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EPIDEMIOLOGIC STUDIES

Lipoprotein Cholesterol, Apolipoprotein A-I and B and Lipoprotein (a) Abnormalities in Men With Premature Coronary Artery Disease

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The prevalence of abnormalities of lipoprotein cholesterol and apolipoproteins A-I and B and lipoprotein (a) [Lp(a)] was determined in 321 men (mean age 50 ± 7 years) with angiographically documented coronary artery disease and compared with that in 901 control subjects from the Framingham Offspring Study (mean age 49 ± 6 years) who were clinically free of coronary artery disease. After correction for sampling in hospital, beta-adrenergic medication use and effects of diet, patients had significantly higher cholesterol levels (224 ± 53 vs. 214 ± 36 mg/dl), triglycerides (189 ± 95 vs. 141 ± 104 mg/dl), low density lipoprotein (LDL) cholesterol (156 ± 51 vs. 138 ± 33 mg/dl), apolipoprotein B (131 ± 37 vs. 108 ± 33 mg/dl) and Lp(a) levels (19.9 ± 19 vs. 14.9 ± 17.5 mg/dl). They also had significantly lower high density lipoprotein (HDL) cholesterol (36 ± 11 vs. 45 ± 12 mg/dl) and apolipoprotein A-I levels (114 ± 26 vs. 136 ± 32 mg/dl) (all $p < 0.005$).

On the basis of Lipid Research Clinic 90th percentile values for triglycerides and LDL cholesterol and 10th percentile values for HDL cholesterol, the most frequent dyslipidemias were low HDL cholesterol alone (19.3% vs. 4.4%), elevated LDL cholesterol (12.1% vs. 9%), hypertriglyceridemia with low HDL cholesterol (9.7% vs. 4.2%), hypertriglyceridemia and elevated LDL cholesterol with low HDL cholesterol (3.4% vs. 0.2%) and Lp(a) excess (15.8% vs. 10%) in patients versus control subjects, respectively ($p < 0.05$). Stepwise discriminant analysis indicates that smoking, hypertension, decreased apolipoprotein A-I, increased apolipoprotein B, increased Lp(a) and diabetes are all significant ($p < 0.05$) factors in descending order of importance in distinguishing patients with coronary artery disease from normal control subjects.

Not applying a correction for beta-adrenergic blocking agents, sampling bias and diet effects leads to a serious underestimation of the prevalence of LDL abnormalities and an overestimation of HDL abnormalities in patients with coronary artery disease. However, 35% of patients had a total cholesterol level <200 mg/dl after correction; of those patients, 73% had an HDL cholesterol level <35 mg/dl.

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The leading cause of morbidity and mortality in the United States is coronary artery disease and its sequelae. The identification of subjects at risk of developing coronary atherosclerosis is an important public health issue. In addition to other risk factors, such as male gender, increasing age, hypertension, diabetes and a family history of premature coronary artery disease, elevated plasma low density lipoprotein (LDL) cholesterol and decreased high density lipoprotein (HDL) cholesterol have been shown to be independent predictors for coronary artery disease in prospective (1–11) and case-control (12–44) epidemiologic studies. In view of the multifactorial etiology of coronary atherosclerosis, no single biochemical variable will identify all patients at risk for developing coronary atherosclerosis. The effects of the various risk factors are clearly cumulative and the identification of major biochemical markers and their interrelations should allow earlier detection of patients at risk (4,45).

Elevated LDL cholesterol and decreased HDL cholesterol concentrations are associated with an increased risk of developing coronary artery disease (1,3,5,8–11). The major apolipoproteins of LDL and HDL particles, namely, apolipoprotein B and apolipoprotein A-I, respectively, are strongly associated with the presence of coronary artery disease. An increased level of LDL apolipoprotein B has been associated with increased risk, as has a low level of apolipoprotein A-I (13–33,36–41,43,44). Lipoprotein (a) [Lp(a)], first identified by Berg (46), has been shown to be increased in patients with angiographically documented coronary artery disease (47–53). Lp(a) consists of one or more

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molecules of apolipoprotein (a) linked by a cysteine bond to the apolipoprotein B moiety of LDL particles. Recently (54,55), the molecular structure and complementary DNA (cDNA) of apolipoprotein (a) have been elucidated and have revealed considerable homology between the plasminogen and apolipoprotein (a) genes. Variant forms of apolipoprotein (a) differ in apparent molecular weight, in part because of varying numbers of kringle-like domains in the molecule (56).

Our ability to measure lipoproteins, apolipoproteins and Lp(a) has been greatly refined in the past few years. These variables are used in many studies to provide an assessment of cardiovascular risk in a given population. Most of these variables, however, are closely interrelated and their measurement may not improve our ability to predict risk. The present study was undertaken to determine lipid, lipoprotein and apolipoprotein concentrations and the prevalence of abnormalities in lipoprotein cholesterol, apolipoproteins A-I and B and Lp(a) in men with premature coronary artery disease. We also corrected for confounding variables that affect lipid and lipoprotein levels, such as the effect of sampling in the hospital (57,58), the use of medication—especially beta-adrenergic blocking drugs (59–62)—and the role of dietary changes in our patients (63). We also studied the interrelations among the variables and determined the most discriminant variables for the presence of coronary artery disease.

