High-Density Lipoprotein: Relations to Metabolic Parameters and Severity of Coronary Artery Disease

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The regulation of plasma high-density lipoprotein (HDL) cholesterol level by the joint influence of plasma lipoprotein lipids, lipoprotein lipase (LPL), hepatic lipase (HL), cholesteryl ester transfer protein (CETP), oral glucose tolerance, and postload plasma insulin and proinsulin levels was investigated in young postinfarction patients and healthy population–based control subjects. In addition, the association between HDL cholesterol and the number and severity of coronary stenoses previously reported in this cohort of young postinfarction patients was further investigated by analyzing the determinants and angiographic relations of HDL subclasses measured by gradient gel electrophoresis. The following parameters showed significant univariate relations with HDL cholesterol level in the patient group: very–low-density lipoprotein (VLDL) cholesterol and triglyceride, low-density lipoprotein (LDL) triglyceride, and postload plasma insulin concentrations, preheparin plasma LPL mass, and postheparin plasma HL activity. In the control group, significant correlations with HDL cholesterol concentration in addition to those noted among the patients were found for body mass index (BMI), LDL cholesterol level, postload plasma intact proinsulin concentration, and LPL activity in postheparin plasma. In contrast to the patients, no significant relations were noted for postload plasma insulin level and preheparin plasma LPL mass. Multiple stepwise regression analysis showed that 42% of the variability of HDL cholesterol in the patients could be accounted for by VLDL cholesterol concentration (29%), LDL triglyceride level (7%), and postheparin plasma HL activity (6%), whereas the corresponding figure in controls was 35% (VLDL cholesterol concentration [9%] and postheparin plasma HL activity [26%]). The strength of the relationships of HDL cholesterol and HDL subclasses to the coronary stenosis score was similar and statistically significant (r = .25 to .36). When the metabolic parameters that correlated with HDL cholesterol and HDL subclass concentrations in univariate analysis were used as covariates, all relations to the coronary stenosis score disappeared. This clearly indicates that the influence of triglyceride-rich lipoproteins and lipolytic enzymes needs to be considered when assessing the association between HDL cholesterol and coronary artery disease (CAD).

HIGH-DENSITY LIPOPROTEIN (HDL) cholesterol concentration has been linked to the risk of future coronary heart disease (CHD) in prospective epidemiological studies, and to the presence, severity, and rate of progression of coronary artery disease (CAD) as documented by angiography.[1] However, the mechanisms behind the inverse relationship between HDL cholesterol and CAD remain obscure. Efflux of free cholesterol from cells to HDL particles or to its structural protein, apolipoprotein A-I, has been described,[2] suggesting that increased HDL cholesterol reflects an effective reverse cholesterol transport. Other possibilities are that a high HDL cholesterol level reflect the efficiency of the metabolism of other potentially atherogenic lipoproteins such as very–low-density lipoprotein (VLDL)[3] and chylomicrons,[4] or that a substantial number of HDL particles increases the resistance to oxidative modification of VLDL and low-density lipoprotein (LDL) by carrying larger amounts of antioxidative substances.[5]

Several enzymes are involved in the regulation of HDL metabolism. Lipoprotein lipase (LPL) and hepatic lipase (HL) hydrolyze triglycerides and phospholipids of triglyceride-rich lipoproteins (chylomicrons and VLDL), which reduces particle size, and subsequently, residual surface material such as phospholipids and free cholesterol is taken up by HDL, whereby cholesteryl esters are formed by the action of lecithin cholesterol acyl-transferase.[6,7] A further mechanism regulating HDL cholesterol concentration is the transfer of cholesteryl esters from HDL to triglyceride-rich lipoproteins in exchange for triglycerides, by cholesteryl ester transfer protein (CETP).[8] Triglycerides transferred to HDL may, in turn, be hydrolyzed by HL, a process generating smaller HDL particle species.[6]

Men with a myocardial infarction before the age of 45 comprise a group of CAD patients known to have disturbances in the metabolism of triglyceride-rich lipoproteins and low HDL cholesterol levels,[9] which are related to the number and severity of coronary stenoses determined by angiography.[10] The present study extends the previously reported relationships between the preheparin plasma LPL mass and postheparin plasma LPL activity, and the HDL cholesterol concentration in young postinfarction patients and healthy population–based control subjects, respectively,[11] by also including HL, CETP, oral glucose tolerance, and plasma insulin and proinsulin levels to further investigate the determinants of HDL cholesterol level. In addition to the data on preheparin and postheparin plasma LPL

