ELSEVIER

Atherosclerosis 140 (1998) 71–79

ATHEROSCLEROSIS

Elevated plasma cholesteryl ester transfer in NIDDM: relationships with apolipoprotein B-containing lipoproteins and phospholipid transfer protein

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Received 23 December 1997; received in revised form 27 March 1998; accepted 15 April 1998

Abstract

Lecithin:cholesteryl acyl transferase (LCAT) and cholesteryl ester transfer protein (CETP) are key factors in the esterification of cholesterol and the subsequent transfer of cholesteryl ester from high density lipoproteins (HDL) towards very low and low density lipoproteins (VLDL + LDL). Phospholipid transfer protein (PLTP), lipoprotein lipase (LPL) and hepatic lipase (HL) are involved in plasma phospholipid and triglyceride metabolism and also affect HDL. Equivocal changes in plasma cholesteryl ester transfer have been reported in non-insulin-dependent diabetes mellitus (NIDDM). In 16 NIDDM men with plasma triglycerides ≤ 4.5 mmol/l and cholesterol ≤ 8.0 mmol/l, plasma cholesteryl ester transfer (CET), cholesterol esterification rate, LCAT and PLTP activity levels were higher ($P < 0.05$ to $P < 0.02$) in conjunction with higher plasma triglycerides ($P < 0.01$) and lower HDL cholesterol and cholesteryl ester levels ($P < 0.05$) compared to 16 matched healthy men. Multiple stepwise regression analysis demonstrated that CET was positively related to VLDL + LDL cholesterol ($P < 0.001$), triglycerides ($P = 0.001$), PLTP activity ($P = 0.007$) and CETP activity ($P = 0.008$, multiple $r = 0.94$). NIDDM had no effect on CET, independently from these parameters. HDL cholesteryl ester was negatively related to CET ($P = 0.017$), HL activity ($P = 0.033$) and NIDDM ($P = 0.047$) and positively to LCAT activity levels ($P = 0.034$, multiple $r = 0.68$). It is concluded that the elevated CET in plasma from NIDDM patients is associated with higher plasma triglycerides and PLTP activity levels. Furthermore, our data suggest that in normo- and moderately dyslipidaemic subjects PLTP and CETP activity levels per se may influence the rate of cholesteryl ester transfer in plasma. Plasma cholesteryl ester transfer appears to be a determinant of HDL cholesteryl ester, but other factors are likely to contribute to lower HDL cholesteryl ester levels in NIDDM. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Non-insulin-dependent diabetes mellitus; Cholesteryl ester transfer; Cholesterol esterification; High density lipoproteins; Lecithin:cholesterol acyl transferase; Cholesteryl ester transfer protein; Phospholipid transfer protein; Lipoprotein lipase; Hepatic lipase

1. Introduction

The elevated cardiovascular risk in non-insulin-dependent diabetes mellitus (NIDDM) can in part be attributed to plasma lipoprotein abnormalities of which low high density lipoprotein (HDL) cholesterol and high plasma triglycerides are most widely recognized [1–3]. HDL plays a crucial role in the process of reverse cholesterol transport whereby cholesterol is transported from peripheral cells to the liver where it is taken up and subsequently excreted as cholesterol and bile acids in the stool [4–6].

HDL metabolism and remodelling is regulated in a complex way by several factors including lecithin:cholesterol acyl transferase (LCAT), cholesteryl

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PII S0021-9150(98)00111-7

ester transfer protein (CETP), phospholipid transfer protein (PLTP) and lipase activities [5–10]. LCAT has the ability to esterify free cholesterol in HDL, whereas CETP enables the subsequent transfer of cholesteryl ester from HDL towards very low and low density lipoproteins (VLDL and LDL). PLTP, a lipid transfer protein that facilitates the transfer and exchange of phospholipids between lipoproteins, may enhance CETP-mediated transfer of cholesteryl ester between isolated HDL and apolipoprotein (apo) B-containing lipoproteins in vitro [9,11]. Recent studies have shown that PLTP also has the ability to convert HDL_3, the dense HDL fraction, into larger and smaller HDL particles [10,12], and during this HDL remodelling preβ-HDL particles are generated [13,14]. Such particles are thought to represent the initial acceptors of free cholesterol from cell surfaces as well as the primary LCAT substrate [5,15]. During lipoprotein lipase (LPL)-mediated hydrolysis of triglycerides in chylomicrons and VLDL, free cholesterol and phospholipids are liberated and these lipid constituents are incorporated in HDL particles [7]. Hepatic lipase (HL) is thought to enhance hepatic HDL cholesterol uptake, thus explaining the negative relationship between HL activity and HDL cholesterol [1,16].