Methods

Subjects studied. Patients (n = 321) underwent elective cardiac catheterization and coronary angiography for the diagnosis and determination of the extent of coronary artery disease at the New England Medical Center Hospital. The referral base of the hospital includes Greater Boston and Eastern Massachusetts. All patients were white men <60 years of age (mean ± SD 50 ± 7) at the time of coronary angiography. All were studied between July 1985 and December 1987. Patients with acute myocardial infarction, surgery or trauma in the 6 weeks preceding admission were excluded, as were those taking lipid-lowering medications. Information on other risk factors—hypertension (defined as a history of high blood pressure ≥150/95 mm Hg, treated or not), diabetes (history of diabetes or treatment with an oral hypoglycemic agent or insulin) and smoking (≥10 cigarettes/day in the year preceding the procedure), as well as medications (especially diuretic drugs, beta-adrenergic blocking agents and calcium channel blocking drugs)—was noted by direct interview and review of the patient's medical chart.

The degree of coronary artery disease was determined by two independent cardiologists unaware of the patient's inclusion in the study. The presence of coronary artery disease, defined as >50% stenosis of a major coronary artery, was identified on multiple projections (>75% cross-sectional area stenosis). Patients with minimal disease (<50% stenosis) or with normal angiograms (n = 25) were excluded from the analysis. The study protocol was reviewed and accepted by the Human Investigation Review Committee of the New England Medical Center.

Men (n = 901) 49 ± 6 years of age from the offspring cohort of the Framingham Heart Study were used as control subjects. These control subjects were free of clinical manifestations of cerebrovascular, peripheral vascular or coronary artery disease and had no history of myocardial infarction. Information on risk factors and medication as for the study patients was ascertained. Subjects with clinically documented cardiovascular disease (angina, definite or suspected myocardial infarction, electrocardiographic evidence of myocardial infarction, peripheral vascular disease or cerebrovascular disease) were excluded. Control subjects taking lipid-lowering medications were excluded.

Lipid, lipoprotein, apolipoprotein and Lp(a) measurements. Plasma total cholesterol, triglycerides and HDL cholesterol levels were determined enzymatically, with HDL cholesterol determined after dextran-magnesium precipitation. LDL cholesterol was calculated by the method of Friedewald et al. (64) unless the triglyceride concentration was >400 mg/dl, in which case, cholesterol was measured in the d > 1.006 infranate after ultracentrifugation (65,66). LDL cholesterol was then calculated as infranate cholesterol minus HDL cholesterol. Lipid analyses were performed at the Lipid Metabolism Laboratory of the U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, and at the Framingham Heart Study Core Laboratory. Both laboratories use identical procedures and equipment. Our laboratory meets the performance criteria of the Centers for Disease Control Lipid Standardization Program (65). Multiple aliquots of plasma were frozen at −80°C for later analysis of apolipoproteins.

Apolipoprotein A-I and apolipoprotein B were measured by noncompetitive enzyme-linked immunosorbent assays (ELISA) (67,68). Normal ranges for apolipoproteins A-I and B were determined in 3,541 participants (men and women) from the Framingham Offspring Study. Apolipoprotein immunoassays were standardized with use of purified apolipoproteins subjected to amino acid analysis. Lp(a) was determined by ELISA, with use of a monoclonal anti-Lp(a) antibody with no cross-reactivity to plasminogen and a polyclonal antibody directed at the apolipoprotein (a) portion of Lp(a) [Macra Lp(a), Terumo Corp.]. This assay was standardized with use of purified Lp(a), with the mass corresponding to the entire particle. Lp(a) levels were determined in 760 male control subjects and 256 men with coronary artery disease; lack of plasma samples accounts for the missing values. The 90th percentile for Lp(a), based on the control group, was determined to be 38.8 mg/dl. All apolipoprotein and Lp(a) determinations were performed at the Lipid Metabolism Laboratory at Tufts University. Intra- and interrun coefficients of variance for these assays were <10%.

Diet effects. We attempted to correct for a possible diet effect by analyzing the nutrient intake (as a percent of calories) in 43 men with coronary artery disease and 96 Framingham control subjects by using food frequency ques-

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tionnaires (69). The coronary artery disease group had slightly lower total fat consumption than did the control group (29.3% vs. 32.1%), with the following differences in saturated, monosaturated and polyunsaturated fats: 10.2% vs. 12.9%; 11.4% vs. 13.3% and 8.6% vs. 5.9%, respectively. The daily cholesterol intake was 105 vs. 150 mg/1,000 kcal. By applying the formula of Hegsted et al. (70) to determine the change in total cholesterol, patients with coronary artery disease would be expected to have a 6.6% decrease in total cholesterol. On the basis of this subset analysis, we extrapolated the effects of diet to the coronary artery disease group and made the following assumptions: the effect of the diet was the same for all patients (that is, a decrease of 6.6% in total cholesterol due to a decrease in LDL cholesterol), apolipoprotein B changed to the same degree as LDL cholesterol and there was no significant effect overall on triglyceride HDL cholesterol apolipoprotein A-I or Lp(a) levels. Body mass index was calculated as weight (kg)/height^2 (cm).

