Plasma Lipoproteins and Progression of Coronary Artery Disease Evaluated by Angiography and Clinical Events

N.R. Phillips, PhD; D. Waters, MD; R.J. Havel, MD

Background. There is considerable evidence that remnants of triglyceride-rich lipoproteins may be particularly atherogenic.

Methods and Results. Levels of lipoprotein lipids and of apolipoprotein B in low-density lipoproteins were measured in 335 men and women enrolled in a study in which quantitative coronary angiography was carried out at 2-year intervals. Clinical events related to coronary disease occurred in 129 patients during the trial and in the subsequent follow-up period of 4 to 6 years. In multivariate analysis controlled for a number of nonlipid risk factors, high-density lipoprotein cholesterol was inversely related to the mean percentage increase in coronary artery stenosis in both men and women. Neither plasma triglycerides nor low-density lipoprotein cholesterol, triglycerides, or apolipoprotein B was related to change in stenosis, but a measure of remnants of triglyceride-rich lipoproteins, which included cholesterol in intermediate-density lipoproteins, was directly related to lesion progression. The same relations for these measures of plasma lipoprotein concentrations were found to hold for clinical events related to coronary artery atherosclerosis.

Conclusions. In patients with established coronary heart disease, increased levels of remnants of triglyceride-rich lipoproteins and decreased levels of high-density lipoproteins appear to promote progression of coronary artery atherosclerosis, which in turn may lead to an untoward clinical event. No such relation could be shown for the level of components of low-density lipoproteins. These and other observations call for reevaluation of relations between particular species of lipoproteins containing apolipoprotein B and the pathogenesis of coronary artery atherosclerosis and coronary heart disease.

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Key Words • lipoproteins • heart disease • apolipoproteins • triglycerides • atherosclerosis

A relation between the concentration of components of plasma lipoproteins and the risk of coronary heart disease (CHD) is well established. Intervention trials have demonstrated that reducing the levels of low-density lipoprotein (LDL) cholesterol in particular can reduce CHD risk1 and the rate of progression of coronary artery stenosis, assessed angiographically.2 High-density lipoprotein (HDL) cholesterol levels are inversely related to CHD risk,3 but the significance of this relation is confounded by the close association between triglyceride-rich lipoprotein levels and those of HDL.4 High levels of triglyceride-rich lipoproteins are also associated with a reduced size of LDL particles,5 and recent studies have shown that a lipoprotein pattern characterized by increased levels of triglyceride-rich very-low-density lipoproteins (VLDL), reduced levels of HDL cholesterol, and reduced size of LDL is associated with increased CHD risk.6

VLDL are generally considered to include those lipoproteins with buoyant densities of less than 1.006 g/mL. Although LDL was originally defined to include lipoproteins with buoyant densities between 1.019 and 1.063 g/mL,7 in the great majority of studies of the relation between LDL and CHD risk, LDL have been defined operationally to include particles with buoyant densities between 1.006 and 1.063 g/mL.8 Such lipoproteins include not only LDL as originally defined but also intermediate-density lipoproteins (IDL), with densities between 1.006 and 1.019 g/mL.9 IDL, and some VLDL particles, constitute a group of lipoproteins representing partially lipolyzed forms of VLDL, also called VLDL remnants.10 There is considerable evidence that VLDL remnants may be particularly atherogenic, whereas more nascent, usually larger VLDL particles may have little atherogenicity per se.11 In the early studies of Gofman and associates,12 lipoproteins now considered to be VLDL remnants were considered to be more atherogenic than LDL, but this interpretation was challenged in an early multicenter study in which serum cholesterol levels and those of LDL and a fraction comprising small VLDL particles were evaluated as predictors of CHD events.13 More recently, several studies have again raised the possibility that IDL and small VLDL particles may be particularly associated with CHD risk.14-19

This report presents analyses of plasma lipoproteins, including IDL and an estimate of VLDL remnants, in

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From the Cardiovascular Research Institute and Department of Medicine (N.R.P., R.J.H.), University of California, San Francisco; and Montréal Heart Institute (D.W.), Montréal, Canada.
Reprints requests to Dr Richard J. Havel, Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA 94143-0130.
patients undergoing serial quantitative coronary angiography who were entered into a trial of the effects of the calcium channel blocker nicardipine on the progression of coronary atherosclerotic lesions.\textsuperscript{20} The number of patients who completed the trial (335) provided greater power of discrimination of the association of lipoprotein levels with CHD progression than has been obtained in reported studies, and very few of the patients received hypolipidemic drugs that could perturb this relation. Clinical assessment during and after completion of the trial has also permitted an evaluation of the relation between plasma lipoproteins and clinical events related to coronary atherosclerosis. The data show a consistent relation between the concentration of cholesterol in remnant lipoproteins and HDL and the two measures of progression of coronary atherosclerotic disease. By contrast, no such relation could be documented between these measures and the concentration or size of LDL.