Despite intensive study, it remains controversial whether plasma cholesteryl ester transfer between HDL and VLDL + LDL is abnormal in NIDDM. Early studies in severely hyperglycaemic, untreated NIDDM patients showed net cholesteryl ester mass transfer from HDL towards apo B-containing lipoproteins to be diminished [17,18]. More recently, plasma cholesteryl ester transfer was found to be increased [19–23] or unaltered in NIDDM [24]. In some of these studies abnormalities in plasma cholesteryl ester transfer were attributed to alterations in the composition and concentration of cholesteryl ester acceptor lipoproteins, i.e. VLDL and LDL [19,21–23], or in HDL [25], whereas the plasma CETP level itself was not increased [19,24,26]. Altered lipoprotein composition may also be the consequence rather than the cause of altered rates of cholesteryl ester transfer in NIDDM [27]. Of interest, plasma PLTP activity levels are positively related to plasma triglycerides, obesity and insulin resistance in non-diabetic subjects [28–30], thus raising the possibility that PLTP activity is increased in NIDDM.

There are no studies in which plasma cholesteryl ester transfer as well as the activity levels of LCAT, CETP and PLTP and lipases have been measured together in NIDDM. The purpose of the present study was to compare plasma cholesteryl ester transfer in NIDDM and healthy subjects and to determine which of the above mentioned factors contribute to plasma cholesteryl ester transfer in conjunction with plasma levels of apo B-containing lipoproteins. Furthermore, it was evaluated whether the HDL cholesteryl ester level is related to plasma cholesteryl ester transfer.

2. Patients and methods

The study was approved by the medical ethics committee of our hospital and all participants gave their written informed consent. NIDDM patients and healthy subjects were recruited by advertisement. In the NIDDM patients diabetes mellitus was diagnosed according to National Diabetes Data group criteria [31]. NIDDM was diagnosed after 40 years of age and none of the patients had experienced ketoacidotic periods. They were treated with either diet alone or with a combination of diet and oral hypoglycaemic drugs but not with insulin. In the healthy subjects, diabetes mellitus was excluded by a 75-g oral glucose tolerance test with a fasting venous blood glucose level of 6.7 mmol/l and a 2-h level of 7.8 mmol/l as cut-off values. To avoid effects of the menstrual cycle and of smoking on lipid levels only non-smoking men participated. Fasting total cholesterol > 8.0 mmol/l and/or plasma triglycerides > 4.5 mmol/l were exclusion criteria and thus only normolipidaemic and moderately dyslipidaemic subjects were included. Subjects with clinically manifest cardiovascular disease, hypertension (systolic blood pressure > 160 mmHg and diastolic blood pressure > 95 mmHg), microalbuminuria (urinary albumin excretion > 20 μg/min), liver function abnormalities, thyroid dysfunction, alcohol use of more than three drinks per day and the use of any other medication than oral hypoglycaemic drugs were also excluded. Body mass index (BMI) was calculated as weight divided by height squared and a BMI > 27 kg/m^2 was considered to indicate obesity. The waist/hip ratio (WHR) was measured as the ratio of the smallest circumference between rib cage and iliac crest and the largest circumference between waist and thigh [32].

Sixteen NIDDM patients and 16 healthy subjects participated in the study. They were individually matched by age (within 5 years) and the presence of obesity. The clinical characteristics of the study participants are given in Table 1. Age, BMI, waist/hip ratio and mean arterial pressure were not different in NIDDM and healthy subjects. Alcohol consumption was similarly moderate in both groups. HbA_{1c} and fasting blood glucose levels were higher in NIDDM compared to healthy subjects. Two NIDDM patients were treated with diet alone, eight were treated with diet and sulphonylurea, five with diet, sulphonylurea and metformin, and one patient with diet and metformin. Venous blood was obtained at 08:00 h after the subjects were fasting for 12 h. Blood was taken when the subjects were in supine rest for 30 min. They were urged not to change dietary habits in the week preceding the study. On separate occasions, most of the subjects also participated in studies aimed at evaluating the effect of hyperinsulinaemia on free fatty acids (FFA) and lipoprotein metabolism.

2.1. Laboratory measurements

Venous blood was collected into ethylene diaminetetracetic acid-containing (1.5 mg/ml) tubes and was placed on ice immediately. Plasma was obtained within 30 min by centrifugation at 3000 rpm for 15 min at 4°C. Samples were frozen at −70°C until analysis. Lipids were measured in plasma and in the HDL-containing supernatant fraction after removal of apolipoprotein B (apo B)-containing lipoproteins with polyethylene glycol-6000 [33]. VLDL + LDL were calculated as the difference between whole plasma and the supernatant fraction. Total cholesterol was measured by gas chromatography. In plasma and in the HDL fraction free cholesterol was assayed by a modification of this method in which the hydrolysis step was omitted [34]. Cholesteryl ester was calculated as the difference between total and free cholesterol. Triglycerides were measured enzymatically [28]. Apo A-I and apo B were measured by immunoturbidimetry (kits from Boehringer Mannheim, Germany, catalogue nos. 726478 and 726494, respectively). Plasma FFA were measured enzymatically (kit from Wako, Germany, catalogue no. 994-754-75409).