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mass and activity,[11] group differences in postload glucose, insulin, and proinsulin concentrations have been presented previously[12] in these cohorts of postinfarction patients and controls, whereas the data on postheparin plasma HL and plasma CETP activities have not been published elsewhere. Furthermore, the inverse association between HDL cholesterol concentration and the number and degree of coronary stenoses in this cohort of young postinfarction patients[10] was investigated by analyzing the determinants and angiographic relations of HDL subclasses measured by gradient gel electrophoresis.[13]

SUBJECTS AND METHODS

Patients and Controls

A total of 64 male patients who had suffered a first myocardial infarction before the age of 45 were investigated consecutively 3 to 6 months after the acute event as part of an ongoing study of mechanisms underlying myocardial infarction at a young age. The patients had initially been admitted to one of 10 hospitals in Stockholm County with a coronary or intensive care unit between April 1989 and October 1990. During this period, 104 patients were considered eligible for the study. Patients with insulin-dependent diabetes mellitus, large cerebral infarctions, severely impaired renal function, and heterozygous familial hypercholesterolemia were excluded. Other reasons for exclusion were death, late referral, insufficient laboratory capacity, or unwillingness to participate (for further details, see Tornvall et al[10]). Healthy men with an age distribution similar to that of the patients were recruited by random selection from the general population. A total of 164 men born between 1947 and 1956 and living in Stockholm County were invited to participate between August 1991 and September 1992. Of these, 129 agreed to participate in the program, which included blood sampling and an interview to exclude individuals with a history of myocardial infarction, angina pectoris, or any other severe illness. The first 95 control subjects participating in the study also underwent an oral glucose tolerance test and formed the control group for the present report.

There was no clinical or laboratory evidence of thyroid dysfunction or other conditions leading to secondary hyperlipidemia in any of the patients or controls studied. None of the patients or controls were on lipid-lowering drugs at the time of study. All patients 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 as carbohydrate. The ratio of saturated to monounsaturated to polyunsaturated fat was 1:1:1.

Blood Sampling

Blood samples 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 from symptoms of infectious disease at the time of blood sampling. Venous blood for lipoprotein separation and isolation of lipoprotein-depleted plasma (LPDP) was drawn into precooled vacutainer tubes containing disodium EDTA and placed in an ice bath. Plasma was then recovered by low-speed centrifugation (1,400 × *g* for 20 minutes). Blood samples were also taken into vacutainer tubes containing sodium heparin (29 U/mL) before and 15 minutes after injection of 100 U heparin/kg body weight. Plasma samples for analysis of LPL mass and activity and HL activity were prepared and immediately frozen at −70°C, and later transported on dry ice to Umeå University. On a separate occasion 1 or 2 weeks later, venous blood was also taken before and during an oral glucose tolerance test for determination of glucose, insulin, intact proinsulin.

Lipoprotein Fractionation and Lipid Analysis

The major plasma lipoproteins (VLDL, LDL, and HDL) were determined by a combination of preparative ultracentrifugation and precipitation of apolipoprotein B–containing lipoproteins,[14] followed by cholesterol and triglyceride analysis using chemical methods[15,16] after extraction with methylene chloride-methanol.[17] Lipoprotein phenotyping was based on the 90th percentiles of VLDL triglycerides (1.79 mmol/L) and LDL cholesterol (4.83 mmol/L) in all controls, after exclusion of three individuals on β-blockers due to hypertension and two individuals with known non–insulin-dependent diabetes mellitus. Three VLDL and 13 LDL subclasses were isolated by density gradient ultracentrifugation followed by enzymatic analysis of triglyceride, free and esterified cholesterol, and phospholipid concentrations.[10]

Determination of Lipases in Preheparin and Postheparin Plasma

LPL mass and activity were determined in fasting and postheparin plasma in patients and controls analyzed together, as previously described.[11] Briefly, LPL mass was determined with a sandwich enzyme-linked immunosorbent assay, and LPL activity by incubating a labeled triglyceride emulsion after preincubation with antibodies to HL. Postheparin plasma HL activity was determined as the salt-resistant lipolytic activity.[18] Lipase measurements were performed in 62 patients and 68 controls.

CETP Activity Assay

LPDP was recovered by ultracentrifugation, after adjustment of the plasma to density 1.21 kg/L by NaBr, for 48 hours, at 1°C in a Beckman (Palo Alto, CA) 50.3 Ti rotor at 50,000 rpm. LPDP was frozen at −70°C after dialysis against 0.15 mol/L NaCl and 1 mmol/L EDTA. CETP activity in LPDP was measured, with patients and controls analyzed together, as transfer of HDL_3-bound labeled cholesteryl esters to an acceptor substrate consisting of native VLDL, as described in detail previously.[19] CETP activity was determined in 56 patients and 55 controls.