Methods

Sample Selection

All persons undergoing coronary angiography at the Montréal Heart Institute between June 1984 and December 1986 were screened for eligibility for the clinical trial. The inclusion and exclusion criteria have been reported in detail elsewhere.\textsuperscript{20,21} Briefly, the eligibility criteria were age between 21 and 65 years, without child-bearing potential if female, and stenoses of 5\% to 75\% of the lumen diameter in at least four of the 15 coronary segments defined in the Coronary Artery Surgery Study.\textsuperscript{22}

Of the 8915 persons screened, 449 met the inclusion criteria and had none of the exclusion criteria. Of these persons, 383 gave written informed consent and were enrolled into the study within 1 month of the screening angiogram.

Coronary Angiography

Both the angiographic procedure and the method of quantifying lesions have been fully described elsewhere.\textsuperscript{20,21} The Cardiovascular Angiographic Analysis System (CAAS) developed by Reiber et al\textsuperscript{23} was used to measure the paired lesions quantitatively. We have found the standard deviation for repeat measurements of diameter stenosis with CAAS, determined from paired angiograms taken 1 to 6 months apart, to be 6.7\%.\textsuperscript{24}

Paired angiograms were obtained on 335 persons (87.5\%). Repeat angiography was to be performed 24 months after enrollment but was done earlier on 37 of these persons for the following reasons: myocardial infarction in 12 cases, unstable or worsening angina in 21 cases, and a persistent and unacceptable level of stable angina in 4 cases. Of the 48 persons without repeat angiography, 5 died during the study, and 43 either dropped out of the study or declined to have the second angiogram at the end of the study.

Plasma Lipoproteins

Plasma, separated from fasting blood samples treated with EDTA (1 mg/mL) and containing sodium azide (0.5 mg/mL), gentamycin sulfate (0.1 mg/mL), and dithiobis-(nitrobenzoic acid) (1.4 mmol/L) as preservatives, was shipped on ice to San Francisco by air and processed for lipoprotein analysis within 72 hours of blood collection. Under these conditions, lecithin–cholesterol acyltransferase is inhibited more than 90\%, and transfer of cholesteryl esters between lipoprotein classes is undetectable (C. Fielding, personal communication). Fasting lipid levels in whole plasma and in VLDL, IDL, LDL, and HDL were measured at the Cardiovascular Research Institute, University of California, San Francisco, on admission to the study and again on termination, ie, 24 months later unless terminated earlier. Whole plasma and HDL lipid levels were also measured at 12 months unless the subject was no longer in the study. The level of LDL apoprotein B was measured on admission and again at termination. Apoprotein E phenotype was determined on admission.

Lipoprotein fractions were separated as follows. Five milliliters of plasma was centrifuged at 35 000 rpm for 16 hours at 16°C in a 40.3 rotor of a Beckman ultracentrifuge at a nonprotein solvent density of 1.006 g/mL to separate VLDL. An additional 5 mL of plasma was centrifuged under the same conditions at a nonprotein solvent density of 1.019 g/mL to separate IDL and VLDL from LDL and HDL.\textsuperscript{25} HDL was separated by precipitation of LDL in the 1.019-g/mL density infrate.\textsuperscript{25} The concentration of total cholesterol and triglycerides in whole plasma and separated fractions was measured by an automated method.\textsuperscript{26} IDL lipid level was calculated as the difference between the measured values in the d<1.019 g/mL and d<1.006 g/mL fractions; LDL cholesterol level was calculated as the difference between the measured values in the d>1.019 g/mL and HDL fractions.

The level of LDL apoprotein B was determined by rate immunonephelometry of the 1.019-g/mL infrate.\textsuperscript{27} Apoprotein E phenotype was determined by isoelectric focusing.\textsuperscript{28}

The level of cholesterol in the VLDL fraction carried by remnant particles was estimated by calculating the expected level of VLDL cholesterol given the VLDL triglyceride level and then subtracting the expected level from the observed level. The expected level of VLDL cholesterol was calculated as the antilog of \[a+b (\log \text{VLDL triglycerides})\]. The coefficients \(a\) and \(b\) were derived by regressing log VLDL cholesterol on log VLDL triglycerides and an apoprotein E phenotype represented by a set of dummy variables with the E3/3 phenotype serving as the reference group. The slope of the regression common to all phenotypes was used as the value of \(b\); the intercept for the E3/3 phenotype reference group was used as the value for \(a\). When measured VLDL cholesterol was less than expected, the estimated value for remnant VLDL cholesterol was negative. Adding a small constant to all estimated values would remove the negative values without affecting the variance of the estimate or its covariance with other variables but would serve no analytical purpose. Therefore, estimated remnant VLDL cholesterol was analyzed without correcting for negative values.

Smoking

Persons who smoked cigarettes during all or part of the interval between angiograms were classified as smokers. Persons who had stopped smoking before enrollment and did not resume smoking were classified, together with never smokers, as nonsmokers.
**Blood Pressure**

Blood pressure was taken with subjects in the supine position and recorded on all visits.

