infrequently are spuriously large in models based on a small sample.

Hypertriglyceridemia is a common metabolic disturbance in young postinfarction patients, and the hypertriglyceridemic patients had higher coronary angiographic scores than the normotriglyceridemic patients. Nevertheless, no consistent relations were found between VLDL lipid concentrations and coronary scores. However, the cholesteryl ester content of the VLDL subtraction containing the smallest particles, the VLDL$_2$ subtraction, related to the global coronary atherosclerosis and stenosis scores, particularly in the hypertriglyceridemic patients. The increased number of cholesteryl ester molecules in small VLDL was not related to the plasma VLDL triglyceride concentration and could therefore explain the nonlinear relation between hypertriglyceridemia and CAD. The reason for the relative enrichment of small VLDL with cholesteryl esters is unclear but could be either decreased catabolism of VLDL with a resulting increased number of VLDL remnant particles or increased activity of cholesteryl ester transfer protein, or both. Since small VLDL particles contain more than twice as many cholesteryl ester molecules per particle as LDL particles do, VLDL particles are likely to have a higher atherogenic potential per particle. Also, the potential for oxidation and subsequent uptake by the scavenger receptor would be larger for VLDL particles with their increased fatty acid content than for LDL particles. However, the mechanism linking VLDL particles enriched in cholesteryl esters to formation of atherosclerotic lesions remains unknown.

A preponderance of small and dense LDL particles has been demonstrated in subjects with manifest CAD. Furthermore, a relation has been noted between the triglyceride content of LDL and global severity as well as rate of progression of CAD determined by coronary angiography. The cause of the accumulation of dense LDL particles, which are relatively richer in triglyceride, is not well defined. Possible mechanisms are the combined action of lipases and transfer proteins on triglyceride-rich lipoproteins, decreased catabolism of dense LDL, and genetic control or a combination thereof. The triglyceride concentration in dense LDL correlated significantly with the plasma concentrations of apolipoprotein B, HDL cholesterol, and the number of cholesteryl ester molecules in small VLDL. However, since these associations accounted for <50% of the variation in dense LDL triglyceride, it seems reasonable to assume that the genetic component is a strong determinant of a dense LDL pattern.

The reason why increased amounts of dense LDL particles are associated with more severe CAD is also unclear. Either dense LDL particles are atherogenic per se, or their presence in plasma reflects changes in the metabolism of other potentially atherogenic lipoproteins. The first hypothesis is supported by the relation found between the susceptibility of the total LDL fraction to in vitro oxidation and coronary atherosclerosis in a small group of young postinfarction patients and the increased susceptibility of dense LDL to in vitro oxidation, which in turn could lead to increased uptake by the scavenger receptor. Alternatively, changes in the VLDL and HDL fractions are associated with increased amounts of triglyceride in dense LDL as well as with more severe CAD. Another, less plausible explanation would be that increased lipoprotein particle concentrations in the density range 1.040 to 1.063 kg/L are partly accounted for by lighter particle species of lipoprotein Lp(a). However, a recent study of young postinfarction patients was neither able to show any relations between the lipoprotein Lp(a) concentration and coronary atherosclerosis or stenosis scores nor an association between the levels of Lp(a) and the concentration of triglyceride or apolipoprotein B in dense LDL.
A weak but significant inverse relation was noted only between the plasma HDL cholesterol concentration and the stenosis score. The negative association between HDL cholesterol and the coronary atherosclerosis score was nearly significant, however, and with a larger study group a significant relation could have been obtained.

The clustering of hypertriglyceridemia with low HDL, hypertension, central obesity, and insulin resistance called syndrome X by Reaven and the association of hyperinsulinemia with jointly disturbed VLDL, LDL, and HDL levels have attained considerable interest in the past few years. Insulin resistance with ensuing hyperinsulinemia may well have central and even etiological roles in promoting a sequence of events leading to premature coronary atherosclerosis and myocardial infarction in this patient group.

The question arises whether the complex metabolic disturbances found in this group of patients could have been partly explained by the use of β-adrenergic blocker medication. Recent studies in young postinfarction patients argue against a major influence of these drugs on the plasma levels of major lipoprotein lipids, and the use of β-blockers actually marginally decreased the strength of the associations between LDL lipids and coronary atherosclerosis or stenosis scores in one study. The role of β-blockers in the distribution and concentration of LDL subclasses is not clear. One study using density gradient ultracentrifugation as the separation technique showed no major differences in the distribution of lipid and protein in LDL between postinfarction patients with and without β-blockers, whereas another study using density gel electrophoresis showed a change in LDL particle distribution toward smaller particles in patients using β-blockers. However, in the second study, CAD patients still had smaller LDL particles than control subjects when the confounding effect of β-blocker medication was controlled for. Other factors that probably could influence the concentrations of lipoprotein lipids would be the diet instructions given to the patients. However, dietary intervention would be