HDL Gradient Gel Electrophoresis

HDL subclasses were determined in the patients by a modification of the gradient gel electrophoresis method described by Blanche et al.[20] Briefly, HDL with density 1.070 to 1.21 kg/L was first separated from plasma by two consecutive runs in a 50.3 Ti Beckman fixed-angle rotor (Beckman L 8-80 ultracentrifuge; 20 hours at density 1.070 kg/L and 65 hours at density 1.21 kg/L, 50,000 and 45,000 rpm, respectively, at +1°C) followed by protein analysis by the Lowry method. Gradient gel electrophoresis was then performed within 24 hours after completion of HDL separation. After precooling the electrophoresis equipment (GE 2/4 LS; Pharmacia, Uppsala, Sweden), the polyacrylamide gradient gels (PAA 4/30; Pharmacia) were installed and prerun at 70 V for 20 minutes; 10 μL isolated HDL was mixed with equal parts of space solution (1.21 kg/L NaBr with 8% sucrose) before application to the gel. Electrophoresis was performed for 16 hours at 150 V. The gels were then stained for protein with amido black. The stained gels were scanned at 570 nm with a dual scanner (Shimadzu CS 930, Kyoto, Japan), and the protein concentration in each HDL subclass was calculated by multiplying the percentage of distribu-

tion by the protein concentration for the total HDL fraction. Particle diameter (d) ranges for the HDL subclasses were defined (HDL_{2b}, 9.71 < d < 12.9 nm; HDL_{2a}, 8.77 < d < 9.71 nm; HDL_{3a}, 8.17 < d < 8.77 nm; HDL_{3b}, 7.76 < d < 8.17 nm: and HDL_{3c}, 7.21 < d < 7.76 nm). The location of these limits on the scanning curves was obtained by plotting the known Stokes diameters of the protein standards with known hydrated diameters against their respective migration distance in the gels.

Oral Glucose Tolerance Test and Insulin and Insulin Propeptide Assays

Glucose was ingested after an overnight fast 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, 90, and 120 minutes after glucose intake. Blood glucose level was measured by a glucose oxidase method (Ektachem; Eastman Kodak, Rochester, NY) in all samples, whereas insulin, des 31,32proinsulin, and intact proinsulin levels were only measured in the first 62 patients and 41 controls in one analysis at the Wynn Department of Metabolic Medicine. Total immunoreactive insulin was determined by radioimmunoassay with polyclonal antisera supplied by Guildhay (Guildford, UK). Immunoradiometric assays were used to measure intact proinsulin and des 31,32proinsulin levels using the murine monoclonal antibodies PEP001 and 3B1 obtained from Novo Nordisk (Bagsvaerd, Denmark) and Serono Diagnostics (Woking, UK), respectively. True insulin was obtained from the insulin radioimmunoassay by subtraction of intact proinsulin and corrected des 31,32proinsulin. Specificity, precision, and reliability of the analytical methods have been described in detail.[21]

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 who was without knowledge of the patients' clinical characteristics or biochemical profiles. The presence and severity of coronary stenoses was determined using a semiquantitative classification system in 15 proximal coronary arterial segments.[10,22] 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. A global coronary stenosis score was subsequently calculated by dividing the sum of all segmental stenosis scores by the number of segments accessible to evaluation. The method for determining the coronary stenosis core and statistical analyses of the reliability of this system have been described in detail elsewhere.[22]

Calculations and Statistical Methods

Conventional methods were used for calculation of the median and range (skewed parameters), mean ± SD, Pearson correlation coefficients, and partial correlation coefficients. Coefficients of skewness were calculated to test deviations from a normal distribution. Logarithmic transformations were performed to obtain a normal distribution before parametric statistical computations on the following parameters: plasma triglycerides, VLDL cholesterol and triglycerides, and insulin areas under the curve (AUCs). Differences in continuous variables between groups were tested by Student's unpaired two-tailed *t* test or by ANOVA. Multiple stepwise linear regression analysis was performed to analyze the independent relationships between different variables. 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 greater.

RESULTS

Basic Characteristics of the Patients and Controls

The patients had a considerably higher body mass index (BMI) than the controls, whereas smoking habits did not differ at the time of the metabolic study. All except three patients were on treatment with cardioselective β-adrenergic blockers (mainly metoprolol) as part of their postinfarction regimen, whereas only three controls had β-blockers as treatment for hypertension. One fifth of the patients but only three of 95 controls had a prior diagnosis of hypertension. About one third of the patients and the majority of controls had a normal lipoprotein phenotype. Hypertriglyceridemic (HTG) lipoprotein phenotypes dominated among hyperlipoproteinemias in both patients and controls (Table 1). In the group comparison, patients had higher VLDL and LDL lipid concentrations and a higher HDL triglyceride level than controls, whereas HDL cholesterol was lower among patients (Table 2).