**Clinical Events**

Persons admitted to the clinical trial have continued to be monitored for the following events: cardiac death, myocardial infarction, coronary artery bypass surgery, and percutaneous transluminal coronary angioplasty. A death where the decedent was observed during the hour before death and either had symptoms compatible with acute myocardial infarction or had no symptoms was classified as a sudden cardiac death.\(^{21}\) Myocardial infarction was diagnosed by predetermined standard criteria.\(^{21}\)

**Statistical Analysis**

The within-person difference in mean percentage diameter stenosis between the first and second angiograms was chosen as the measure of change in coronary atherosclerosis because it uses all of the data on each person and also circumvents the classification problem presented by opposing directional changes in two or more lesions within the same person. The correlation between paired angiograms in mean percentage diameter stenosis was high \((r = .85)\), and the simple difference score was essentially independent of the baseline value \((r = -.05, P = .32)\).

The partial associations between the difference score and lipid levels within lipoprotein classes were assessed by multiple regression while also taking into account the major nonlipid risk factors for coronary artery disease either as possible confounders or as extraneous sources of variation. The nonlipid risk factors taken into account were sex, age, cigarette smoking, blood pressure, and diabetes mellitus. The possible effect of sex was initially controlled by sex-specific analyses. The sex-specific results were then pooled when no statistically significant heterogeneity was found \((F < 1.0)\). Because progression of initially small lesions (diameter stenosis \(< 20\%\)) was previously found to be less frequent in persons assigned nicardipine than in those assigned placebo,\(^{20}\) group assignment was also taken into account as an extraneous source of variation in the difference score.

Variation among apoprotein E phenotypes in lipoprotein lipid levels and angiographic results was assessed by ANOVA or, when controlled for a covariate, ANCOVA. When the \(F\) ratio was statistically significant, the predominant E3/3 phenotype served as the reference group against which the other phenotypes were compared, using Dunnett's modified \(t\) tables to control the \(\alpha\) error for the set of possible contrasts.

The expected distribution of the six phenotypes, E3/3, E4/3, E3/2, E4/4, E4/2, and E2/2, was calculated as \(r^2, 2pr, 2qr, p^2, 2pq,\) and \(q^2\), where \(r, p,\) and \(q\) are the respective relative frequencies of the E3, E4, and E2 alleles found in the sample. The observed and expected phenotypic distributions were compared with a \(\chi^2\) test for goodness of fit. Because two degrees of freedom were spent in estimating the allelic frequencies from the sample, the \(\chi^2\) test had three degrees of freedom.

Event-free follow-up time was computed as the number of days from the date of admission into the study to the date of the first event (either cardiac death, myocardial infarction, bypass surgery, or angioplasty, whichever occurred first) for persons who experienced an event or to the date of death for persons who died from noncardiac causes with no prior event or to the date of last contact for all other persons. The Cox proportional hazards regression model\(^{29}\) was used to relate the event rate to lipoprotein lipid levels. The univariate association between the event rate and each lipoprotein lipid was determined first. A set of independent prognostic lipids was then selected from the lipid covariate candidates in a stepwise fashion. The variable having the smallest \(P\) value was selected first. If no variable, or no remaining variable, was significant at \(P < .10\), the selection process was stopped. When a variable was added, each previously selected variable had to retain significance at \(P < .15\) to remain in the model. The estimated rate ratio corresponding to a 1-mg/dL difference in the lipid's level is found as the antilog (to the base \(e\)) of the Cox coefficient.

**Results**

**Angiographic Results**

Paired angiograms were obtained on 272 men, aged 29 to 65 years, and 63 women, aged 32 to 65 years. Table 1 gives the number of lesions, the within-person mean percentage diameter stenosis at baseline, and the within-person difference on the second angiogram by sex. The within-person mean percentage diameter stenosis was computed from all lesions regardless of size and ranged from 14% to 71% at baseline. The within-person difference in mean percentage diameter stenosis ranged from −14 percentage points to +40 percentage points. Women tended to have smaller lesions at baseline than men. Lesion progression was greater overall in men than in women, although the mean difference \((0.55 \text{ percentage points})\) was not statistically significant \((P = .52)\).

Only 16 persons were taking a hypolipidemic medication. Table 2 gives lipoprotein lipid levels by sex. The values in Table 2 for plasma cholesterol and triglycerides and for HDL cholesterol were based on the mean of three determinations, at entry, at 1 year, and at termination. The values for the other lipoprotein variables were based on the mean of two measurements, one made at entry and the other at termination. Women had higher levels of cholesterol in the LDL and HDL fractions, but lower levels of VLDL cholesterol and triglycerides, than men.

![Table 1. Number of Lesions, Within-Person Mean Percentage Diameter Stenosis at Baseline, and Within-Person Difference in Mean Percentage on Second Angiogram in 335 Persons With Paired Angiograms by Sex](image-url)
TABLE 2. Mean Plasma Lipoprotein Lipid and Apoprotein Levels in 335 Persons With Paired Angiograms by Sex

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Plasma CH, mg/dL</td>
<td>252.6</td>
<td>41.6</td>
</tr>
<tr>
<td>VLDL CH, mg/dL</td>
<td>37.0</td>
<td>21.6</td>
</tr>
<tr>
<td>IDL CH, mg/dL</td>
<td>16.0</td>
<td>8.2</td>
</tr>
<tr>
<td>LDL CH, mg/dL</td>
<td>147.2</td>
<td>34.6</td>
</tr>
<tr>
<td>LDL apo B, mg/dL</td>
<td>108.2</td>
<td>22.7</td>
</tr>
<tr>
<td>HDL CH, mg/dL</td>
<td>41.7</td>
<td>9.2</td>
</tr>
<tr>
<td>Plasma TG, mg/dL</td>
<td>217.7</td>
<td>121.5</td>
</tr>
<tr>
<td>VLDL TG, mg/dL</td>
<td>153.8</td>
<td>101.6</td>
</tr>
</tbody>
</table>

CH indicates cholesterol; TG, triglycerides; VLDL, very-low-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins; and apo, apoprotein.