Plasma newly synthesised cholesteryl ester transfer, designated cholesteryl ester transfer (CET) [22], was assayed essentially as described previously [35]. In brief, [^{3}H]cholesterol was incorporated in an albumin emulsion and was equilibrated overnight with plasma free cholesterol at 4°C, followed by incubation of the sample at 37°C for 3 h. Subsequently, VLDL + LDL were precipitated by addition of phosphotungstate/$MgCl_2$. Lipids were extracted from the precipitate and the cholesteryl esters were separated on silica columns and radioactivity was then counted. This assay system is not influenced by cholesterol esterification on LDL [35]. Since it has been demonstrated that CET is strongly correlated with net cholesteryl ester mass transfer from HDL towards VLDL and LDL in the absence of active LCAT, CET can be regarded as an accurate estimate of cholesteryl ester mass transfer in plasma [20]. Plasma cholesterol esterification rate (EST) was determined by measurement of [^{3}H]cholesterol esterification following equilibration of added [^{3}H]cholesterol at 4°C, as described above and incubated at 37°C for 1 h [35]. Plasma LCAT activity level was measured using excess exogenous substrate containing [^{3}H]cholesterol as described [28,36]. Plasma LCAT activity levels vary linearly with the amount of plasma used in the incubation. CETP activity level was determined after removal of VLDL + LDL from each sample [28,37]. The isotope assay measures the transfer of [1-^{14}C-oleate]-cholesteryl ester from labelled LDL to an excess of unlabelled pooled normal HDL, while LCAT is inhibited with dithiobis-2-nitrobenzoic acid. CETP activity was calculated as the bidirectional transfer between labelled LDL and HDL. The LCAT and CETP activity levels obtained by these methods are strongly correlated with their mass concentrations in plasma [38,39]. Plasma PLTP activity level was measured in a liposome vesicles-HDL system as described [28,40]. Plasma samples were incubated with [^{3}H]phosphatidylcholine labelled liposomes and an excess of pooled normal HDL, followed by precipitation of the liposomes with a mixture of NaCl, $MgCl_2$ and heparin (final concentrations of 230 mmol/l, 92 mmol/l and 200 U/ml, respectively). The measured PLTP activities are linearly correlated with the amount of plasma used in the incubations. The method is not influenced by the phospholipid transfer promoting properties of CETP [40]. All these assays were performed using the same batches of substrates. CET and EST are expressed in nmol/ml/h. Plasma LCAT, CETP and PLTP activity levels were related to human pool plasma and are expressed in arbitrary units (AU, which corresponds to the percentages of the activities in the pool plasma). The within assay coefficients of variation of CET, EST, LCAT, CETP and PLTP, are 7.1, 5.4, 4.5, 2.7, and 3.5%, respectively. Plasma for LPL and HL was obtained 20 min after intravenous injection of 50 IU/kg of heparin. This test was done after collection of plasma for the other lipoprotein variables. LPL and HL were assayed as described previously [41].

Blood glucose was analysed with a Yellow Springs Glucose Analyzer (model 23A, Yellow Springs, Yellow Springs, OH). Glycated haemoglobin (HbA_{1c}) was measured by high performance liquid chromatography (Bio-Rad, Veenendaal, Netherlands; normal range 4.6–6.1%).

Table 1
Clinical characteristics and parameters of metabolic control in non-insulin-dependent diabetic (NIDDM) and healthy subjects

	NIDDM patients ($n = 16$)	Healthy subjects ($n = 16$)
Age (years)	54 (42–66)	55 (40–65)
Diabetes duration (years)	4 (1–25)	
BMI (kg/m^2)	26.9 (22.1–43.4)	27.0 (20.9–31.3)
Waist/hip ratio	0.99 (0.89–1.12)	0.98 (0.79–1.04)
Mean arterial pressure (mmHg)	104 (87–117)	101 (94–110)
HbA_{1c} (%)	7.0 (6.0–10.5)[a]	5.5 (4.0–6.6)
Fasting blood glucose (mmol/l)	7.8 (3.8–14.4)[a]	4.9 (4.1–5.9)
Alcohol consumption (drinks/day)	0 (0–3)	0.2 (0–2)

Data in median (range). BMI, body mass index.
[a] $P < 0.001$ from healthy subjects.