Lipolytic Enzymes, CETP Activity, and Postload Glucose and Insulin Concentrations

Since postheparin plasma LPL activity, the classic measure of lipolytic capacity, was highly correlated with postheparin plasma LPL mass,[11] only the former is presented (Table 3). Patients had higher preheparin plasma LPL activity and postheparin plasma HL activity than controls, whereas no difference was noted in preheparin plasma LPL mass, postheparin plasma LPL activity, or CETP activity (Table 3). When normotriglyceridemic ([NTG] lipoprotein phenotypes normal and II-A) and HTG (lipoprotein phenotypes II-B and IV) subgroups of patients and controls were

Table 1. Characteristics of Patients and Controls at the Time of the Metabolic Study

Characteristic	Patients (n = 64)	Controls (n = 95)
Age (yr)	39.5 ± 4.0	40.2 ± 2.8
BMI	28.2 ± 4.1	24.5 ± 3.1
β-Blocker medication	61 (95)	3 (3)
Hypertension*	14 (22)	3 (3)
Smoking status†		
Nonsmokers	9 (14)	62 (65)
Former smokers	36 (56)	2 (2)
Current smokers	19 (30)	28 (30)
Lipoprotein phenotypes‡		
Normal	21 (33)	79 (83)
II-A	9 (14)	4 (4)
II-B	12 (19)	4 (4)
IV	22 (34)	8 (8)

NOTE. Results are the mean ± SD or the no. (%).

*A history of hypertension before the myocardial infarction (patients) or the metabolic investigation (controls).

†Subjects who had stopped smoking > 2 years before the metabolic investigation were considered nonsmokers. Former smokers had stopped smoking during the past 2 years, generally at the time of the myocardial infarction (patients). Data on smoking are missing for 3 controls.

‡Lipoprotein phenotypes were defined by the 90th percentiles for VLDL triglycerides (1.79 mmol/L) and LDL cholesterol (4.83 mmol/L) in the control group.

Table 2. Plasma Concentrations of Lipids, Lipoprotein Lipids, and HDL Subclasses

Parameter	Patients (n = 64)	Controls (n = 95)
Plasma (mmol/L)		
Cholesterol	6.24 ± 1.25*	5.22 ± 1.01
Triglycerides	2.52 (0.65-14.25)*	1.13 (0.44-5.57)
VLDL (mmol/L)		
Cholesterol	0.75 (0.11-6.17)*	0.35 (0.04-2.22)
Triglycerides	1.94 (0.27-13.50)*	0.76 (0.15-4.04)
LDL (mmol/L)		
Cholesterol	4.30 ± 1.08*	3.57 ± 0.86
Triglycerides	0.46 ± 0.16*	0.32 ± 0.11
HDL (mmol/L)		
Cholesterol	0.90 ± 0.23*	1.17 ± 0.32
Triglycerides	0.13 ± 0.04*	0.11 ± 0.04
HDL subclass (g/L)		
2b	0.08 ± 0.07	ND
2a	0.25 ± 0.11	ND
3a	0.39 ± 0.11	ND
3b	0.35 ± 0.09	ND
3c	0.17 ± 0.10	ND

NOTE. Values are the mean ± SD or median (range).
Abbreviation: ND, not determined.
*$P < .001$, Student's unpaired t test.

compared, no differences were found in preheparin plasma LPL activity. The difference in postheparin plasma HL activity between all patients and controls was accounted for by the HTG patients and NTG controls. As previously described,[12] the AUCs during the oral glucose tolerance test for glucose, insulin, des 31,32proinsulin, and intact proinsulin concentrations (data not shown) were significantly higher in patients than in controls. Both NTG and HTG patients had higher glucose and insulin AUCs than NTG controls (Table 3).

Univariate Correlations With HDL Cholesterol Level and HDL Subclass Protein Concentrations

Strong inverse relations were found between VLDL cholesterol and triglyceride concentrations and HDL cholesterol level in patients and controls. LDL triglyceride and cholesterol concentrations correlated inversely with HDL cholesterol level in patients and controls, respectively. When NTG and HTG subgroups of patients and controls were considered separately, relations between VLDL lipids and HDL cholesterol were accounted for by HTG patients and NTG controls, respectively (Table 4). Postload insulin levels, but not des 31,32proinsulin (data not shown), intact proinsulin, or glucose levels, correlated weakly and inversely with HDL cholesterol concentration in patients, whereas a weak negative correlation was found between postload intact proinsulin and HDL cholesterol level in controls. The negative correlation between postload insulin and HDL cholesterol in patients was accounted for by the NTG patients (Table 4).