Table 3 gives the zero-order coefficients of correlation among lipoprotein variables and the difference in mean percentage diameter stenosis. On univariate analysis, the difference score varied with VLDL and IDL, but not LDL, cholesterol and varied inversely with HDL cholesterol. VLDL triglycerides and VLDL cholesterol were highly correlated, and both were inversely correlated with HDL cholesterol; yet there was no statistically significant association between lesion progression and triglyceride levels. IDL cholesterol covared with VLDL cholesterol and, to a lesser degree, with LDL cholesterol. There was no statistically significant association between IDL and HDL cholesterol.

There was an insignificant trend for lesion progression to be greater among smokers and insulin-dependent (but not non–insulin-dependent) diabetics and to covary with diastolic (but not systolic) blood pressure. HDL cholesterol levels were lower among smokers and insulin-dependent diabetics; VLDL, cholesterol, and triglyceride levels covaried with diastolic blood pressure.

Taken together, sex, age, cigarette smoking, insulin-dependent diabetes mellitus, diastolic blood pressure, and treatment group accounted for a statistically insignificant proportion of the variance in the difference in mean percentage diameter stenosis ($R^2=.022$, $F=1.46$, $P=.20$). With diastolic blood pressure taken into account, treatment group accounted for none of the variance and was dropped as an extraneous factor. The other factors were retained as possible confounders of the relation between lesion progression and lipoprotein lipid levels.

Table 4 gives the partial coefficients of the regression of the within-person difference in mean percentage diameter stenosis on lipoprotein lipid levels, controlled for the nonlipid risk factors specified above. Two models are presented. Model A of Table 4 relates mean lesion progression to the cholesterol level in each of the four separated lipoprotein classes: VLDL, IDL, LDL, and HDL.

<table>
<thead>
<tr>
<th>Model</th>
<th>Lipids, mg/dL</th>
<th>b</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model A</td>
<td>VLDL CH</td>
<td>−.009</td>
<td>.019</td>
<td>.654</td>
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<tr>
<td></td>
<td>IDL CH</td>
<td>.088</td>
<td>.046</td>
<td>.057</td>
</tr>
<tr>
<td></td>
<td>LDL CH</td>
<td>.000</td>
<td>.010</td>
<td>.965</td>
</tr>
<tr>
<td></td>
<td>HDL CH</td>
<td>−.116</td>
<td>.038</td>
<td>.002</td>
</tr>
<tr>
<td>Model B</td>
<td>Log$_10$ VLDL TG</td>
<td>−1.802</td>
<td>1.542</td>
<td>.244</td>
</tr>
</tbody>
</table>

Sum of IDL and remnant

|        | VLDL CH | .054 | .023 | .019   |
|        | LDL CH  | .002 | .009 | .812   |
|        | HDL CH  | −.130 | .039 | .001   |

VLDL indicates very-low-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins; CH, cholesterol; and TG, triglycerides. Nonlipid risk factors: smoked cigarettes during all or part of interval, insulin-dependent diabetes mellitus, diastolic blood pressure, age, and sex.

Table 4. Partial Coefficients (b) of the Regression of the Within-Person Difference in Mean Percentage Diameter Stenosis on Lipoprotein Lipid Levels in 335 Persons With Paired Angiograms, Controlled for Nonlipid Risk Factors

TABLE 3. Coefficients of Correlation Among Lipoprotein Components and the Difference in Mean Percentage Diameter Stenosis for 335 Persons With Paired Angiograms