Table 2
Plasma lipids, (apo)lipoproteins and free fatty acids in non-insulin-dependent diabetic (NIDDM) and healthy subjects

	NIDDM patients ($n=16$)	Healthy subjects ($n=16$)
Plasma total cholesterol (mmol/l)	5.03 (3.83–7.38)	4.80 (3.60–6.67)
Plasma triglycerides (mmol/l)	1.66 (0.72–3.69)***	0.90 (0.50–1.74)
VLDL+LDL cholesterol (mmol/l)	4.00 (2.02–5.92)	3.60 (2.33–5.75)
HDL cholesterol (mmol/l)	1.00 (0.53–1.81)*	1.20 (0.85–1.75)
HDL cholesteryl ester (mmol/l)	0.82 (0.48–1.49)*	1.04 (0.71–1.65)
HDL triglycerides (mmol/l)	0.21 (0.13–0.43)****	0.15 (0.11–0.23)
Apolipoprotein B (g/l)	0.99 (0.60–1.23)**	0.73 (0.56–1.25)
Apolipoprotein A-I (g/l)	1.22 (0.77–1.78)	1.23 (1.06–1.55)
Plasma free fatty acids (mmol/l)	0.64 (0.40–0.94)***	0.46 (0.27–0.64)

Data in median (range).
* $P<0.05$, from healthy subjects.
** $P<0.02$, from healthy subjects.
*** $P<0.01$, from healthy subjects.
**** $P<0.001$ from healthy subjects.

2.2. Statistical analysis

Data are expressed in median (range). Between-group differences in parameters were compared by using Mann–Whitney U tests. Bivariate relationships were evaluated by Spearman's rank correlation analysis (R_s). Multiple stepwise logistic regression analysis was used to analyze the independent relationships between parameters. A two-sided P-value <0.05 was considered significant.

3. Results

Fasting plasma triglycerides, apo B and FFA levels were higher in NIDDM than in healthy subjects, whereas HDL cholesterol was lower in the NIDDM patients (Table 2). The lower HDL cholesterol in NIDDM patients was due to a lower HDL cholesteryl ester concentration. HDL triglycerides, in contrast, were higher in NIDDM patients. No significant differences in plasma total and VLDL + LDL cholesterol and in apo A-I levels were present between the groups.

As shown in Table 3, CET and EST were higher in plasma from NIDDM patients compared to healthy subjects. Plasma LCAT and PLTP activity levels were also higher in NIDDM patients but no significant between-group differences in plasma CETP activity as well as in LPL and HL activity levels in postheparin plasma were present. A total of six patients had plasma triglycerides ≥ 2.0 mmol/l. When these subjects were excluded from the between-group comparisons, no significant differences in plasma CET, EST, LCAT and PLTP activity were present (all $P>0.10$) but HDL cholesteryl ester still tended to be lower and HDL triglycerides remained higher in NIDDM compared to healthy subjects (0.84 (0.64–1.49) vs. 1.04 (0.71–1.65) mmol/l, $P<0.08$ and 0.20 (0.13–0.25) vs. 0.15 (0.11–0.23) mmol/l, $P<0.05$, respectively).

Multiple stepwise regression analysis was performed in the combined subjects to determine which factors contributed to CET. CET was positively related to VLDL + LDL cholesterol ($P<0.001$), plasma triglycerides ($P=0.001$), plasma PLTP activity ($P=0.007$) and CETP activity ($P=0.008$, multiple $r=0.94$) without significant contributions of plasma LCAT activity ($P=0.09$) as well as of LPL and HL activity, the plasma FFA level, BMI and the presence of NIDDM as categorial covariate (or alternatively the HbA_{1c} level) ($P>0.20$ for all). This analysis thus demonstrated that the higher plasma CET levels in NIDDM were related to higher plasma triglycerides in combination with higher plasma PLTP activity levels. The bivariate correlations of CET with VLDL + LDL cholesterol, plasma triglycerides, plasma PLTP and CETP activity levels in the combined subjects and in NIDDM patients and healthy subjects separately are shown in Fig. 1. Plasma PLTP activity was correlated with triglycerides in the combined subjects ($R_s=0.57$, $P<0.01$), whereas the correlations in the NIDDM patients ($R_s=0.45$, $P<0.10$) and healthy subjects ($R_s=0.27$, ns) did not reach significance. There was no significant relationship between plasma CETP and PLTP activity levels in the combined

Table 3
Plasma cholesteryl ester transfer (CET), cholesterol esterification rate (EST), lecithin:cholesterol acyl transferase (LCAT), cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP) activities, and postheparin lipoprotein lipase (LPL) and hepatic lipase (HL) activities in non-insulin-dependent diabetic (NIDDM) and healthy control subjects

	NIDDM patients ($n=16$)	Healthy subjects ($n=16$)
CET (nmol/ml/h)	28.5 (9.6–49)*	19.2 (7.2–43.3)
EST (nmol/ml/h)	76.7 (47.8–117.2)*	61.2 (35.8–101.4)
LCAT activity (AU)	95 (71–128)*	86 (68–105)
PLTP activity (AU)	96 (56–127)**	76 (60–107)
CETP activity (AU)	72 (51–157)	76 (52–110)
LPL (U/l)	142 (58–194)	109 (66–196)
HL (U/l)	436 (260–952)	425 (131–723)

Data in median (range).
* $P<0.05$, from healthy subjects.
** $P<0.02$ from healthy subjects.

