Net Mass Transfer of Plasma Cholesteryl Esters and Lipid Transfer Proteins in Normolipidemic Patients With Peripheral Vascular Disease

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The role of plasma cholesteryl ester transfer and lipid transfer proteins in atherosclerosis is unclear. Recent data suggest both antiatherogenic and atherogenic properties for cholesteryl ester transfer protein (CETP). The overall effect of CETP on atherosclerosis may thus vary depending on individual lipid metabolism. To test whether lipid transfer parameters are of importance even in patients without major lipid risk factors for atherosclerosis, CETP mass and activity, net mass transfer of cholesteryl esters between endogenous lipoproteins (CET), and phospholipid transfer protein (PLTP) activity were determined in plasma from 18 normolipidemic male patients with peripheral vascular disease and 21 controls. Furthermore, lecithin: cholesterol acyltransferase (LCAT) activity was tested. The results show that CETP mass, CETP activity, and LCAT activity are not different between patients and controls. However, specific CETP activity (CETP activity/CETP mass) is lower in the patients ($P < .02$). On the contrary, higher CET is observed in patients' plasma ($P < .001$). Increased plasma PLTP activity ($P = .052$) is demonstrable in the patients. If the data of all subjects are combined, CET correlates positively with triglycerides ([TG], $r = .45$, $P < .001$) and with PLTP activity ($r = .32$, $P < .05$) but negatively with specific CETP activity ($r = -.37$ $P < .05$). CET and specific CETP activity remain significantly different in TG-matched patients and controls and are more strongly interrelated ($r = -.71$, $P < .001$), suggesting a higher and selective influence of lipid transfer inhibitor(s) on CET and CETP activity in the patients. CET allows the best discrimination between patients and controls in univariate and multivariate analysis. Eighty-eight percent of the subjects are correctly classified by CET as a single parameter. The results suggest that increased CET in the patients may reflect atherogenic alterations in TG metabolism and/or in lipid transfer protein activities despite normal fasting lipoprotein levels.

THE ROLE OF cholesteryl ester transfer protein (CETP)-mediated cholesteryl ester transfer between antiatherogenic and atherogenic plasma lipoproteins in atherosclerosis is not clear.[1-2] Conflicting results have been obtained in animals and in man. Expression of the simian or human CETP gene in mice caused more severe atherosclerosis,[3,4] suggesting that CETP is in fact an atherogenic protein. However, CETP expression diminished the development of early atherosclerotic lesions in hypertriglyceridemic human apolipoprotein (apo) CIII transgenic mice.[4] On the other hand, inhibition of CETP expression by antisense oligonucleotides in rabbits decreased the CETP concentration and atherosclerosis.[5] In man, carriers of CETP mutations leading to decreased CETP mass showed a higher prevalence of coronary heart disease,[6] whereas a common variant of the CETP gene was associated with elevated CETP concentrations and increased progression of coronary heart disease.[7] Further, a direct relationship of CETP mass to carotid artery wall thickness has been described in one study.[8]

Possibly, the overall effect of CETP in vascular disease is dependent on individual lipoprotein metabolism. Mann et al[9] have shown that CETP is not rate-limiting for cholesteryl ester transfer in normolipidemic individuals. Therefore, measurement of cholesteryl ester transfer with endogenous lipoproteins may provide more information about the role of CETP in vivo than a determination of CETP itself. In several studies, an increased net mass transfer of high-density lipoprotein (HDL) cholesteryl esters to endogenous apo B–containing lipoproteins (CET) has been observed in (hyperlipidemic) patients with atherosclerosis.[10-12] Decreased CET was reported in hyperlipidemic plasma of subjects with documented vascular disease on one occasion.[13] Whether these findings reflect differences in endogenous lipoproteins and/or in CETP mass or activity is not fully understood. To our knowledge, there are no studies reporting concurrent measurements of CETP mass, CETP activity, and CET in patients with vascular disease.