<table>
<thead>
<tr>
<th></th>
<th>Plasma CH</th>
<th>VLDL CH</th>
<th>IDL CH</th>
<th>LDL CH</th>
<th>LDL apo B</th>
<th>LDL CH/apo B</th>
<th>HDL CH</th>
<th>Plasma TG</th>
<th>VLDL TG</th>
<th>Difference in Mean % Stenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma CH</td>
<td>1.00</td>
<td>.35</td>
<td>.54</td>
<td>.77</td>
<td>.75</td>
<td>.24</td>
<td>.11</td>
<td>.34</td>
<td>.26</td>
<td>.05</td>
</tr>
<tr>
<td>VLDL CH</td>
<td>1.00</td>
<td>.46</td>
<td>−.12</td>
<td>.02</td>
<td>−.33</td>
<td>−.44</td>
<td>.90</td>
<td>.92</td>
<td>.11</td>
<td>.11</td>
</tr>
<tr>
<td>IDL CH</td>
<td>1.00</td>
<td>.16</td>
<td>.23</td>
<td>.07</td>
<td>.07</td>
<td>.35</td>
<td>.28</td>
<td>.12</td>
<td>.01</td>
<td>.01</td>
</tr>
<tr>
<td>LDL CH</td>
<td>1.00</td>
<td>.88</td>
<td>.50</td>
<td>.11</td>
<td>−.10</td>
<td>−.14</td>
<td>−.01</td>
<td>.04</td>
<td>.00</td>
<td>.01</td>
</tr>
<tr>
<td>LDL apo B</td>
<td>1.00</td>
<td>.06</td>
<td>.03</td>
<td>.04</td>
<td>.00</td>
<td>.01</td>
<td>.01</td>
<td>.08</td>
<td>.07</td>
<td>.08</td>
</tr>
<tr>
<td>LDL (CH/apo B)</td>
<td>1.00</td>
<td>.22</td>
<td>−.32</td>
<td>−.32</td>
<td>−.46</td>
<td>−.46</td>
<td>−.18</td>
<td>−.04</td>
<td>.08</td>
<td>.08</td>
</tr>
<tr>
<td>HDL CH</td>
<td>1.00</td>
<td>−.45</td>
<td>−.46</td>
<td>−.18</td>
<td>−.18</td>
<td>−.18</td>
<td>−.18</td>
<td>−.18</td>
<td>−.18</td>
<td>−.18</td>
</tr>
<tr>
<td>Plasma TG</td>
<td>1.00</td>
<td>.94</td>
<td>.04</td>
<td>.04</td>
<td>.04</td>
<td>.04</td>
<td>.04</td>
<td>.04</td>
<td>.04</td>
<td>.04</td>
</tr>
<tr>
<td>VLDL TG</td>
<td>1.00</td>
<td>.94</td>
<td>.04</td>
<td>.04</td>
<td>.04</td>
<td>.04</td>
<td>.04</td>
<td>.04</td>
<td>.04</td>
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</tr>
</tbody>
</table>

CH indicates cholesterol; TG, triglycerides; VLDL, very-low-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins; and apo, apolipoprotein. Correlations with an absolute value $\geq .11$ are statistically significant ($P \leq .05$) on a two-tailed test.
and HDL. When this set of lipid variables was combined with the set of nonlipid risk factors, the proportion of explained variance increased to .058 (F=2.23, P<.02). The increment of .036 in R² was statistically significant (F=3.15, P<.02). In this model, lesion progression was inversely and significantly related to HDL cholesterol level and tended to covary with IDL cholesterol level. With IDL and HDL cholesterol taken into account, there was no apparent independent association between either VLDL or LDL cholesterol level and lesion progression.

Both IDL and remnant VLDL particles are intermediate products of VLDL catabolism. The estimated level of remnant VLDL cholesterol, calculated as described in “Methods,” and the measured level of IDL cholesterol were correlated (r=.47), and lesion progression correlated well as well with remnant VLDL cholesterol (r=.12, P=.03) as with IDL cholesterol (r=.12) on univariate analysis. Therefore, estimated remnant VLDL cholesterol and IDL cholesterol were added together. In model B of Table 4, the sum of remnant VLDL and IDL cholesterol replaced IDL cholesterol alone, and VLDL triglycerides replaced VLDL cholesterol as an indicator of VLDL concentration; LDL and HDL cholesterol were retained as before. This set of lipid variables combined with the set of nonlipid variables yielded an R² of .067 (F=2.59, P<.01). The increment of .045 in R² was also statistically significant (F=3.22, P<.02). The partial coefficient for the sum of remnant VLDL and IDL cholesterol, as well as that for HDL cholesterol, was statistically significant. There was no statistically significant independent association between VLDL triglyceride level and lesion progression. The model B substitutions had no appreciable effect on the partial coefficients for LDL and HDL cholesterol.

LDL apoprotein B was highly correlated with LDL cholesterol (r=.88, Table 3). Replacement of LDL cholesterol with LDL apoprotein B (not presented) had no appreciable effect on the partial coefficients for the other lipoprotein lipids in either model A or model B and no effect whatsoever on R². The ratio of cholesterol to apoprotein B in LDL is an indicator of particle size.2 To consider particle size as well as concentration, both LDL cholesterol and LDL apoprotein B were simultaneously included in the multiple regression equation (not presented). If lesion progression were related to increasing concentrations of small LDL particles, the partial coefficient for LDL cholesterol would be negative in sign, whereas that for LDL apoprotein B would be positive. Just the opposite result was realized. The inclusion of both LDL cholesterol and LDL apoprotein B had no essential effect on the coefficients for the lipid levels in the other lipoprotein fractions in either model A or model B and no effect at all on R². LDL triglyceride level was only moderately correlated with LDL cholesterol level (r=.38). But again, when LDL triglyceride level was substituted for LDL cholesterol in both model A and model B, the coefficients for the other lipoprotein lipids were little affected and R² was unchanged.

Apoprotein E Phenotype

The phenotype frequencies found among persons admitted to the study agreed well with those expected given the allelic frequencies found in this group (χ² with 3 df=3.2, P>.25). Thus, these data gave no internal evidence that the study subjects were selected with respect to apoprotein E phenotype.