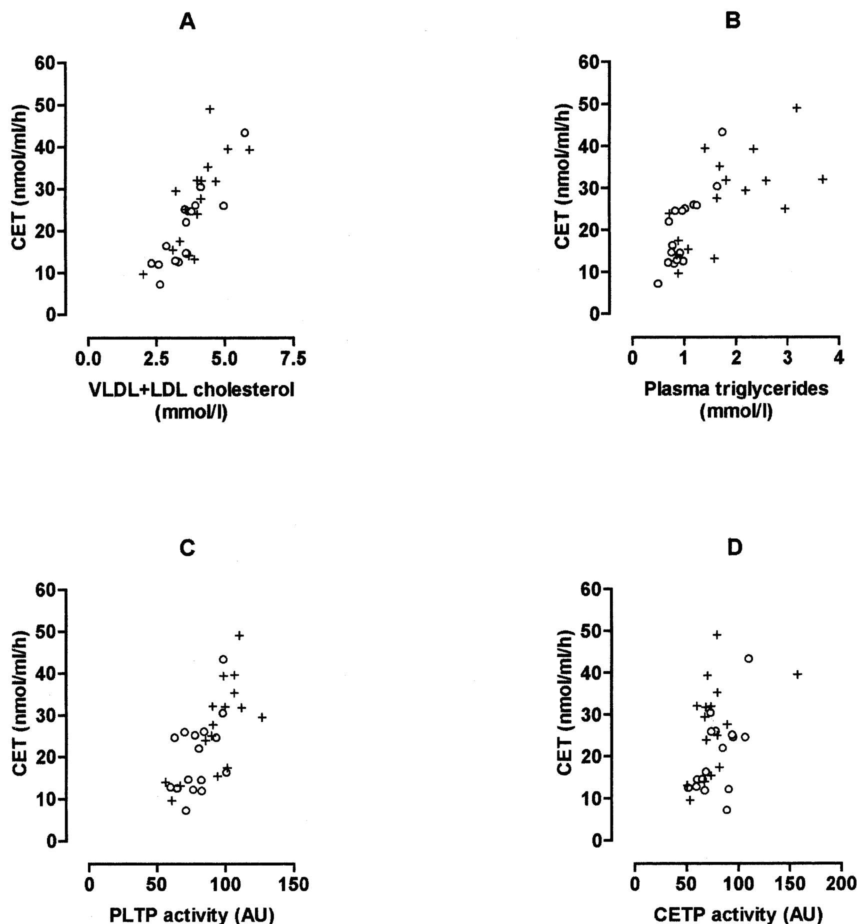

Fig. 1. Relationships of plasma cholesteryl ester transfer (CET) with VLDL + LDL cholesterol (A), plasma triglycerides (B), plasma phospholipid transfer protein (PLTP) activity (C) and cholesteryl ester transfer protein (CETP) activity (D) in 16 NIDDM (+) and 16 healthy subjects (○). Combined subjects: (A) $R_s = 0.83$, $P < 0.001$; (B) $R_s = 0.77$, $P < 0.001$; (C) $R_s = 0.72$, $P < 0.001$; and (D) $R_s = 0.38$, $P < 0.05$. NIDDM patients: (A) $R_s = 0.78$, $P < 0.01$; (B) $R_s = 0.64$, $P < 0.02$; (C) $R_s = 0.69$, $P < 0.01$; and (D) $R_s = 0.45$, $P < 0.10$. Healthy subjects: (A) $R_s = 0.89$, $P < 0.001$; (B) $R_s = 0.78$, $P < 0.01$; (C) $R_s = 0.42$, $P < 0.10$; and (D) $R_s = 0.44$, $P < 0.10$.