**TABLE 6. Multiple Stepwise Regression Analysis of the Relations of Plasma Lipoproteins to Coronary Angiography Scores and Triglyceride Levels in Dense LDL**

<table>
<thead>
<tr>
<th></th>
<th>Atherosclerosis Score, All Patients (HTG Patients)</th>
<th>Stenosis Score</th>
<th>Dense LDL TG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Partial Correlation Coefficient*</td>
<td>Final Model† Increase in Multiple $R^2$</td>
<td>Partial Correlation Coefficient*</td>
</tr>
<tr>
<td>Age</td>
<td>0.27§ (0.41§)</td>
<td>0.13 (0.34)</td>
<td>0.28§</td>
</tr>
<tr>
<td>BMI</td>
<td>0.22 (0.16)</td>
<td>0.40</td>
<td>0.12</td>
</tr>
<tr>
<td>log VLDL TG</td>
<td>0.18 (−0.17)</td>
<td>0.19</td>
<td>0.34§</td>
</tr>
<tr>
<td>LDL chol</td>
<td>0.37§ (0.42§)</td>
<td>0.22</td>
<td>0.39§</td>
</tr>
<tr>
<td>HDL chol</td>
<td>−0.23 (0.04)</td>
<td>−0.28§</td>
<td>−0.40§</td>
</tr>
<tr>
<td>Plasma apo B</td>
<td>0.43</td>
<td>(0.35§)</td>
<td>0.31§</td>
</tr>
<tr>
<td>light LDL TG</td>
<td>0.37§ (0.36§)</td>
<td>0.19</td>
<td>0.51</td>
</tr>
<tr>
<td>dense LDL TG</td>
<td>0.44§ (0.41§)</td>
<td>0.16</td>
<td>0.41§</td>
</tr>
<tr>
<td>VLDL₃ no. mol CE</td>
<td>0.24 (0.45§)</td>
<td>0.33§</td>
<td>0.33§</td>
</tr>
<tr>
<td>Multiple $R^2$ for final model</td>
<td>0.29 (0.49)</td>
<td>0.44</td>
<td>0.43</td>
</tr>
</tbody>
</table>

HTG indicates hypertriglyceridemic; LDL, low-density lipoprotein; TG, triglycerides; BMI, body mass index; VLDL, very-low-density lipoprotein; chol, cholesterol; HDL, high-density lipoprotein; apo, apolipoprotein; mol, molecules; CE, cholesteryl ester.

*Correlation coefficients when age had first been entered in the regression model.
†Variables included in the last step of the multiple stepwise regression analysis and their respective contribution to the value of multiple $R^2$.
§$P<.05$, §$P<.01$, ||$P<.001$. 

**Fig 2. Scattergrams showing the relations between the number of cholesteryl ester molecules in very-low-density lipoprotein 3 (VLDL III) particles and (A) coronary atherosclerosis ($r=.269$, $P<.05$) and (B) stenosis ($r=.340$, $P<.01$) scores. o, Normotriglyceridemic patients; e, hypertriglyceridemic patients (n=57).**
expected to weaken any existing relations between plasma lipoprotein fractions and coronary scores rather than producing spurious associations.

The high prevalence of an isolated elevation of VLDL in the present study group is in accordance with the findings of several previous studies of postinfarction patients\(^\text{48-50}\) in which a raised VLDL cholesterol concentration proved to be the most pronounced alteration of the major lipoprotein fractions. Similarly, an elevated concentration of whole serum or VLDL triglycerides has been shown to be the best discriminant between subjects with and without CAD in an older British population, even when cholesterol and triglyceride concentrations in VLDL, LDL, and HDL as well as serum apolipoproteins A-I and B were included in the analysis.\(^\text{51}\)

In summary, of the major lipoproteins and apolipoproteins, the plasma apolipoprotein B concentration, which reflects the number of VLDL and LDL particles in plasma, seems to be the best predictor of CAD severity. Addition of the plasma concentration of triglyceride in dense LDL increases the ability to predict the global coronary atherosclerosis and stenosis scores in young postinfarction patients. With respect to possible atherogenic mechanisms, compositional changes and alterations in the subclass distribution of apolipoprotein B-containing lipoproteins were found, linking disturbances of triglyceride-rich lipoproteins to CAD. A novel finding is the positive relation between the cholesteryl ester content of small VLDL and CAD in hypertriglyceridemic patients. This could represent a pathogenic mechanism in this subset of patients.

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**References**


Relation of plasma levels and composition of apolipoprotein B-containing lipoproteins to angiographically defined coronary artery disease in young patients with myocardial infarction.
P Tornvall, P Båvenholm, C Landou, U de Faire and A Hamsten

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