There was no statistically significant variation among phenotypes in either the number of coronary artery lesions, the within-person mean percentage diameter stenosis at baseline, or the within-person difference on the second angiogram.

There was no statistically significant variation among phenotypes in either VLDL cholesterol or VLDL triglyceride level. The level of VLDL cholesterol relative to the level of VLDL triglycerides, however, was higher among the 32 persons with the E3/2 phenotype than among the 213 persons with the E3/3 phenotype. The mean difference in the estimated levels of remnant VLDL cholesterol was 4.8 mg/dL (t=3.65, P<.01). Persons with the E3/2 phenotype also tended to have higher levels of IDL cholesterol than those with the E3/3 phenotype, although the mean difference (3 mg/dL) was not statistically significant. Compared with persons with the E3/3 phenotype, persons with the E3/2 phenotype had lower levels of both LDL cholesterol (−20 mg/dL, t=3.04) and LDL apoprotein B (−13 mg/dL, t=2.95, P<.05 for both using Dunnett’s tables). The 9 persons with the E4/4 phenotype tended to have higher values than persons with the E3/3 phenotype, although neither contrast was statistically significant given the small number of persons with E4/4. There was no statistically significant variation among phenotypes in HDL cholesterol level.

The partial coefficients of the regression of the within-person difference in mean percentage diameter stenosis on lipoprotein lipid levels (both models A and B) were computed within E phenotype for the three largest phenotypes (E3/3, E4/3, and E3/2) and tested for heterogeneity. No significant interaction was found (F<1.0).

Clinical Events

When last contacted between April 22 and July 3, 1991, 129 of the 383 persons admitted to the study had experienced one or more of the following events: cardiac death, myocardial infarction, coronary artery bypass surgery, and percutaneous transluminal coronary angioplasty. Two others had died from noncardiac causes (lung cancer), and another 10 had been lost to follow-up, 4 during the clinical trial phase of the study. The censored exposure time of persons withdrawn from surveillance because of a noncardiac death or loss to follow-up was included in the analyses of clinical events presented here. Of the 129 first events since admission to the study, 8 were cardiac deaths (all sudden and out of hospital), 39 were myocardial infarctions, and the remaining 82 were revascularization procedures.

The association between lipoprotein lipid levels and a clinical event was evaluated by the Cox proportional hazards regression model (Table 5). On univariate analysis, the sum of IDL and remnant VLDL cholesterol, IDL cholesterol alone, and HDL cholesterol were statistically significant prognostic factors. Of these three factors, the sum of IDL and remnant VLDL cholesterol was the most significant and was the first variable entered into the regression equation. Needless to say, IDL cholesterol alone was no longer a significant prognostic factor once the sum of IDL and remnant VLDL cholesterol was taken into account. HDL cholesterol,
TABLE 5. Association Between Lipoprotein Lipid Levels and a Clinical Event

<table>
<thead>
<tr>
<th>Lipid Variate, mg/dL</th>
<th>Univariate Analysis</th>
<th>Stepwise Multivariate Analysis</th>
<th>Rate Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\chi^2$</td>
<td>$P$</td>
<td>Cox Coefficient</td>
</tr>
<tr>
<td>Plasma CH</td>
<td>0.01</td>
<td>.915</td>
<td>- .0108</td>
</tr>
<tr>
<td>VLDL CH</td>
<td>0.08</td>
<td>.783</td>
<td>- .0248</td>
</tr>
<tr>
<td>IDL CH</td>
<td>6.73</td>
<td>.009</td>
<td>- -</td>
</tr>
<tr>
<td>LDL CH</td>
<td>0.22</td>
<td>.636</td>
<td>- -</td>
</tr>
<tr>
<td>HDL CH</td>
<td>5.16</td>
<td>.023</td>
<td>- -</td>
</tr>
<tr>
<td>Plasma TG</td>
<td>0.39</td>
<td>.530</td>
<td>- -</td>
</tr>
<tr>
<td>VLDL TG</td>
<td>0.55</td>
<td>.460</td>
<td>- -</td>
</tr>
<tr>
<td>Sum of IDL and remnant VLDL CH</td>
<td>8.45</td>
<td>.004</td>
<td>.0210</td>
</tr>
</tbody>
</table>

CH indicates cholesterol; TG, triglycerides; VLDL, very-low-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; and HDL, high-density lipoproteins.

No. of events=129.

however, was an independent prognostic factor. Although neither plasma triglycerides nor VLDL lipid levels were significant on univariate analysis (Table 5), they became so after HDL cholesterol was entered into the equation, with VLDL cholesterol being the most significant ($P=.015$). With VLDL cholesterol added to the equation, no remaining lipid variable was statistically significant ($P>.46$).