Statistical analysis. The data were stored on a VAX 11/780 computer (Digital Equipment Corp.) with use of the database RS/1 (BBN Software). The normality of continuous lipoprotein measures was tested by using the Kolmogorov-Smirnoff test. Triglycerides and Lp(a) levels were transformed by using $\log_{10}$ to better approximate a normal distribution. The grouped t test was used to compare patient and control groups for these variables. Unpaired two-tailed t tests were used to evaluate the differences between mean values for variables having a parametric distribution. Log_{10} transformation of nonparametric variables was performed and the t test was then used. Chi-square analysis was used to evaluate the differences in smoking, diabetes, hypertension and use of beta-blockers, as well as differences in prevalence of lipid disorders. Multiple regression analyses were conducted by using the Statistical Analysis Software (SAS) package to determine correlation coefficients between the clinical data and lipid analyses. We corrected for noted beta-blocker effect, in-hospital sampling bias and diet effect and calculated the expected changes in lipid, lipoprotein cholesterol and apolipoprotein A-I and B levels. The Spearman correlation coefficients were used for variables not having a normal distribution. Stepwise discriminant analysis was performed by using a forward/backward procedure with hypertension, smoking, diabetes, triglycerides and lipoprotein cholesterol levels or apolipoprotein values entered into the statistical model.

Results

Clinical data (Table 1). The mean age of the coronary artery disease and control groups was virtually identical, although a statistically significant difference was detected (50 ± 7 vs. 49 ± 6 years, patients vs. control subjects, $p = 0.046$). The prevalence of hypertension was higher in the coronary artery disease group (41% vs. 20%, $p < 0.001$), as was the frequency of diabetes mellitus (12% vs. 3.2%, $p < 0.001$) and smoking (67% vs. 28%, $p < 0.001$). Body mass index was 27.79 ± 4.08 in the coronary artery disease group versus 27.14 ± 3.66 in the control group ($p = 0.024$). After correction for multiple t tests (Bonferroni correction), age and body mass index were no longer significantly different.

Table 1. Clinical Characteristics of Patient and Control Groups

	Patients With CAD (n = 321)	Control Subjects (n = 901)	p Value	p' Value
Age (yr)	50 ± 7	49 ± 6	0.046	0.276
% Male	100%	3%	—	—
Beta-blocker	65%	7%	<0.001	0.001
Hypertension	41%	20%	<0.001	0.001
Diabetes	12%	3.2%	<0.001	0.001
Smokers	67%	28%	<0.001	0.001
BMI	27.79 ± 4.08	27.14 ± 3.66	0.024	0.144

BMI = body mass index (weight [kg]/height [cm]^2); CAD = coronary artery disease; p' = corrected for multiple t tests (Bonferroni correction).

Because of our previous finding (57) that sampling in patients in the hospital can lead to a bias in lipoprotein levels, especially for HDL cholesterol, we performed prospective resampling in 72 patients after hospital discharge and ≥6 weeks after cardiac catheterization. No significant effect on total, LDL and VLDL cholesterol, plasma triglycerides or apolipoprotein B concentrations was noted in the out of hospital state compared with the hospital sampling. However, as we have previously seen, HDL cholesterol and apolipoprotein A-I were lower at the time of the catheterization than in the out of hospital state (33 ± 9 vs. 37 ± 8 mg/dl, $p < 0.001$ and 105 ± 23 vs. 117 ± 24 mg/dl, $p < 0.001$, respectively). On the basis of this sample (58) and previously reported data (57), we believed that a correction factor was necessary to compare HDL cholesterol and apolipoprotein A-I values in the patient and control groups. The increase in HDL and apolipoprotein A-I concentrations observed out of hospital was proportional to the initial (in-hospital) values. HDL cholesterol was thus increased by a factor of 1.0916 and apolipoprotein A-I by 1.101 for patients with coronary artery disease who underwent sampling in the hospital at the time of cardiac catheterization.

Medication effect was also evaluated in patients with coronary artery disease (Table 2). Beta-blockers are known to exert an effect on plasma lipoprotein levels (59–62). Of the 321 patients 113 (35%) were not and 208 (65%) were taking a beta-blocker. There were no statistically significant differences in total cholesterol or apolipoprotein B values in these two subgroups; however, patients taking a beta-blocker had lower LDL cholesterol, HDL cholesterol and apolipoprotein A-I concentrations and higher triglyceride levels than did patients who were not taking such medication ($p < 0.05$) (Table 2). Because the effects of beta-blockers on plasma lipoprotein concentrations are significant and two-thirds of our patients were taking this class of medication, we analyzed the patients with coronary artery disease as a

Table 2. Lipoprotein and Apolipoprotein Levels in Control Subjects and Patients With Coronary Artery Disease: Effects of Beta-Adrenergic Blocking Agents

	Control Group (n = 901)	Coronary Artery Disease Group					
		Overall* (n = 321)	p[1]	Beta-Blocker Treatment		p[3]	p[4]
				Off (n = 113)	On (n = 208)		
T chol	214 ± 36	211 ± 49	0.343	218 ± 56	208 ± 45	0.059	0.18
Tg	141 ± 104	189 ± 96	<0.001	177 ± 95	195 ± 96	0.046	<0.001
VLDL	28 ± 21	38 ± 19	<0.001	35 ± 19	39 ± 19	0.037	<0.001
LDL	138 ± 33	141 ± 46	0.853	148 ± 48	137 ± 40	0.034	<0.01
HDL	45 ± 12	35 ± 10	<0.001	37 ± 12	34 ± 9	0.023	<0.001
Apo B	108 ± 33	123 ± 33	<0.001	135 ± 34	123 ± 35	0.336	<0.001
Apo A-I	136 ± 32	111 ± 25	<0.001	114 ± 27	108 ± 24	0.016	<0.001
Lp(a)	14.9 ± 17.5	19.9 ± 21.5	<0.002	21.1 ± 16	17.8 ± 19	0.378	<0.01