Of special interest is the role of lipid transfer parameters in patients without major lipid risk factors for atherosclerosis. Therefore, we measured CET, CETP mass, and CETP activity in normolipidemic patients with peripheral atherosclerosis and controls. Further, phospholipid transfer protein (PLTP) activity was tested. PLTP may facilitate phospholipid transfer from triglyceride (TG)-rich lipoproteins into HDLs during lipolysis,[14] and phospholipid-enriched postprandial HDL may cause an increase in CETP activity.[15] Stimulation of CETP-mediated cholesteryl ester transfer by PLTP has been demonstrated in vitro.[16] PLTP may also be involved in the regulation of HDL particle size.[17] Elevated CETP and PLTP activities were demonstrated in obese men[18] and in smokers.[19]

The present study was performed to investigate whether lipid transfer parameters allow a better discrimination between normolipidemic patients with vascular disease and controls than the usual plasma lipid parameters. The results show that differences in lipid transfer parameters are indeed demonstrable even in patients and controls with comparable plasma lipid concentrations in the normal range.

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Submitted September 9, 1998; accepted May 12, 1999.

Supported by grants from the Deutsche Forschungsgemeinschaft (Ru 552/1-1) and The Netherlands Heart Foundation (89.094).

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0026-0495/99/4811-0024$10.00/0

SUBJECTS AND METHODS

Subjects

Eighteen normolipidemic male patients with peripheral vascular disease of the legs and 21 normolipidemic male controls were investigated. Normolipidemia was defined as total plasma cholesterol less than 6.46 mmol/L, HDL cholesterol greater than 0.9 mmol/L, and TG less than 1.71 mmol/L. Patients and controls were matched for total, free, and esterified cholesterol in plasma, in HDL, and in very–low-density lipoprotein plus low-density lipoprotein (VLDL + LDL), as well as for apo A-I and apo B. All subjects were sedentary men. Patients and controls were also matched for body mass index and smoking and drinking habits. No differences in dietary habits were present between patients and controls. None of the subjects had diabetes or any other severe illness except for vascular disease. Patients using β-blockers or lipid-lowering agents were excluded from the study. Peripheral vascular disease was diagnosed by intermittent claudication and by electronic oscillography and, in most cases, also by ultrasonography, venous occlusion plethysmography, or angiography. The control group consisted of asymptomatic individuals who were not on medication and had normal findings on electronic oscillography, ultrasonography, and venous occlusion plethysmography. Informed consent was obtained from all subjects.

Sampling

After an overnight fast, blood was obtained by venipuncture and collected into ice-chilled tubes containing EDTA (1 mg/mL blood) or sodium heparinate (Weddel, London, UK; 5,000 U/mL, final concentration 7 U/mL blood). Samples were kept on ice. The plasma was separated by centrifugation at 4°C and 3,000 rpm for 10 minutes. Determinations of CET and total and free plasma cholesterol were performed immediately after plasma separation. Samples for determination of CETP mass, CETP activity, PLTP activity, lecithin:cholesterol acyltransferase (LCAT) activity, and apolipoproteins were stored at −80°C and transported on dry ice. Plasma for TG and phospholipid determination was stored at −20°C for less than 2 months.

Determination of Lipids and Apolipoproteins

Total and free plasma cholesterol and HDL-cholesterol (HDL-C) were determined enzymatically using the catalase method,[20] with prolonged incubation (2 hours) to complete cholesteryl ester hydrolysis. Under this condition, we found no difference versus the stated values of the commercial control sera Precilip E.L. and Precinorm L (Boehringer, Mannheim, Germany). Esterified cholesterol (EC) was calculated as the difference between total and free cholesterol (FC). HDLs were separated by phosphotungstate/$MgCl_2$ precipitation.[21] VLDL+LDL-cholesterol (VLDL + LDL-C) was calculated by difference (total plasma cholesterol minus HDL-C). TG, choline-containing phospholipids (PL), and apo A-I and B were determined with test kits from Boehringer. All determinations were performed at least in duplicate. Quality control was performed for each assay using Precilip E.L. and Precinorm L (Boehringer). The interassay coefficients of variation were 2.4% for total cholesterol, 4.3% for HDL-C, 3.8% for TG, 5.7% for apo A-I, and 2.5% for apo B.