As previously described,[11] preheparin plasma LPL protein concentration correlated strongly with HDL cholesterol level in the patients but not in the controls (Table 5). These relations were similar in NTG and HTG patients. Conversely, a strong positive relation was found between postheparin plasma LPL activity and HDL cholesterol concentration in the controls but not in the patients. This correlation was similar in NTG and HTG controls, but did not reach the level of statistical significance in the latter group, due to the small numbers (Table 5). Postheparin plasma HL activity, on the other hand, correlated inversely with HDL cholesterol level in patients and controls (Table 5). In contrast, no consistent relations were found between CETP activity and HDL cholesterol level in patients and controls (Table 5).

Table 3. LPL Mass and Activity, HL Activity, CETP Activity, and Postload Glucose and Insulin Levels

Parameter	Patients			Controls		
	NTG	HTG	All	NTG	HTG	All
Preheparin plasma						
LPL activity (mU/mL)	2.0 ± 1.5	2.0 ± 1.0	2.0 ± 1.2†	1.5 ± 1.0	1.1 ± 0.8	1.4 ± 0.9
LPL protein (ng/mL)	99 ± 45	82 ± 34	90 ± 40	96 ± 38	82 ± 25	94 ± 37
	(n = 28)	(n = 34)	(n = 62)	(n = 61)	(n = 10)	(n = 71)
Postheparin plasma						
LPL activity (mU/mL)	248 ± 98	290 ± 75	270 ± 87	302 ± 113	307 ± 84	303 ± 109
HL activity (mU/mL)	313 ± 129	376 ± 112§	349 ± 123*	301 ± 109	331 ± 106	305 ± 108
	(n = 26)	(n = 34)	(n = 60)	(n = 58)	(n = 10)	(n = 68)
CETP activity (mU/mL)	2.6 ± 1.0	2.5 ± 0.9	2.5 ± 1.0	2.5 ± 0.9	2.2 ± 0.9	2.4 ± 0.9
	(n = 24)	(n = 32)	(n = 56)	(n = 46)	(n = 9)	(n = 55)
AUC						
Glucose (mmol/L)	9,308 ± 2,654‖	10,122 ± 3,019¶	9,741 ± 2,861‡	7,757 ± 1,473	8,620 ± 2,006	7,879 ± 1,561
	(n = 30)	(n = 34)	(n = 64)	(n = 82)	(n = 12)	(n = 94)
Insulin (pmol/mL)	44,013‖	57,719¶	52,186‡	23,676	35,383	26,514
	(17,434-144,774)	(9,318-154,043)	(9,318-154,043)	(9,292-187,009)	(25,498-56,752)	(9,292-187,009)
	(n = 30)	(n = 34)	(n = 64)	(n = 35)	(n = 6)	(n = 41)

NOTE. Values are the mean ± SD or median (range).
Student's unpaired t test: *$P < .05$, †$P < .01$, ‡$P < .001$: all patients v all controls.
ANOVA for differences between NTG and HTG patients and NTG controls: §$P < .05$, HTG patients v NTG controls; ‖$P < .05$, NTG patients v NTG controls; ¶$P < .001$, HTG patients v NTG controls.

Table 4. Correlation Coefficients for Relations of BMI, VLDL and LDL Lipid Concentrations, and Postload Glucose, Insulin, and Proinsulin Levels With HDL Cholesterol Concentration

Parameter	Patients			Controls		
	NTG	HTG	All	NTG	HTG	All
BMI	−.11	−.12	−.13	−.19	−.30	−.22*
	(n = 29)	(n = 34)	(n = 63)	(n = 82)	(n = 12)	(n = 94)
VLDL						
Cholesterol	−.44*	−.45†	−.50‡	−.57‡	−.37	−.53‡
Triglycerides	−.30	−.43†	−.48‡	−.56‡	−.46	−.54‡
	(n = 29)	(n = 34)	(n = 63)	(n = 83)	(n = 12)	(n = 95)
LDL						
Cholesterol	.14	.17	.13	−.30†	−.32	−.28†
Triglycerides	−.09	−.36*	−.35†	−.22*	−.14	−.21*
	(n = 29)	(n = 34)	(n = 63)	(n = 83)	(n = 12)	(n = 95)
AUC						
Glucose	−.28	.00	−.19	−.11	−.28	−.15
	(n = 29)	(n = 34)	(n = 63)	(n = 82)	(n = 12)	(n = 94)
Insulin	−.46*	.00	−.27*	−.10	.45	−.14
Proinsulin	−.23	.21	−.13	−.29	.62	−.32*
	(n = 29)	(n = 34)	(n = 63)	(n = 35)	(n = 6)	(n = 41)

*$P < .05$.
†$P < .01$.
‡$P < .001$.