*Overall results (expressed in mg/dl) corrected only for hospital effect. High density lipoprotein (HDL) cholesterol increased from 32 ± 10 to 35 ± 11 mg/dl (HDL × 1.0916). Lipoprotein (a) [Lp (a)] was determined in 760 male control subjects and 256 male patients with coronary artery disease. Apolipoprotein A-I (Apo A-I) increased from 100 ± 22 to 111 ± 25 mg/dl (Apo A-I × 1.101) to compensate for hospital effect (see text for details). Apo B = Apolipoprotein B; LDL = low density lipoprotein cholesterol; p[1] = control subjects vs. patients with coronary artery disease; p[2] = patients with coronary artery disease not taking a beta-adrenergic blocker vs. those taking such medication; p[3] = control subjects vs. patients with coronary artery disease not taking a beta-blocker; T chol = total cholesterol; Tg = triglycerides; VLDL = very low density lipoprotein cholesterol.

group, then separated those who were and were not receiving beta-blocker therapy. We also adjusted triglyceride, LDL cholesterol, HDL cholesterol, apolipoprotein B and apolipoprotein A-I levels in patients taking a beta-blocker to those patients who were not taking a beta-blocker. We assumed that the differences observed between patients with and without beta-blocker therapy were solely due to the use of such medication.

Lipid, lipoprotein and apolipoprotein levels (Tables 2 and 3). To correct for a diet effect, total cholesterol, LDL cholesterol and apolipoprotein B levels were increased by 6.6%, as discussed. Uncorrected lipid, lipoprotein and apolipoprotein levels are shown in Table 2.

After adjustment for confounding variables, lipid and lipoprotein cholesterol and apolipoprotein levels in patients and control subjects were compared (Table 3). The patients with coronary artery disease had a 4.7% higher total cholesterol level (224 ± 53 vs. 214 ± 36 mg/dl, $p < 0.001$), 34% higher triglyceride level (189 ± 95 vs. 141 ± 104 mg/dl, $p < 0.001$) and 13% higher LDL cholesterol level (156 ± 51 vs. 138 ± 33 mg/dl, $p < 0.001$); they had a 22% lower HDL cholesterol concentration (36 ± 11 vs. 45 ± 12 mg/dl, $p < 0.001$), 16% lower apolipoprotein A-I level (114 ± 26 vs. 136 ± 32 mg/dl, $p < 0.001$), 21% higher apolipoprotein B level (131 ± 37 vs. 108 ± 33 mg/dl, $p < 0.001$) and 34% higher Lp(a) level (19.9 ± 19 vs. 14.9 ± 17.5 mg/dl, $p < 0.003$). Not correcting for beta-blocker and diet effects significantly alters the classification of lipid disorders in patients with coronary artery disease on such medication (Table 2).

Prevalence of lipoprotein abnormalities (Table 4). The cut points used for lipoprotein abnormalities were the 90th percentiles for age and gender according to the Lipid Research Clinics data for total and LDL cholesterol and triglyceride levels and the 10th percentile for HDL choles-

Table 3. Lipoprotein and Apolipoprotein Levels in Control Subjects and Patients With Coronary Artery Disease

	Control Group (n = 901)	Coronary Artery Disease Group (n = 321)			
		Overall*	p[1]	After ADJ†	p[2]
T chol	214 ± 36	211 ± 49	0.343	224 ± 53	<0.001
Tg	141 ± 104	189 ± 96	<0.001	189 ± 95	<0.001
VLDL	28 ± 21	38 ± 19	<0.001	38 ± 19	<0.001
LDL	138 ± 33	141 ± 46	0.853	156 ± 51	<0.001
HDL	45 ± 12	35 ± 10	<0.001	36 ± 11	<0.001
Apo B	108 ± 33	123 ± 33	<0.001	131 ± 37	<0.001
Apo A-I	136 ± 32	111 ± 25	<0.001	114 ± 26	<0.001
Lp(a)	14.9 ± 17.5	19.9 ± 21.5	<0.002	19.9 ± 19	<0.003

*As in Table 2. †Total cholesterol (T chol), low density lipoprotein (LDL) cholesterol, apolipoprotein A-I (Apo A-I) and apolipoprotein b (Apo B) adjusted (ADJ) for beta-adrenergic blocker use and effects of diet (see text). p[2] = control subjects vs. patients with coronary artery disease after adjustment for beta-adrenergic blockers and diet effects. Abbreviations as in Table 2.