Net Mass Transfer of HDL-EC

The method followed the principle of Fielding et al,[13] with some modifications. After LCAT inhibition with iodoacetate, the change in the plasma HDL-EC concentration during incubation at 37°C was determined. Briefly, to ice-chilled plasma samples, a 1:10 vol of buffered saline (154 mmol/L NaCl) containing Tris hydrochloride, iodoacetate, and EDTA (final concentrations, 10 mmol/L Tris hydrochloride, 2 mmol/L iodoacetate, and 1 mmol/L EDTA, pH 7.4) was added. After preincubation for 15 minutes in an ice bath, complete LCAT inhibition was achieved as controlled in samples from six donors using the method of Patsch et al.[22] Samples were incubated at 37°C in a metabolic shaker. Before and after 1, 2, and 4 hours of incubation, aliquots were cooled on ice and HDLs were separated in quadruplicate by phosphotungstate/$MgCl_2$ precipitation. Total and free HDL-C was measured in one analytical run, and the change in HDL-EC was calculated. To improve the sensitivity of the test, the sample volume was increased up to 10-fold. Within-assay coefficients of variation were 1.1% for total HDL-C and 2.0% for HDL-EC.

Iodoacetate was used to inhibit LCAT because dithiobis-2-nitrobenzoic acid (DTNB) disturbed the enzymatic cholesterol determination, causing high blank values. To test the possible influence of iodoacetate on cholesteryl ester transfer, plasma samples without LCAT inhibition were incubated in parallel, and the change in VLDL+LDL-EC was determined. VLDL+LDL-EC increased to the same extent that HDL-EC decreased after LCAT inhibition. Consequently, no influence of iodoacetate on cholesteryl ester transfer was detectable.

Determination of CETP Mass

Plasma CETP mass was measured according to the method of Marcel et al[23] by solid-phase competitive radioimmunoassay in the presence of Triton and the specific monoclonal antibody TP-2. The antibody was purified from a TP-2–producing hybridoma cell line provided by A.R. Tall, MD (New York, NY). In brief, plastic wells were coated with recombinant CETP and saturated with bovine serum albumin. Diluted samples and a limiting dilution of iodine 125–labeled antibody TP-2 were preincubated and added to the coated wells. The wells were incubated and washed, and the bound radioactivity was measured. Determinations were made in triplicate.

In the present study, CETP concentrations were in the range of 1.7 to 6.0 μg/mL plasma. These values are higher than those reported by others[23-25]; however, Seip et al[26] found CETP concentrations in a comparable range (1.2 to 5.2 μg/mL) using the same method as in the present study.

Assay of Plasma CETP, PLTP, and LCAT Activity

CETP activity was measured in each plasma sample after removal of endogenous VLDL+LDL by phosphotungstate/$MgCl_2$ precipitation.[27,28] The isotope assay detects the transfer of radioactive cholesteryl ester between exogenous [1-^{14}C-oleate]-cholesteryl ester–labeled LDL and an excess of unlabeled pooled normal HDL. DTNB was added to the incubation mixture to inhibit LCAT. Incubations were performed for 16 hours at 37°C. The reaction was stopped by cooling the tubes to 4°C, followed by precipitation of LDL with Mn^{2+} ions. CETP activity was calculated as the bidirectional transfer of cholesteryl ester between radiolabeled LDL and HDL, and was linear in time during the 16-hour incubation.[27] During this time, less than 5% of the substrates were used.

PLTP activity was assayed using a phospholipid vesicles–HDL system.[28] Briefly, plasma samples were incubated with [^{3}H]-phosphatidylcholine–labeled vesicles and normal HDL for 1.5 hours at 37°C. The reaction was stopped by chilling the tubes on ice. The vesicles were then precipitated with a mixture of NaCl, $MgCl_2$, and heparin (final concentrations, 230 mmol/L, 92 mmol/L, and 200 U/mL, respectively). The measured PLTP activities are linear with the amount of plasma added to the assay system. The method is specific for PLTP activity and is not influenced by the phospholipid transfer–promoting properties of CETP.[28]