subjects ($R_s = 0.29$, $P = 0.10$, $n = 32$). Bivariate correlation analysis showed that plasma EST was strongly correlated with CET in the combined subjects ($R_s = 0.84$, $P < 0.001$), as well as in NIDDM patients ($R_s = 0.89$, $P < 0.001$ and in healthy subjects ($R_s = 0.78$, $P < 0.01$). In multiple regression analysis EST was positively related to plasma LCAT activity ($P = 0.001$), plasma triglycerides ($P = 0.016$), plasma CETP activity ($P = 0.018$) and PLTP activity ($P = 0.032$, multiple $r = 0.89$). Again no independent associations with LPL and HL activity levels as well as with the plasma FFA level, BMI and the presence of NIDDM were demonstrated (all $P > 0.15$). The bivariate correlations of EST with plasma LCAT activity, triglycerides, CETP activity and PLTP activity in the combined subjects and in the separate groups of NIDDM and healthy subjects are shown in Fig. 2. Multiple regression analysis also demonstrated that HDL cholesteryl ester concentration was negatively related to CET ($P = 0.017$), HL activity ($P = 0.033$) and to the presence of NIDDM ($P = 0.047$), and positively to plasma LCAT activity ($P = 0.034$, multiple $r = 0.68$). No associations with BMI, plasma FFA levels and LPL activity were found (all $P > 0.15$). HDL cholesteryl ester was significantly correlated with CET ($R_s = -0.44$, $P < 0.02$) and with HL activity ($R_s = -0.44$, $P < 0.02$) in the combined subjects. Such bivariate correlations were also present in the healthy subjects ($R_s = -0.73$, $P < 0.01$ for CET, and $R_s = -0.71$, $P < 0.01$ for HL activity), but not in the NIDDM patients ($R_s = -0.04$, ns and $R_s = -0.24$, ns, respectively). The bivariate relationship with LCAT activity was not significant ($R_s = 0.29$, $P = 0.10$, $n = 32$).

4. Discussion

In the present study, we have demonstrated that CET is higher in plasma from NIDDM patients compared to healthy control subjects in conjunction with higher plasma triglycerides and PLTP activity levels. Moreover, CET was related to VLDL + LDL cholesterol

and plasma triglyceride concentrations as well as to the plasma activity levels of PLTP and CETP. Thus, our data support the possibility that in normolipidaemic and moderately dyslipidaemic subjects plasma cholesteryl ester transfer is not only governed by the constellation of apo B-containing lipoproteins that accept cholesteryl ester from HDL but also by the plasma activity levels of lipid transfer proteins per se.

Elevated plasma levels of triglyceride-rich lipoproteins are important in accelerating cholesteryl ester transfer [8,42,43], as supported by the lack of difference in CET after exclusion of subjects with high triglyceride levels from the present analysis. This is in keeping with previous findings in NIDDM [20,21], although even in the absence of hypertriglyceridaemia lipoprotein compositional changes have been shown to accelerate cholesteryl ester transfer in NIDDM [19,22]. The rate of cholesteryl ester transfer in plasma from normolipidaemic subjects has been demonstrated to correlate with the FFA level [44]. Our present study suggests that FFA levels as well as lipolytic enzyme activities do not significantly influence cholesteryl ester transfer, independently from the other factors involved. Enhanced apolipoprotein glycation has been shown to accelerate cholesteryl ester transfer in vitro [45]. This mechanism may not be important in diabetic patients with acceptable metabolic control, since CET was not different in NIDDM and control subjects after exclusion of hypertriglyceridaemic individuals from the analysis. The influence of apolipoprotein glycation on CET in poorly controlled NIDDM awaits further study. The absence of an effect of diabetes on the plasma level of CETP activity, as an estimate of CETP mass, is in accordance with other studies in NIDDM [19,24,26] and uncomplicated IDDM [46], and indicates that plasma cholesteryl ester transfer can be accelerated without an increase in the plasma CETP concentration.

A potentially important novel finding was that PLTP activity levels are higher in NIDDM. Correlation analysis suggested that PLTP may accelerate cholesteryl ester