Table 4. Prevalence of Lipoprotein Abnormalities in Patients With Premature Coronary Artery Disease

	Control Group (n = 901)	Coronary Artery Disease Group (n = 321)	
		Overall	After ADJ*
HDL	4.4%	27.1%†	19.3%†
Tg+HDL	4.2%	13.7%†	9.7%‡
Tg	8.5%	9.7%	9.7%
LDL	9.0%	4.7%‡	12.1%‡
LDL+HDL	0.3%	4.7%‡	3.7%‡
Tg+LDL	0.4%	1.6%‡	3.1%‡
Tg+LDL+HDL	0.2%	0.9%	3.4%‡
Dyslipidemic	27.0%	62.4%†	61.1%†
Normal	73.0%	37.8%†	38.9%†
All HDL	9.0%	46.1%†	36.1%†
All Tg	13.3%	25.9%†	25.9%†
All LDL	9.900%	11.9%	22.4%†
Apo B	11.6%	24.1%†	36.4%†
Apo A-I	10.7%	37.0%†	36.4%†
Lp(a)	10.0%	15.8%‡	15.8%‡

*After adjustment for sampling, beta-adrenergic blockers and diet. Triglyceride (Tg) level >90th percentile. Low density lipoprotein (LDL) cholesterol >90th percentile and high density lipoprotein (HDL) cholesterol <10th percentile [>90th percentile for age and sex matched LRC values for Tg and LDL cholesterol; <10th percentile for HDL; apolipoprotein B [Apo B] >90th percentile. Apolipoprotein A-I [Apo A-I] <10th percentile, based on the Framingham Heart Study). Presence of familial hypercholesterolemia in 4 of 321 (frequency 0.0125). †p < 0.005; ‡p < 0.05. Abbreviations as in Table 2.

terol (71). For apolipoproteins B and A-I, the values were derived from our control group of 901 men from the Framingham Heart Study free of clinical manifestations of coronary artery disease. The 90th percentile for apolipoprotein B and the 10th percentile for apolipoprotein A-I were chosen as cut points. Based on a slightly smaller sample of control subjects (n = 760), the 90th percentile for Lp(a) was determined at 38.8 mg/dl.

The most frequent abnormality observed was low HDL cholesterol (hypoalphalipoproteinemia) after correction for hospital and medication effects. The next most common phenotype was elevated LDL cholesterol either alone or in combination with elevated triglycerides or reduced HDL cholesterol, or both. The combination of hypertriglyceridemia and decreased HDL cholesterol was also significantly higher in patients with coronary artery disease. The relation between hypertriglyceridemia and low HDL cholesterol has long been known (5). The prevalence of elevated Lp(a) >38.8 mg/dl was higher in patients (15.8%) than in control subjects (10%, $p < 0.05$). In the coronary artery disease group, four cases of heterozygous familial hypercholesterolemia with tendinous xanthomas were noted (estimated frequency 0.0125). In the control group, two patients had a cholesterol level >350 mg/dl with LDL cholesterol level >95th percentile.

Based on the 90th percentile for triglycerides and LDL cholesterol and the 10th percentile for HDL cholesterol, 38.9% of patients had no abnormality compared with 73% of control subjects ($p < 0.001$) (Table 4). A slightly higher proportion of patients had a significant lipid abnormality while taking a beta-blocker than while not taking such medication (64.4% vs. 57.5%, data not shown). The correlations among the lipid variables are shown in Table 5.

The relation between LDL cholesterol and plasma apolipoprotein B on a scattergram (data not shown) reveals that in some patients with coronary artery disease, elevation of apolipoprotein B occurs without a proportional elevation in LDL cholesterol. This observation has been previously made by Sniderman et al. (19) for LDL apolipoprotein B (72) and LDL cholesterol. The prevalence of elevated apolipoprotein B with normal LDL cholesterol (using the 90th percentile for both LDL and apolipoprotein B levels) was 19.8% compared with 8.4% in the control group ($p < 0.005$). However, only 10.7% of the patients with coronary artery disease had elevated apolipoprotein B with normal (that is, <90th percentile) levels of triglycerides and LDL cholesterol. This provides an index of the frequency of hyperapobetalipoproteinemia (19) in this cohort.

Discriminant analysis (Table 6). Discriminant analysis reveals that conventional risk factors allow for an excellent discrimination between patients and control subjects (Table 6A). When apolipoprotein variables were entered into the statistical model and triglycerides, total cholesterol, LDL cholesterol and HDL cholesterol were removed, apolipoprotein B conferred better discrimination than did LDL choles-

Table 5. Correlation Matrix of Lipoproteins and Apolipoproteins in Patients With Premature Coronary Artery Disease (n = 321) and Control Subjects (n = 901)

	T Chol	Tg	VLDL	LDL	HDL	Apo B	Apo A-I	Lp(a)*
T Chol		0.262†	0.257†	0.923†	0.183*	0.720†	0.279†	−0.030
Tg	0.310†		0.997†	−0.078	−0.263**	0.317†	0.007	−0.166
VLDL	0.346†	0.970†		−0.085	−0.258**	0.315†	0.003	−0.166
LDL	0.857†	−0.102*	−0.077		0.096	0.644†	0.153*	−0.006
HDL	0.005	−0.420†	−0.429†	−0.089†		−0.040	0.670†	0.093
Apo B	0.673†	0.346†	0.387†	0.589†	−0.293†		0.073	−0.160
Apo A-I	0.135†	−0.189†	−0.186†	−0.008	0.755†	−0.093*		0.056
Lp(a)	0.042	−0.147	−0.147	0.135	0.055	0.041	0.039	

*Lipoprotein (a) [Lp (a)] based on 760 control subjects and 256 patients with coronary artery disease. *p < 0.01, †p < 0.001. Data are Spearman correlation coefficients. Lower left = control subjects; upper right = patients with coronary artery disease. Abbreviations as in Table 2.