LCAT activity was determined using excess exogenous substrate containing [^{3}H]-cholesterol.[29] The test is linear in time for at least 6 hours and less than 5% of the substrates are used. Plasma samples were incubated for 6 hours at 37°C. The reaction was stopped by addition of cold ethanol to the incubation mixture. Lipids were extracted twice with hexane. Free and esterified cholesterol were separated using disposable silica columns, with [^{3}H]-cholesteryl ester eluted with hexane:

diethylether (6:1 vol/vol).[30] The measured LCAT activities were linear with the amount of plasma used. Assays for CETP, PLTP, and LCAT activities were performed in duplicate. The within-assay coefficients of variation are 2.7%, 3.5%, and 4.5% for CETP, PLTP, and LCAT, respectively. The measured activities reflect the activities of the lipid transfer proteins and the enzyme as such and are independent of the endogenous lipoproteins present in each plasma. The activities of CETP, PLTP, and LCAT were related to the activities of these factors measured in the human reference plasma that was included in each assay. CETP activity is expressed as nanomoles of cholesteryl ester per milliliter of plasma per hour, PLTP activity as micromoles of phosphatidylcholine per milliliter of plasma per hour, and LCAT activity as nanomoles of esterified cholesterol per milliliter of plasma per hour.

Statistics

Results are expressed as the mean ± SD. Statistical evaluation was performed by Student's *t* test for paired or unpaired data. Linear regression analysis was used to calculate correlation coefficients. Stepwise linear discriminant analysis was performed with the SAS system (SAS Institute, Cary, NC). All significance testing was two-tailed, and a P level less than .05 was considered significant.

RESULTS

Clinical characteristics and plasma lipid parameters of the study subjects are given in Table 1. Patients and controls differed only by age and plasma TG concentration.

PLTP activity was increased by 12% ($P = .052$) in the patients. CETP mass and activity, as well as LCAT activity, were not different between patients and controls. Although a strong positive correlation was found between plasma CETP mass and activity ($r = .85$, $P < .001$), specific CETP activity (CETP activity/CETP mass) was lower in the patients ($P < .02$) (Table 2).

On the contrary, considerably higher CET was observed in the patients (Fig 1). The difference was significant after 1, 2, and 4 hours of incubation. With a 4-hour incubation, 2.4-fold more HDL-EC was transferred in patient plasma. HDL-EC decreased by 10% during 4 hours of incubation in the patients and by only 4% in the controls. Notably, no significant CET occurred in the controls during 1 hour of incubation. In 10 controls (but only one patient), an increase in HDL-EC was observed after 1 hour, and in three controls also after 4 hours, of incubation. In these 10 controls, a small but significant ($P < .05$) decrease in VLDL+LDL-EC was also found if plasma without LCAT inhibitor was incubated for 1 hour at 37°C.

Table 1. Clinical Characteristics and Plasma Lipid Parameters in Normolipidemic Patients and Controls

Variable	Patients (n = 18)	Controls (n = 21)
Age (yr)	62.5 ± 8.7*	52.9 ± 3.9
Body mass index (kg/m²)	24.9 ± 4.0	24.1 ± 1.6
Plasma total C (mmol/L)	5.24 ± 0.81	5.48 ± 0.53
Plasma FC (mmol/L)	1.54 ± 0.21	1.60 ± 0.20
Plasma EC (mmol/L)	3.71 ± 0.63	3.87 ± 0.37
Plasma TG (mmol/L)	1.32 ± 0.27*	0.97 ± 0.26
Plasma PL (mmol/L)	2.76 ± 0.36	2.90 ± 0.25
HDL-C (mmol/L)	1.23 ± 0.19	1.31 ± 0.26
HDL-FC (mmol/L)	0.30 ± 0.06	0.32 ± 0.08
HDL-EC (mmol/L)	0.93 ± 0.15	0.99 ± 0.19
VLDL + LDL-C (mmol/L)	4.02 ± 0.78	4.18 ± 0.57
VLDL + LDL-FC (mmol/L)	1.24 ± 0.22	1.28 ± 0.18
VLDL + LDL-EC (mmol/L)	2.78 ± 0.58	2.88 ± 0.41
Apo A-I (g/L)	1.35 ± 0.15	1.37 ± 0.21
Apo B (g/L)	0.78 ± 0.12	0.79 ± 0.10

Abbreviations: C, cholesterol; FC, free cholesterol; EC, esterified cholesterol; PL, phospholipids.

*$P < .001$ *v* controls.