The ratio of HDL_2 subclass protein concentrations to total HDL protein was related to the metabolic variables in a similar fashion as HDL cholesterol level, with two exceptions. There was a strong negative relation between the ratio of HDL_2 protein to total HDL protein mass and BMI ($r = -.37$, $P < .01$), which was similar in NTG and HTG patients. The relation between the ratio of HDL_2 to total HDL protein concentration and LPL mass in preheparin plasma was weaker ($r = .28$, $P < .05$) and confined to HTG patients.

Multivariate Analysis of Determinants of HDL Cholesterol Concentration and HDL Size

Only variables within each group that showed statistically significant ($P < .05$) univariate correlations with HDL cholesterol were included in the multiple stepwise regression analysis (Table 6). In patients, 42% of the variation of HDL cholesterol could be predicted by VLDL cholesterol concentration (29%), LDL triglyceride level (7%), and postheparin plasma HL activity (6%), whereas in controls, 35% of the variation of HDL cholesterol could be predicted by VLDL cholesterol concentration (9%) and postheparin plasma HL activity (26%). In NTG patients, 47% of the variation of HDL cholesterol was accounted for by preheparin plasma LPL mass (25%) and insulin AUC (22%). In HTG patients, on the other hand, 41% of the variation of HDL cholesterol could be accounted for by preheparin plasma LPL mass (32%) and CETP activity (9%). Subgroup analysis of the control group was only possible for NTG controls, due to the small number of HTG controls. A total of 39% of the variation of HDL cholesterol among NTG controls could be predicted by VLDL cholesterol concentration (31%) and postheparin plasma LPL activity (8%).

A total of 54% of the variation in HDL size as determined from the ratio of HDL_2 subclass protein to total HDL protein concentrations could be predicted by VLDL cholesterol concentration (34%), postheparin plasma HL

Table 5. Correlation Coefficients for the Relations of Lipase Levels and CETP Activity With HDL Cholesterol Concentration

Parameter	Patients			Controls		
	NTG	HTG	All	NTG	HTG	All
Preheparin plasma						
LPL activity	.03	.02	.02	−.09	.36	.01
LPL mass	.50†	.54‡	.54‡	.10	.40	−.15
	(n = 27)	(n = 34)	(n = 61)	(n = 61)	(n = 10)	(n = 71)
Postheparin plasma						
LPL activity	.12	.06	−.02	.44‡	.36	.42‡
HL activity	−.27	−.41*	−.39†	−.35†	−.60*	−.39‡
	(n = 25)	(n = 34)	(n = 59)	(n = 58)	(n = 10)	(n = 68)
CETP	.35	−.36*	.02	.00	−.31	−.03
	(n = 23)	(n = 32)	(n = 55)	(n = 46)	(n = 9)	(n = 55)

*$P < .05$.
†$P < .01$.
‡$P < .001$.

Table 6. Results of Multiple Stepwise Regression Analysis on HDL Cholesterol

Parameter	Patients			Controls	
	NTG	HTG	All	NTG	All
BMI					
VLDL cholesterol			.29	.31	.09
LDL cholesterol/triglycerides			.07		
Preheparin LPL mass	.25	.32			
Postheparin LPL activity				.08	
Postheparin HL activity			.06		.26
CETP activity		.09			
AUC insulin	.22				
Sum of R^2	.47	.41	.42	.39	.35

NOTE. Values are increased in multiple R^2 contributed by variables included in the regression equation. The multivariate analysis was based on 57 patients (25 NTG and 32 HTG) and 35 controls (26 NTG) who had complete data for all included variables.

activity (8%), LDL triglyceride concentration (7%), and BMI (5%).

Relationships of HDL Cholesterol and HDL Gradient Gel Electrophoresis Subclass Protein Concentrations to Coronary Angiography Scores

As described previously in this cohort of patients,[10] HDL cholesterol concentration related inversely to the coronary stenosis score (model 1, Table 7). Similarly, HDL subclasses 2b, 2a, and 3a correlated inversely with the coronary stenosis score, whereas HDL subclasses 3b and 3c showed positive correlations with the stenosis score. When partial correlation coefficients were calculated between HDL cholesterol, HDL subclasses, and the coronary stenosis score, controlling for the influence of BMI, VLDL cholesterol, and postheparin plasma HL activity, in addition to the number of cholesteryl ester molecules in small VLDL and dense LDL triglycerides, which have been shown to be strong determinants of the coronary stenosis score,[10] all significant correlations between HDL variables and CAD severity disappeared (model 2, Table 7).