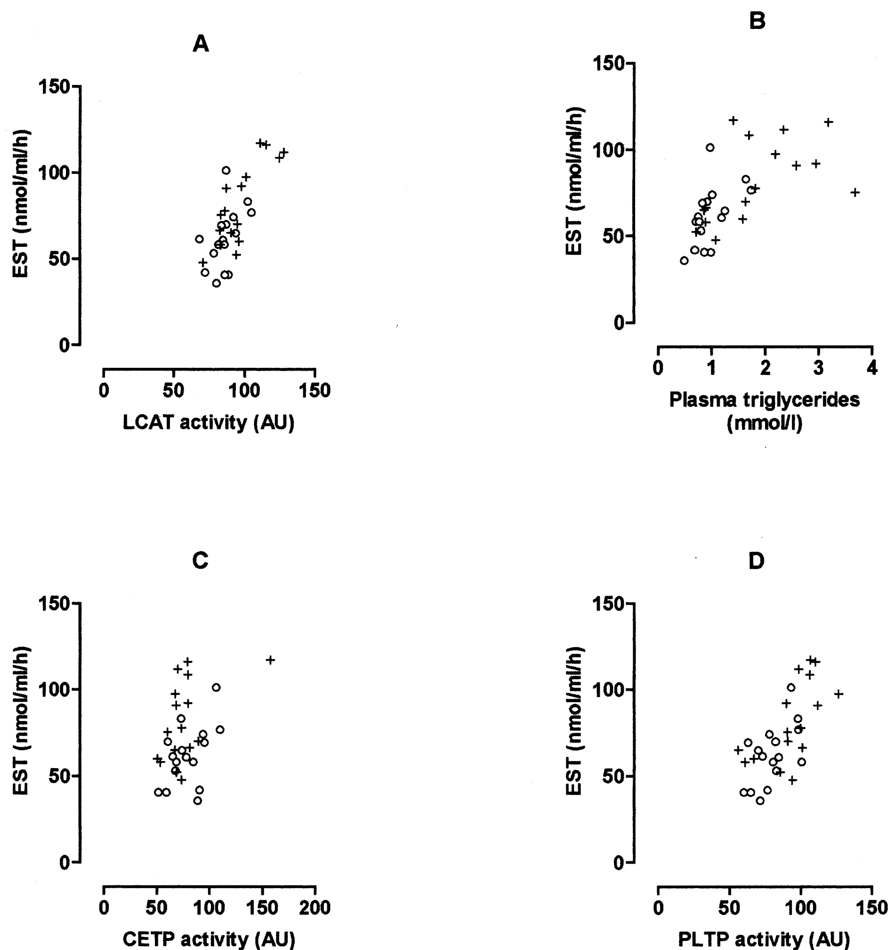

Fig. 2. Relationships of plasma cholesterol esterification rate (EST) with plasma lecithin:cholesterol acyl transferase (LCAT) (A), plasma triglycerides (B), cholesteryl ester transfer protein (CETP) activity (C) and phospholipid transfer protein activity (D) in 16 NIDDM (+) and 16 healthy subjects (○). Combined subjects: (A) $R_s = 0.69$, $P < 0.001$; (B) $R_s = 0.75$, $P < 0.001$; (C) $R_s = 0.40$, $P < 0.05$; and (D) $R_s = 0.67$, $P < 0.001$. NIDDM patients: (A) $R_s = 0.76$, $P < 0.01$; (B) $R_s = 0.64$, $P < 0.02$; (C) $R_s = 0.43$, $P < 0.10$; and (D) $R_s = 0.68$, $P < 0.01$. Healthy subjects: (A) $R_s = 0.57$, $P < 0.05$; (B) $R_s = 0.62$, $P < 0.02$; (C) $R_s = 0.46$, $P < 0.10$; and (D) $R_s = 0.48$, $P < 0.10$.

transfer in plasma. A recent evaluation of PLTP in NIDDM showed that PLTP action on exogenous lipoproteins in vitro was decreased in plasma from NIDDM patients compared to control subjects [47]. However, in that study the phospholipid transfer promoting effect of PLTP was not separated from that of CETP, making a direct comparison between the assay systems difficult. In another study, plasma PLTP activity was not found to be altered in NIDDM [48]. The higher plasma PLTP activity levels in NIDDM are in keeping with our hypothesis that PLTP activity is higher in insulin resistant states [28–30]. Since plasma PLTP is lowered by endogenous hyperinsulinaemia in healthy subjects [30], it is tempting to speculate that this effect of insulin is impaired in insulin resistant states.

The rate of plasma cholesterol esterification was also accelerated in NIDDM, in contrast with earlier data [22,49,50]. In those reports plasma apo A-I was lower [22] and triglycerides were not significantly higher [49,50] in the NIDDM groups. Apo A-I is an important cofactor for LCAT [7] and hypertriglyceridaemia stimulates cholesterol esterification [51]. These points could explain part of the discrepancy. Our results support a role of plasma triglycerides as well as of LCAT, CETP and PLTP activity levels in plasma cholesterol esterification. Among other mechanisms, this may be due to the ability of PLTP and CETP to generate preβ-HDL particles during HDL remodelling [5,6,13–15,52]. In this respect it is important that variability in plasma PLTP expression influences preβ-HDL generation in vivo [53,54]. Interestingly, preβ-HDL levels are elevated in hypertriglyceridaemia [55], a phenomenon which could be due to a link between hypertriglyceridaemia and high PLTP activity levels.