Table 2. Plasma Lipid Transfer Proteins and LCAT in Normolipidemic Patients With Peripheral Vascular Disease and Controls

Parameter	Patients (n = 18)	Controls (n = 21)
CETP mass (μg/mL)	3.33 ± 0.90	3.12 ± 0.60
CETP activity (nmol/mL · h^{-1})	168 ± 46.4	176 ± 38.9
Specific CETP activity (nmol/μg · h^{-1})	50.9 ± 6.82†	56.4 ± 6.33
PLTP (μmol/ml · h^{-1})	14.0 ± 3.17*	12.5 ± 1.31
LCAT (nmol/mL · h^{-1})	35.9 ± 6.30	34.1 ± 4.92

*$P = .052$, †$P < .02$ *v* controls.

Considering the combined data of all subjects (Table 3), CET correlated positively with plasma TG and with PLTP activity. Whereas no significant correlations were found between CET and CETP mass or CETP activity, specific CETP activity was negatively related to CET. CETP mass and CETP activity correlated directly with VLDL+LDL-EC. TG was related inversely to HDL-C, and plasma phospholipids correlated positively with HDL-C and with VLDL+LDL-EC. No significant correlation was found between CET and age.

Because patients and controls differed in plasma TG, increased CET in the patients could simply reflect an elevated concentration of TG-rich acceptor lipoproteins. Therefore, CET was also compared in TG-matched patients and controls (Table 4). The difference in CET and in specific CETP activity remained highly significant. Notably, CET and specific CETP activity showed an even stronger negative correlation in this subgroup ($r = -.71$, $P < .001$).

CET, age, and TG were the most important parameters in multivariate stepwise discriminant analysis. The ranking of the selected parameters and their contribution to the average squared canonical correlation is shown in Table 5. Ninety-eight percent of the patients and controls were correctly classified by the combination of these six selected parameters. CET allowed the best discrimination between patients and controls of all lipid parameters tested. Eighty-eight percent of the normolipidemic individuals were correctly classified by CET as a single parameter. In 72% of the subjects, TG allowed a correct discrimination, and specific CETP activity in 67% (Table 6).

DISCUSSION

The present study was performed to investigate (1) whether the increased net mass transfer of cholesteryl esters previously described in (hyperlipidemic) patients with vascular disease[10-12] may result from changes in lipid transfer proteins and/or endogenous lipoproteins, (2) whether changes in lipid transfer parameters are also demonstrable in normolipidemic patients with peripheral vascular disease, and (3) whether lipid transfer parameters allow a better discrimination between normolipidemic patients and controls than the usual plasma lipid parameters.

The results show that the net mass transfer of HDL-EC to endogenous apo B–containing lipoproteins (CET) is indeed increased in normolipidemic patients with peripheral vascular