Table 6. Stepwise Discriminant Analysis

Step	Variable	Partial r^2	Model r^2	p Value
	A: Lipids and Lipoproteins*			
1	Smoking	0.371	0.371	0.001
2	Hypertension	0.114	0.485	0.001
3	HDL	0.094	0.579	0.001
4	Diabetes	0.050	0.629	0.001
5	LDL	0.008	0.637	0.002
6	Lp(a)	0.003	0.640	0.041
	B: Lipids, Lipoproteins and Apolipoproteins*			
1	Smoking	0.372	0.372	0.001
2	Apo A-I	0.107	0.479	0.001
3	Hypertension	0.094	0.573	0.001
4	Apo B	0.049	0.622	0.001
5	Diabetes	0.030	0.652	0.001
6	Lp(a)	0.003	0.655	0.040

*Based on 760 control subjects and 256 men with coronary artery disease. Abbreviations as in Table 2.

terol between patients and control subjects (Table 6B). Triglyceride concentrations appear to offer significant univariate discrimination between patients and control subjects, but were excluded from the model with multivariate analysis. Mean and median Lp(a) levels were higher in patients than in control subjects; Lp(a) is an independent risk factor for the development of coronary artery disease. The second model that included traditional risk factors revealed that smoking, hypertension, apolipoprotein A-I, apolipoprotein B, Lp(a) and diabetes were all significantly associated with the presence of coronary artery disease.

The frequency distributions for adjusted levels of LDL cholesterol (Fig. 1A), apolipoprotein B (Fig. 1B), HDL cholesterol (Fig. 1C), apolipoprotein A-I (Fig. 1D), triglycerides (Fig. 1E) and Lp(a) (Fig. 1F) are shown for patients and control subjects.

Discussion

Identification of patients at risk for developing coronary artery disease. This poses a daunting problem. Because of the multifactorial etiology of the disorder in which environmental, genetic and nutritional aspects are so closely interrelated, it is becoming increasingly difficult to provide a cost-effective assessment of risk in the general population. The recently published recommendations of the National Cholesterol Education Program (45) have focused on total and LDL cholesterol as a basis for screening and treatment. Our data suggest that total and LDL cholesterol may not be the best discriminants for the presence of coronary artery disease despite the strong association between elevated cholesterol and the development of coronary artery disease in cross-sectional population studies and prospective epidemiologic studies. Although total cholesterol remains a good marker for coronary artery disease between populations (1,2,20), HDL cholesterol appears to be a better predictor within populations.

The present study shows that total and LDL cholesterol do not differ significantly between patients and control subjects if the confounding effects of diet and beta-blockers (75) are not taken into account. LDL cholesterol, however, is higher in patients not taking beta-blockers than in control subjects and patients with coronary artery disease appear to have a healthier diet than normal control subjects.

Triglycerides. The association between triglyceride concentrations and coronary atherosclerosis deserves close scrutiny. We observed a significantly higher triglyceride concentration in patients with coronary artery disease than in control subjects, an observation previously noted (74,75), but not in large, prospective epidemiologic studies. The negative correlation between elevated triglycerides and decreased HDL cholesterol levels makes it difficult to consider triglyceride levels independently. It does appear that high triglyceride levels are associated with increased cardiovascular risk when they are associated with elevated levels of LDL cholesterol or plasma apolipoprotein B (type IIb hyperlipoproteinemia or hyperapobetalipoproteinemia [76]) or low HDL cholesterol levels, alone or in combination. Hypertriglyceridemia alone, however, is not significantly more frequent in patients than control subjects, indicating that it is the combination of hypertriglyceridemia with elevated LDL or reduced HDL cholesterol, or both, that confers additional cardiovascular risk.

The data presented here show the importance of considering confounding variables, not only with respect to individual patients, but when interpreting epidemiologic studies dealing with lipids and coronary atherosclerosis. The high prevalence of dyslipidemias in the coronary artery disease group, irrespective of confounding variables, strongly supports the concept of the role of lipid disorders in the pathogenesis of coronary atherosclerosis. Diverse mechanisms will undoubtedly underlie most lipid disorders; for the purpose of discussion, these disorders will be grouped into disorders of LDL, triglycerides, HDL and Lp(a).

Elevated LDL cholesterol (type IIA hyperlipoproteinemia). In this study, the prevalence of elevated LDL cholesterol in patients was 22.4% vs. 9.9% in control subjects ($p < 0.05$) (Table 4) after correction for confounding variables. If those variables are not taken into account, mean levels of LDL cholesterol and the prevalence of elevated LDL cholesterol are underestimated. The prevalence of elevated LDL cholesterol was 11.9% in patients before correction. The prevalence of LDL cholesterol >90th percentile without other abnormalities was 12.1% vs. 9% in the control group. Heterozygous familial hypercholesterolemia (as defined by markedly elevated LDL cholesterol levels [>95th percentile], the presence of tendinous xanthomas, familial segregation and premature coronary artery disease) was present in 4 of 321 patients (prevalence 0.0125). In the control group, two patients had total cholesterol levels >350 mg/dl with LDL cholesterol >95th percentile (prevalence 0.002). Although