The estimated rate ratio corresponding to a 1-mg/dL difference in the sum of IDL and remnant VLDL cholesterol was 1.021 (Table 5). That is, with a 1-mg/dL difference, the event rate was 2% higher. For a 1-mg/dL difference in HDL cholesterol level, on the other hand, the event rate was 2.5% lower (rate ratio=0.975). When considered in conjunction with HDL cholesterol and the sum of IDL and remnant VLDL cholesterol, increasing VLDL cholesterol level was associated with a decrease in the event rate (Table 5). This result is consistent with the negative partial coefficient for VLDL triglycerides seen in model B of Table 4.

The association between a subsequent clinical event and the prior levels of HDL cholesterol and the sum of remnant VLDL and IDL cholesterol may be more readily appreciated by comparing the event rate (number of events per 100 person-years of follow-up) according to whether both quantities were in the more favorable or in the less favorable half of their respective distributions. For persons in whom the sum of remnant VLDL and IDL cholesterol was below the median level but whose HDL cholesterol level was above the median (n=99), the event rate was 5.1. By contrast, the event rate was 9.1 for persons in whom the sum of remnant VLDL and IDL cholesterol was above the median level but whose HDL cholesterol level was below the median (n=99). Mean lesion progression was also greater for persons with less favorable remnant and HDL cholesterol levels (2.76 percentage points) than for those with the more favorable levels (0.30 percentage points).

Progression of coronary atherosclerosis, defined as an increase of 15 percentage points in any one lesion, was a strong, independent predictor of future clinical events among these study subjects.30 Progression measured by the within-person difference in mean percentage diameter stenosis as used here was also associated with the event rate. The rate ratio corresponding to a difference of one percentage point in the mean difference was 1.049 ($P<.0001$). When the difference in mean percentage stenosis was taken into account, the sum of IDL and remnant VLDL cholesterol was still significantly associated with the event rate (rate ratio = 1.0098; $P=.03$) but less strongly so. HDL cholesterol level, however, was no longer a statistically significant independent prognostic factor ($P=.14$).

Discussion

We found that percentage diameter stenosis of coronary arterial lesions quantitatively measured on angiograms taken 2 years apart increased on average as the level of IDL and remnant VLDL cholesterol increased and as the level of HDL cholesterol decreased. These results support a growing body of evidence that cholesterol-enriched intermediate products of VLDL catabolism are atherogenic,14-19 and an already large body of evidence that HDL particles are antiatherogenic.3 We did not find a clear relation between lesion progression and either the concentration of VLDL particles as indicated by the level of VLDL triglycerides or the concentration of LDL particles as indicated by the level of either LDL cholesterol, LDL apoprotein B, or LDL triglycerides.

LDL cholesterol level is an established risk factor for coronary artery disease. Induced reductions in LDL cholesterol level decrease the risk of coronary artery disease among persons initially free of overt disease and the progression of angiographically measured atherosclerosis. Why, then, was there no apparent association between lesion progression and LDL cholesterol level in our study? In contrast to almost all other studies, we separated LDL from IDL. LDL typically contributes only a small fraction of the cholesterol in the combined IDL-plus-LDL (1.006$<d<1.063 \text{ g/mL}$) fraction (Table 2). Nevertheless, part, if not all, of the association between LDL cholesterol level and coronary artery disease incidence or lesion progression found in earlier studies may be due to the IDL cholesterol included in the measurement of LDL cholesterol.

This possibility was previously raised by Krauss and associates,19 who carried out the only published analysis
in which IDL and LDL particles were distinguished in an intervention study. Based on a subset of 57 men from the Type II Coronary Intervention Study in whom lipoprotein mass concentrations were measured by analytical ultracentrifugation at baseline and again after 2 years, they found that lesion progression was associated with change in IDL mass ($S'$; 12-20) but not with change in LDL mass ($S'$; 0-12). As these authors pointed out, because their sample size was small, one should not construe their results to mean that LDL level or the change in level has no independent effect on disease progression. Although our sample size was much larger, the correlations between lipoprotein lipid levels and lesion progression, even when statistically significant, were of a low order (Table 3). If LDL cholesterol were only somewhat less closely associated with lesion progression than is remnant VLDL and IDL cholesterol, the association might not be displayed in these data. As a further caveat, we should note that the cholesterol level in the combined IDL and LDL fractions of our subjects was not significantly associated with lesion progression (partial $b=.009, P=.30$).

IDL and LDL cholesterol tend to covary (Table 3). When their clearance is delayed, more IDL particles are converted to LDL. Because LDL receptors on liver cells have a greater affinity for lipoproteins containing apoprotein E as well as apoprotein B than for those containing apoprotein B alone, drugs that cause the liver to increase the activity of LDL receptors enhance the removal of remnant VLDL particles, which display normal apoprotein E and thereby lower LDL level not only by enhanced clearance of LDL but also by reduced production of LDL. Furthermore, unless IDL is measured, the effect of an induced reduction in LDL level will be then confounded by the simultaneous reduction in IDL level.

The level of cholesterol in the $d<1.006-g/mL$ fraction is determined by the concentration of a heterogeneous mixture of particles, including that of cholesterol-enriched remnants. Although particles derived from chylomicrons that contain apolipoprotein B-48 are present in fasting plasma, their concentration, except in unusual circumstances, is very small compared with that of particles derived from heptageneric VLDL that contain apoprotein B-100. For example, in normolipemic subjects, more than 95% of particles in fasting plasma with a density less than 1.006 g/mL contain apoprotein B-100.