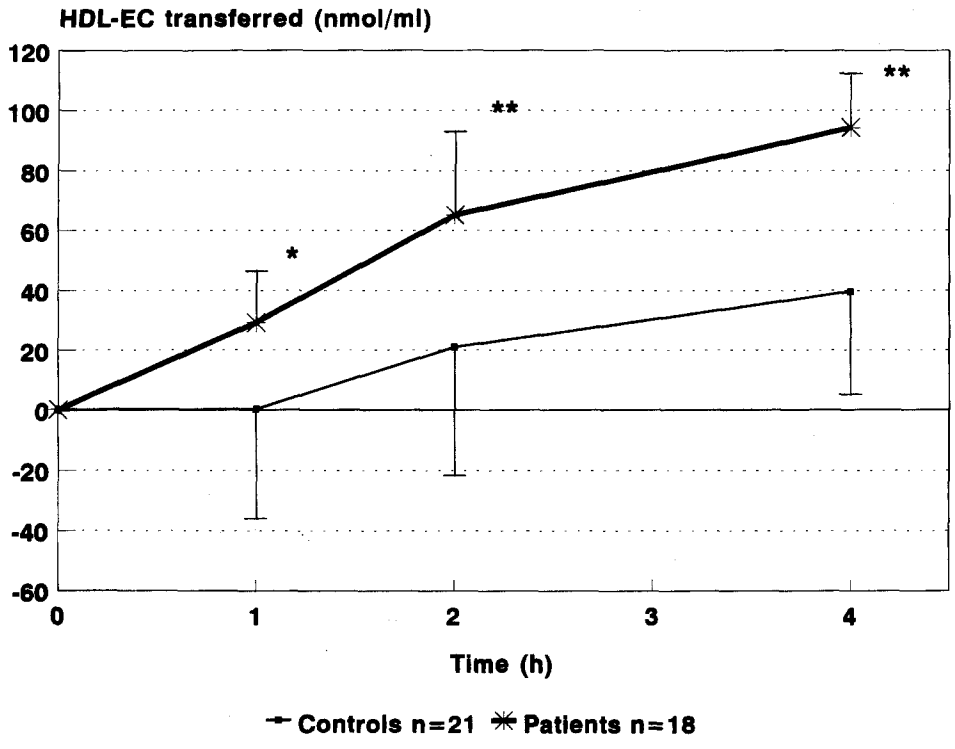

Fig 1. Net mass transfer of HDL-EC to endogenous apo B–containing lipoproteins (mean ± SD) in normolipidemic patients with peripheral vascular disease and controls. *$P < .005$, **$P < .001$. Normolipidemia was defined as plasma total cholesterol <6.45 mmol/L, HDL-C >0.90 mmol/L, and TG <1.71 mmol/L.

disease. They further demonstrate a small increase in PLTP activity in the patients. No significant differences were found between patients and controls in CETP mass, CETP activity, and LCAT activity. However, specific CETP activity was significantly lower in the patients, suggesting an influence of lipid transfer inhibitor protein (LTIP).

There is an apparent discrepancy with the results of Fielding et al.[13] They observed a diminished CET in patients with atherosclerotic disease. However, in contrast to our study, they compared markedly hyperlipidemic patients with normolipidemic controls. Moreover, their results could not be confirmed by others. Tall et al[31] and Bagdade et al[32] found increased CET in hyperlipidemic patients.

To our knowledge, there are no other publications showing specific CETP activity in patients with atherosclerotic disease. Plasma CETP activity measured with exogenous lipoproteins was reported to be unchanged[10,33,34] or decreased[35] in patients with documented vascular disease.

In the present study, CET correlated positively with plasma TG and with PLTP activity but negatively with specific CETP activity (Table 3). All of these factors may contribute to the increased CET in the patients. The patients showed higher TG levels than the controls, albeit in the normal range (Table 1). It is well known that TG-rich lipoproteins are the main acceptors of transferred HDL cholesteryl esters.[9,36] Mann et al[9] have shown that the concentration of TG-rich acceptor lipoproteins is rate-limiting for CET in normolipidemic subjects. In agreement with other studies,[15,37,38] we observed an increase in CET after the addition of an artificial TG emulsion to the test system, as well as after an oral fat load, in normolipidemic healthy male subjects (data not shown). Differences in fasting CET disappeared in the postprandial state. The increase in CET remained significant 9 hours after the fat load, although TG levels were already normalized. Moreover, the postprandial increase in plasma TG was higher in subjects with elevated fasting CET despite comparable fasting TG (Rühling K, John M, unpub-

Table 3. Correlation Matrix: Combined Data of Patients and Controls

	CET	CETP Mass	CETP Activity	Specific CETP Activity	PLTP	HDL-C	VLDL + LDL-EC	TG	PL
CET	—								
CETP mass	.14	—							
CETP activity	−.08	.85‡	—						
Specific CETP activity	−.37*	−.25	.29	—					
PLTP	.32*	.07	.12	.11	—				
HDL-C	−.26	−.07	.08	−.01	.11	—			
VLDL + LDL-EC	−.05	.51‡	.47†	−.11	−.05	−.11	—		
TG	.45†	−.09	−.18	−.15	.25	−.32*	.11	—	
PL	.20	.25	.28	.03	.29	.56‡	.48†	.03	—

*$P < .05$.
†$P < .01$.
‡$P < .001$.