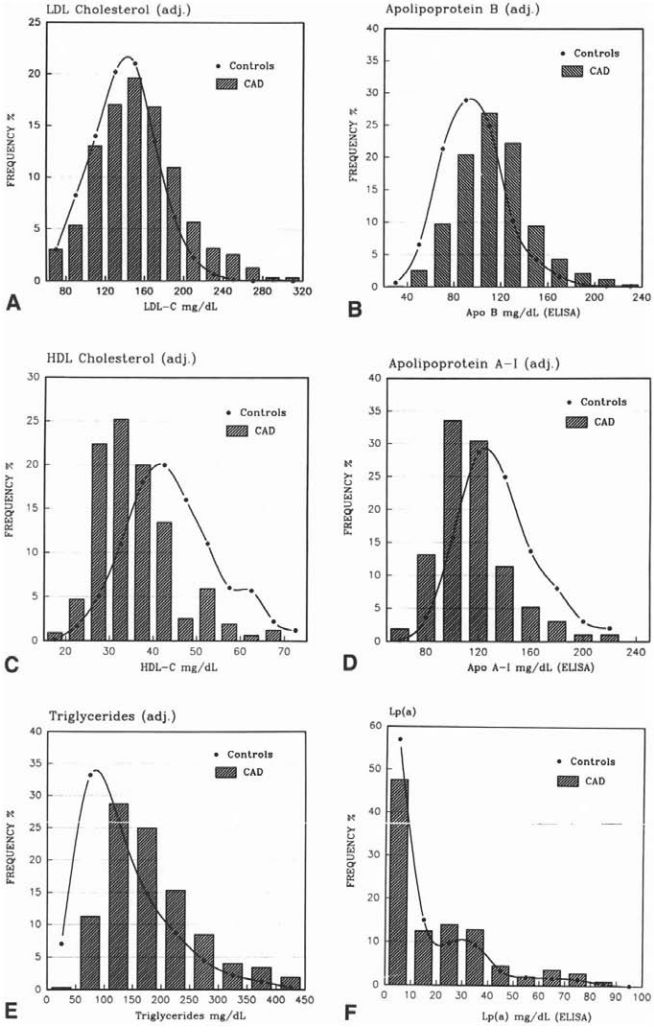
LDL Cholesterol (adj.)
FREQUENCY %
• Controls
CAD
LDL-C mg/dL
A
Apolipoprotein B (adj.)
Apo B mg/dL (ELISA)
B
HDL Cholesterol (adj.)
HDL-C mg/dL
C
Apolipoprotein A-I (adj.)
Apo A-I mg/dL (ELISA)
D
Triglycerides (adj.)
Triglycerides mg/dL
E
Lp(a)
Lp(a) mg/dL (ELISA)
F

Figure 1. Frequency distribution of LDL cholesterol (LDL-C) (**A**), apolipoprotein (Apo) B (**B**), HDL cholesterol (HDL-C) (**C**), apolipoprotein (Apo) A-I (**D**), triglycerides (**E**) and Lp(a) (**F**) in control subjects (lines) and in men with coronary artery disease (CAD) (hatched bars). Data are adjusted (adj.) for diet, sampling and beta-blocker use. ELISA = enzyme-linked immunosorbent assays.

accurate estimates of the prevalence of familial hypercholesterolemia due to a functional abnormality of the LDL receptor do not exist, the frequency varies from 0.002 (1 of 500) in the general population to 0.0037 (1 of 270) in populations with a founder effect, such as the French Canadians or Afrikaaners in South Africa (77,78).

Combined hyperlipidemia (types IIB and IV hyperlipoproteinemias). The frequency of hypertriglyceridemia (without other lipoprotein abnormalities) was 9.7% in the patient group versus 8.5% (close to the expected frequency of 8.1%) in the control group (p = NS). Hypertriglyceridemia without elevated LDL cholesterol or decreased HDL cholesterol was not seen more frequently in patients with premature coronary artery disease in this study. The frequency of elevated triglycerides associated with low HDL cholesterol was higher than expected in the control group if both were independent of each other (which is not the case). Hypertriglyceridemia combined with hypoalphalipoproteinemia is common in patients with coronary artery disease (Table 4). The frequency of elevated triglycerides and LDL cholesterol, with or without decreased HDL cholesterol, was greater in patients on a beta-blocker than those not taking such medication.

Hypoalphalipoproteinemia (low HDL cholesterol). In the present study, the most common abnormality was hypoalphalipoproteinemia, either alone (19.3% vs. 4.4%, $p < 0.001$) or associated with an elevated triglyceride concentration (9.7% vs. 4.2%, patients vs. control subjects, $p < 0.001$). Both the use of a beta-blocker and in-hospital sampling cause an overestimation of the prevalence of hypoalphalipoproteinemia, as does the higher proportion of smokers in our patient group (Table 1). The frequency of "pure" hypoalphalipoproteinemia is lower than the expected level in the control group (4.4%) and the combined disorder of hypertriglyceridemia with low HDL cholesterol is higher than expected (4.2%). These observation underlie the close inverse association of triglycerides and HDL cholesterol. In the coronary artery disease group, 36.1% had low HDL cholesterol alone or in combination with other lipoprotein abnormalities compared with 9% in the control group. This represents a fourfold increase over values in control subjects. Our data are consistent with previously published data. In several studies (14,27,35,37,40,41,43), the mean HDL cholesterol level was lower than reported in the present study. None of these studies has reported the prevalence of low HDL cholesterol in their patients based on the 10th percentile for age and gender. When considering the cut points of the National Cholesterol Education Program (45), 35% (113 of 321) of our patients had a total cholesterol level <200 mg/dl. Of those, 73% (83 of 113) had a HDL cholesterol level <35 mg/dl. Thus, despite a total cholesterol level considered within the desirable range, many patients had a significant dyslipidemia.