DISCUSSION

The results of the present study confirm the findings of multiple previous studies showing increased VLDL lipid and decreased HDL cholesterol concentrations in patients with CHD. Studies using multivariate statistical models to compare CHD patients with healthy controls usually show that VLDL lipids are not independent predictors of CHD.[1] To the best of our knowledge, no statistical method has yet properly taken the close relation between VLDL lipids and HDL cholesterol into account. Other metabolic factors such as lipolytic enzymes also need to be considered in the regulation of HDL cholesterol and its relation to CHD. In the present study, multiple stepwise regression analysis showed that 42% of the variation in HDL cholesterol concentration among patients with premature CAD could be predicted by VLDL cholesterol and LDL triglyceride levels and HL activity in postheparin plasma, whereas in population-based controls, 35% of the variation in HDL cholesterol level could be predicted by VLDL cholesterol concentration and postheparin plasma HL activity. Addition of LPL or CETP activities or glucose, insulin, and proinsulin concentrations during an oral glucose tolerance test added limited information about the regulation of HDL cholesterol. Subgroup analyses revealed interesting differences between HTG and NTG patients. Preheparin plasma LPL mass and postload insulin concentration (the insulin AUC) contributed equally (25% and 22%, respectively) to the variation in HDL cholesterol in NTG patients, whereas in HTG patients, preheparin plasma LPL protein contributed significantly to the variation in HDL cholesterol (32%), with a small contribution by CETP activity (9%). Thus, about half of the variation in HDL cholesterol was accounted for by the metabolic variables included in this study. However, the present data need to be interpreted carefully, bearing in mind the large number of correlation coefficients calculated, many of which are highly intercorrelated, and the fact that the number of individuals included in the multivariate analysis of HDL cholesterol determinants in the control group was small.

Table 7. Partial Correlation Coefficients Between HDL Cholesterol Level, HDL Subclass Protein Concentrations, and Coronary Stenosis Score

	HDL Cholesterol	HDL Gradient Gel Electrophoresis Subclass‡				
		2b	2a	3a	3b	3c
Model 1 (n = 64)	−.35†	−.30*	−.36†	−.25*	.34†	.33†
Model 2 (n = 49)	−.10	−.08	−.16	−.27	−.17	.15

NOTE. In model 1, the age of the patient was used as a forced variable. In model 2, BMI, VLDL cholesterol, number of molecules of cholesteryl esters in small VLDL, dense LDL triglyceride concentrations, and postheparin HL activity were used as forced variables in addition to age.

*$P < .05$.

†$P < .01$.

‡Nomenclature according to Blanche et al.[13]

Separation of HDL into subclasses defined by particle size showed that the relationship between HDL and CAD is more complex than indicated by the inverse relationship between HDL cholesterol concentration and angiographic severity of disease. As expected, significant inverse correlations were obtained with the larger particle species, whereas the smaller HDL_{3b} and HDL_{3c} particles were positively related to the coronary stenosis score. Furthermore, controlling for the influence of metabolic variables on the HDL correlations with CAD severity clearly indicated that triglyceride-rich lipoproteins and factors related to their metabolism strongly affect the associations of HDL cholesterol and HDL gradient gel electrophoresis subclasses with CAD.

The increased postheparin plasma HL activity in patients compared with controls confirms the results of two smaller cross-sectional studies of young male postinfarction patients[19] and older men with asymptomatic myocardial ischemia.[23] The negative relations between postheparin plasma HL activity and HDL cholesterol concentration or HDL particle size have been noted in several studies,[4,23-26] and it has been speculated that this is due to the triacylglycerol-

hydrolyzing effect of HL on HDL triglycerides. The present study confirms the results obtained by Syvänne et al,[26] who showed that HL activity and plasma triglycerides or VLDL lipids are important determinants of HDL particle size. In contrast to that study, fasting plasma insulin did not independently predict HDL particle size, whereas minor contributions were observed for BMI and LDL triglyceride concentration. Further support for the role of HL in the regulation of HDL cholesterol level is that 25% of the interindividual variation in HDL cholesterol can be explained by allelic variation in the gene encoding for HL.[27] Despite the negative univariate relations between postheparin plasma HL activity and HDL cholesterol concentration of similar strength in patients and controls, only a minor degree of the variation in HDL cholesterol could be explained by HL activity in patients, whereas in controls, HL activity accounted for the majority of variation in HDL cholesterol in multivariate analysis. This difference could be explained by the inclusion of different parameters in the statistical model in patients and controls, respectively. However, the influence of confounding factors such as diet and drugs in the patients cannot be excluded entirely (further discussion below).