Table 4. Net Mass Transfer of HDL-EC and Specific CETP Activity in Plasma From Patients and Controls Matched for TGs

Parameter	Patients (n = 12)	Controls (n = 12)
TG (mmol/L)	1.23 ± 0.28	1.24 ± 0.28
CET (nmol/mL · 4 h^{-1})	92.3 ± 16.5*	35.4 ± 26.2
Specific CETP activity (nmol/μg · h^{-1})	48.7 ± 5.16*	57.9 ± 6.02

*$P < .001$ *v* controls.

lished results, July 1998). Therefore, increased CET in the patients of the present study may reflect latent disturbances in TG metabolism, even independently of plasma TG concentrations (Table 4). Bhatnagar et al[12] also found an increased CET in hypertriglyceridemic patients with coronary artery disease compared with TG-matched controls. Another explanation for the increased CET even in patients with comparable plasma TG levels in the present study may be the lower specific CETP activity, suggesting higher lipid transfer inhibitor(s) levels in the patients. This finding and the negative correlation between CET and specific CETP activity predominantly in TG-matched patients and controls are new and surprising results. They may reflect a selective influence of LTIP on CET and CETP activity assays. Morton et al[39,40] have shown that LTIP preferentially inhibits lipid transfer from LDL to VLDL or HDL. Furthermore, the addition of LTIP to normolipidemic whole plasma increased cholesteryl ester transfer from HDL to VLDL but inhibited cholesteryl ester transfer from LDL to VLDL in a dose-dependent manner.[39] The investigators stated that "... in normolipidemic individuals we propose that at higher LTIP levels, HDL are better able to compete for the limited TG pool because LTIP preferentially reduces lipid transfer between LDL and VLDL. Thus HDL loses more CE and gains more TG when LTIP levels are elevated."[40] This suggestion is in agreement with the results of the present study and could explain the stronger inverse correlation between CET and specific CETP activity observed in patients and controls with comparable TG concentrations. More studies are necessary to investigate the role of inhibitor protein(s) in lipid transfer and atherosclerosis.

Table 5. Results of Multivariate Stepwise Discriminant Analysis Ranking of Selected Parameters and Their Contribution to the Average Squared Canonical Correlation (ASCC, n = 39)

Parameter	ASCC
1. CET 4 h	.496
2. Age	.592
3. TG	.638
4. VLDL + LDL-C	.666
5. Specific CETP activity	.691
6. CETP activity	.709

Table 6. Error Rates of the Selected Parameters in Univariate Discriminant Analysis

Parameter	Error Rate (%)
CET 4 h	12.3
Age	21.0
TG	27.8
Specific CETP activity	33.3
CETP activity	40.9
VLDL + LDL-C	46.4

NOTE. Error rates reflect the percentage of misclassified patients and controls (n = 39).

Of further interest is the delayed CET in the controls of the present study. Comparable observations in healthy controls were reported by Bagdade et al.[32,41] The reason for this delay is unknown, but it may be related to the net mass transfer of cholesteryl esters from LDL to HDL described during 1-hour incubations of plasma from normolipidemic individuals.[42] In accordance with this assumption, in about 50% of the controls but in only one of the patients, we found a net mass transfer of cholesteryl esters to the HDL during 1 hour of incubation. A corresponding decrease in VLDL+LDL-EC was also observed if plasma without LCAT inhibitor was incubated for 1 hour.

Further investigations are necessary to clarify the role of PLTP in cholesteryl ester transfer. Tall et al[15] found that phospholipid-enriched postprandial HDLs are a better substrate for CETP. A synergistic interaction between CETP and PLTP was suggested.[1] The observed correlation between CET and PLTP activity levels (Table 3) may support this idea.

CET and TG were the most important lipid parameters in multivariate and univariate discriminant analysis in the present study. This is of special interest because the patients had no severe lipid risk factors and a normal body mass index. CET as a single parameter allowed a correct classification of patients and controls in 88% of the subjects, and TG in 72%. It is noteworthy that TG in the normal range had such a high discriminative value. Although the importance of these results is limited by the relatively small number of subjects, the findings suggest that the differences in lipid transfer parameters and TGs between patients and controls may reflect atherogenic alterations in lipid metabolism despite normal fasting lipoprotein levels. Increased CET in the patients caused a more atherogenic lipoprotein profile, at least in vitro. If similar alterations occur in vivo, eg, in alimentary lipemia, they may contribute to the well-known inverse relationship between HDL-C and TG and to the enrichment of potentially atherogenic lipoproteins with cholesteryl esters.

ACKNOWLEDGMENT

We gratefully acknowledge A. Herzberg, M. Franke, M.M. Geelhoed-Mieras, L.M. Scheek, T. van Gent, and C. Boscher for expert technical assistance.

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