Remnant VLDL particles, which are normally taken up by the liver, were first characterized in functionally hepatomeitized animals. These particles are distinctly smaller than unmodified VLDL and have reduced electrophoretic mobility. The remnants are enriched in cholesteryl esters and apoproteins B and E and are depleted in triglycerides and C apoproteins. Particles with properties like those that accumulate in functionally hepatomeitized animals ($β$-VLDL) are found in large numbers in persons with familial dysbetaiproteinemia who are homozygous for genetically defective apolipoprotein E. Isolated VLDL from persons without the E2/2 phenotype commonly show two bands on electrophoresis. Particles comprising the slower band have properties that also closely resemble those of remnant VLDL found in the functionally hepatomeitized animals. They are smaller than particles comprising the faster band; contain a larger proportion of cholesteryl esters, free cholesterol, and apoproteins B and E; and contain a smaller proportion of triglycerides and C apoproteins. Of the characteristics that define remnant particles, the one that was measured in this study was the ratio of total cholesterol to triglycerides in the $d<1.006-g/mL$ fraction.

We have observed in numerous bodies of data that the ratio of cholesterol to triglycerides in the $d<1.006-g/mL$ fraction is not constant but rather decreases as the level of triglycerides increases and that the relation between the two lipids is well described by expressing VLDL cholesterol as a power function of VLDL triglycerides (unpublished observations). This function was used in estimating the levels of remnant VLDL cholesterol in our study subjects. The estimates thus obtained do not denote the actual concentration of remnant VLDL cholesterol among these persons. Rather, they ranked persons along a continuum that a priori should correlate well with their actual levels were they known. Although the estimates have not been directly validated, we have indirect evidence of their validity. Within this study, they distinguished between groups of persons expected to have different concentrations of remnant VLDL. Thus, the estimated level of remnant VLDL cholesterol was higher in persons with an E2 allele. The prevalence of two electrophoretic bands in the $d<1.006-g/mL$ fraction among persons heterozygous for the E2 allele was twice that among others (data not shown). The estimated levels of remnant VLDL cholesterol in specimens with two electrophoretic bands were also higher than in specimens with a single band within the E3/2 phenotype and within the E3/3 phenotype as well. The estimated level of remnant VLDL cholesterol and the measured level of IDL cholesterol were moderately correlated ($r=.47$), and the two were added together to represent more fully the variation among subjects in accumulated cholesterol-enriched products of VLDL catabolism.

A large proportion (60%) of persons with paired angiograms in this study were taking a $β$-blocker medication during the interval between angiograms. Because these persons were selectively placed on a $β$-blocker, the effect of this class of drugs on the lipoprotein lipid profiles of the persons taking them cannot be fairly assessed from our data. We should note, however, that there was no apparent difference between persons taking a $β$-blocker and other persons in the relation between lesion progression and lipoprotein lipid levels ($F<1.0$). The regression surface for persons assigned the test drug nicardipine was also homogeneous with that for persons assigned placebo ($F<1.0$).

The variation of LDL level found among apoprotein E phenotypes in our sample of persons with coronary artery disease is in accordance with that found in samples from a general population. That is, persons with an E4 allele had slightly higher levels and those with an E2 allele had lower levels than those with two

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*The concentration of LDL cholesterol (including IDL cholesterol) is frequently estimated by the Friedewald formula, based on the levels of total cholesterol, HDL cholesterol, and total triglycerides in whole blood plasma. It is therefore pertinent also to note that when IDL levels are disproportionately increased, LDL cholesterol (1.006<$d<$1.063 g/mL) is generally overestimated.

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E3 alleles. Lenzen and colleagues also found a decrease in LDL cholesterol level going from E4/3 to E3/3 to E3/2 in their sample of 570 male survivors of myocardial infarction with angiographically documented coronary artery lesions. As expected, we found that remnant VLDL particle concentration was higher in persons with an E2 allele. Recently, Hopkins et al have used a different approach to evaluate cholesterol enrichment of VLDL as a function of apoprotein E phenotype in families of patients with heterozygous familial hypercholesterolemia. They found that individuals with this disorder who possessed an E2 allele had a more than threefold increase in the estimated level of “apo-VLDL.”

Our indicator of disease progression—the difference in mean percentage stenosis of all measured lesions within a person—reflected clinically meaningful change in that it was associated with follow-up events: cardiac death, myocardial infarction, and revascularization procedures. The two lipoprotein lipids, HDL cholesterol and the sum of IDL and remnant VLDL cholesterol, related to lesion progression were also related to the follow-up event rate. As with lesion progression, clinical events were unrelated to LDL cholesterol.

Taken together, the results of this study are consistent with the hypothesis that increased levels of remnant VLDL and IDL particles and decreased levels of HDL promote lesion progression, which, in turn, may lead to an untoward clinical event (cardiac death or infarction) or to a critical decrease in myocardial perfusion calling for a revascularization procedure.

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Plasma lipoproteins and progression of coronary artery disease evaluated by angiography and clinical events.
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