To the best of our knowledge, CETP activity has not been compared between patients with CHD and controls, despite the fact that CHD patients consistently have a decreased HDL cholesterol concentration. In the present study, no differences were seen in plasma CETP activity between patients and controls, and CETP activity did not correlate with HDL cholesterol level. In agreement with this finding, one study of patients with different lipoprotein phenotypes showed that subjects with type IV hyperlipidemia, the most common lipoprotein phenotype in our study groups, had similar CETP protein levels compared with normolipidemic controls.[28]

The larger HDL particles showed negative relations and the smaller HDL particles positive relations to the coronary stenosis score, the strength of which was similar to the relation noted between HDL cholesterol and the coronary stenosis score. The biological mechanisms reflected by the relations of HDL cholesterol concentration or HDL particle size distribution to the number and severity of coronary stenoses remain unclear. The potential mechanisms include the virtual shrinking of stenotic lesions by increased efflux of cholesterol and protection by HDL against oxidative modification of LDL. Another possibility would be that the amount of HDL cholesterol in the circulation or the HDL gradient gel electrophoresis particle distribution is a consequence of changes in other, possibly atherogenic, lipoprotein classes or subclasses. The strong inverse relation between VLDL and HDL cholesterol concentrations found in this study is in favor of the latter hypothesis. VLDL lipid levels are also strong determinants of LDL density and size distribution, with increasing density and decreasing particle size accompanying increasing levels of VLDL lipids.[10,29] In fact, only BMI and dense LDL triglycerides, not HDL cholesterol, were found to be independently related to the coronary stenosis score in a recent angiographic study.[10] In the present study, neither HDL cholesterol concentration nor HDL particle size distribution were related to the coronary atherosclerosis score (data not shown). This is surprising, since HDL cholesterol level and particularly HDL_{2b} protein concentration were strongly related to the global severity and rate of progression of coronary atherosclerosis in an previous cohort of young postinfarction patients.[30] One explanation for this discrepancy could be the differences in design of the two studies. In the present study, patients were studied in connection with their myocardial infarction. In the former study, on the other hand, patients were studied 4 to 7 years after their myocardial infarction, which introduces a considerable selection bias.

Some of the differences seen in lipoprotein lipid levels and enzymes involved in lipoprotein metabolism could, to some extent, be explained by the almost universal use of β-blockers among the patients. In general, β-blockers increase VLDL triglyceride and decrease HDL cholesterol.[31] Previously published results regarding lipoprotein lipid effects of different β-blockers have been conflicting, but the effects of the selective β-blocker metoprolol, used by the vast majority of patients in the present study, seems to be less pronounced in comparison to the effects of other β-blockers.[31] It has been speculated that the lipoprotein lipid changes seen during the use of β-blockers are caused by reductions in lipolytic capacity. In fact, two recent reports[32,33] showed that postheparin plasma LPL activity decreased in hypertensive patients during use of the unselective β-blocker propranolol. This change was due to inhibition of skeletal muscle LPL activity.[33] The fact that patients and controls in the present study had similar postheparin plasma LPL activities, together with a report on hypertensive patients showing no change in postheparin plasma LPL activity after 2 weeks' treatment with metoprolol,[34] speaks against a significant effect on lipoprotein lipids of the selective β-blocker metoprolol. However, regional differences in LPL activity caused by metoprolol cannot be excluded. The possibility that the increased postheparin plasma HL activity in patients compared with controls is due to a β-blocker effect is not likely, since studies in hypertensive patients during use of the unselective β-blocker propranolol showed either no effect[33] or a decrease[34] in postheparin plasma HL activity. Another possible confounding factor is the dietary advice given to the patients as part of the routine postinfarction regimen, but not to the controls. Adherence to the dietary instructions given to the patients was not monitored. Theoretically, a good adherence to the prescribed diet would decrease plasma concentrations of cholesterol and triglyceride in the patients, resulting in reduced case-control differences in lipoprotein lipid concentrations and possibly weakened associations with other metabolic parameters.

The corollary to the findings of this study is that factors related to the metabolism of triglyceride-rich lipoproteins, including VLDL lipid concentrations and LPL mass and HL activity, together or alone, explain about half of the variation in HDL cholesterol level. Accordingly, any associa-

tions between HDL and CHD/CAD could just as well be explained by the relations between triglyceride-rich lipoproteins, lipolytic enzymes, and HDL. The ultimate mechanisms underlying the associations of HDL with coronary atherosclerosis and its thrombotic complications cannot be elucidated by the present study, but probably include both atherogenic and prothrombotic actions of triglyceride-rich lipoproteins.

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