Lp(a) excess. No large population norms are as yet available for Lp(a). In this study Lp(a) levels were higher in the patient group than in the control group and a prevalence of Lp(a) >38.8 mg/dl was also higher in the coronary artery disease group. The frequency distribution of Lp(a) is skewed to the right both in patients and in control subjects. The physiologic role of Lp(a) has not been elucidated, but Lp(a) may interfere with intravascular thrombolysis and inhibit the streptokinase-mediated conversion of plasmin from plasminogen. Furthermore, Lp(a) is found within atherosclerotic plaque and may contribute to cholesterol ester accumulation within the plaque (56).

Apolipoprotein B. It has been suggested that apolipoprotein B and apolipoprotein A-I serve as better discriminators for the presence of coronary artery disease than LDL or HDL cholesterol. The level of apolipoprotein B was increased in our patients (Table 3) and the value was not influenced by sampling effect or beta-blocker use. Furthermore, the frequency of elevated apolipoprotein B in the coronary artery disease group is nearly three times that in the control group after correction for confounders. Our assay does not measure LDL apolipoprotein B, but does measure total plasma apolipoprotein B. Therefore, we could not reliably establish the prevalence of hyperapobetalipoproteinemia (elevated apolipoprotein B in LDL) as originally defined (19) in patients with coronary atherosclerosis because our assay for apolipoprotein B measures total plasma apolipoprotein B in comparison with the radial immunodiffusion (RID) assay that measures LDL apolipoprotein B (72). However, in normolipidemic patients (<90th percentile for triglycerides and LDL cholesterol), 10.7% have an elevated apolipoprotein B alone compared with 4.5% in the control group. Apolipoprotein B may reflect the number of apolipoprotein B-containing particles and thus provides better discrimination than the cholesterol content of VLDL and LDL particles.

Apolipoprotein A-I. Levels of apolipoprotein A-I were decreased in patients with coronary artery disease to a degree similar to the reduction in HDL cholesterol. The prevalence of low apolipoprotein A-I (<10th percentile) in patients was nearly 3.5 times that found in control subjects (after correction for biases). Comparing the two by stepwise discriminant analysis, apolipoprotein A-I appears slightly better than HDL cholesterol in differentiating patients from control subjects.

In this case-control study of 321 men with angiographically documented coronary artery disease, plasma levels of cholesterol, LDL cholesterol, triglycerides, apolipoprotein B and Lp(a) were increased and levels of HDL cholesterol and apolipoprotein A-I were decreased compared with a group of healthy middle-aged men with no clinical manifestations of coronary atherosclerosis. The prevalence of lipo-

protein abnormalities was confounded by in-hospital sampling bias, dietary changes and use of beta-blockers. When these were taken into account, the most common abnormalities include hypoalphalipoproteinemia, combined hypertriglyceridemia with hypoalphalipoproteinemia, elevated Lp(a) and elevated LDL cholesterol. The use of beta-blockers in patients results in overestimation of the frequency of hypertriglyceridemia and hypoalphalipoproteinemia and underestimation of the frequency of elevated LDL cholesterol.

Conclusions. This study revealed a high prevalence of dyslipidemias in patients with coronary artery disease. Clinical trials (79–83) have demonstrated that a reduction in elevated LDL cholesterol is associated with a reduction in cardiovascular mortality. The current guidelines of the National Cholesterol Education Program (45) are directed at the screening and treatment of elevated LDL cholesterol levels. The prevalence of HDL cholesterol and triglyceride abnormalities was greater in the coronary artery disease group than in the control group and accounted for >50% of the lipoprotein abnormalities identified in this study. We recommend that adult men with coronary artery disease have a determination of HDL cholesterol, regardless of total cholesterol, and that healthy men have a determination of triglycerides and HDL cholesterol if the total cholesterol is ≥200 mg/dl. In addition, our data suggest that apolipoprotein B, apolipoprotein A-I and Lp(a) are slightly better discriminators between patients and control subjects than are conventional lipoprotein variables.

To our knowledge, no other case-control studies in patients with coronary artery disease have taken into account the effects of sampling biases, medications and differences in diet compared with a control group. However, prospective studies have shown the importance of elevated total and LDL cholesterol in patients with coronary artery disease. Our study suggests that once these variables are taken into account, our results closely match those of prospective studies with regard to lipoprotein cholesterol levels. Moreover, the data point to the concept that in patients with established coronary artery disease more aggressive efforts should be made to lower LDL cholesterol levels to <100 mg/dl and consideration should also be directed to using agents known to raise HDL constituents (for example, niacin, fenofibrate, gemfibrozil, simvastatin, lovastatin and pravastatin).

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