HDL cholesteryl ester was inversely correlated with CET and HL activity in all subjects together and in healthy subjects separately, in keeping with the notion that cholesteryl ester transfer [5–9] and HL [1,16,56] affect HDL core lipid metabolism. The HDL cholesteryl ester lowering in relation to the diabetic state per se, as suggested by multiple regression analysis and the lack of a significant correlation between CET, HL activity and HDL cholesteryl ester in the NIDDM patients, supports the possibility that NIDDM is associated with other abnormalities in HDL metabolism as well. It was recently shown that increased HDL apo A-I catabolism is related to low HDL cholesterol in NIDDM [57]. Accelerated plasma cholesteryl ester transfer has been shown in various hyperlipidaemic and dyslipidaemic conditions associated with increased risk of atherosclerosis [19,22,58]. The higher plasma cholesteryl ester transfer in NIDDM and its inverse relationship with HDL cholesteryl ester is concordant with this notion. Accordingly, both HDL cholesterol lowering and early development of atherosclerosis are observed in transgenic mice expressing simian CETP [59], whereas complete CETP deficiency is associated with extremely high HDL cholesterol levels and longevity in man [60]. On the other hand, the putative role of accelerated cholesteryl ester transfer to promote reverse cholesterol transport would suggest a protective effect of accelerated cholesteryl ester transfer. Intriguingly, CETP expression in transgenic mice with hypertriglyceridaemia mitigates the development of atherosclerosis [61], and cardiovascular risk is elevated in partial human CETP deficiency despite high HDL cholesterol levels [62].

The present study suggests that PLTP has the ability to promote plasma cholesteryl ester transfer in plasma. Its HDL remodelling action suggests a protective role of PLTP in atherogenesis. Our data clearly illustrate the complexity of interrelated mechanisms involved in HDL metabolism.

Acknowledgements

This study is supported by the Dutch Diabetes Foundation (grant 94-130). The expert technical assistance of P. van den Berg, T. van Gent, L.M. Scheek and A. Zonneveld is appreciated. The lipase activities in postheparin plasma were determined in the laboratory of Dr H. Jansen.

References

[1] Taskinen MR. Hyperlipidaemia in diabetes. Baillières Clin Endocrinol Metab 1990;4:743–75.

[2] Bierman EL. Atherogenesis in diabetes. Arterioscler Thromb 1992;12:647–56.

[3] Lehto S, Rönnemaa T, Haffner SM, Pyörälä K, Kallio V, Laakso M. Dyslipidemia and hyperglycemia predict coronary heart disease events in middle-age patients with NIDDM. Diabetes 1997;46:1354–9.

[4] Fielding CJ, Fielding PE. Molecular physiology of reverse cholesterol transport. J Lipid Res 1995;36:211–28.

[5] Bruce C, Tall AR. Cholesteryl ester transfer proteins, reverse cholesterol transport, and atherosclerosis. Curr Opin Lipidol 1995;6:306–11.

[6] Barter PJ, Rye KA. Molecular mechanisms of reverse cholesterol transport. Curr Opin Lipidol 1996;7:82–7.

[7] Eisenberg S. High density lipoprotein metabolism. J Lipid Res 1984;25:1017–58.

[8] Dullaart RPF, Groener JEM, Erkelens DW. Cholesteryl ester transfer between lipoproteins. Diabetes Nutr Metab 1991;14:329–43.

[9] Tall AR. Plasma cholesteryl ester transfer protein. J Lipid Res 1993;34:1255–74.

[10] Tu AY, Nishida HI, Nishida T. High density lipoprotein conversion mediated by human plasma phospholipid transfer protein. J Biol Chem 1993;268:23098–105.

[11] Tollefson JH, Ravnik S, Albers JJ. Isolation and characterization of a phospholipid transfer protein (LTP-II) from human plasma. J Lipid Res 1988;29:1593–602.

[12] Jauhiainen M, Metso J, Pahlman R, Blomqvist S, van Tol A, Ehnholm C. Human plasma phospholipid transfer protein causes high density lipoprotein conversion. J Biol Chem 1993;268:4032–6.

[13] Eckardstein von A, Jauhiainen M, Huang Y, Metso J, Langer C, Pussinen P, Wu S, Ehnholm C, Assmann G. Phospholipid transfer protein mediated conversion of high density lipoproteins generates preβ_1-HDL. Biochim Biophys Acta 1996;1301:255–62.
[14] Lusa S, Jauhiainen M, Metso J, Somerharju P, Ehnholm C. The mechanism of plasma lipid transfer protein-induced enlargement of high-density lipoprotein particles: evidence for particle fusion. Biochem J 1996;313:275–82.
[15] Hennessy LK, Kunitake ST, Kane JP. Apolipoprotein A-I-containing lipoproteins, with or without apolipoprotein A-II, as progenitors of pre-β high-density lipoprotein particles. Biochemistry 1993;32:5759–65.
[16] Jansen H, van Tol H, Hulsmann WC. On the metabolic function of heparin-releasable liver lipase. Biochem Biophys Res Commun 1980;92:53–9.
[17] Fielding CJ, Reaven GM, Fielding PE. Human non-insulin-dependent diabetes: identification of a defect in plasma cholesterol transport normalized in vivo by insulin and in vitro by selective immunoadsorption of apolipoprotein E. Proc Natl Acad Sci USA 1982;79:6365–9.
[18] Fielding CJ, Reaven GM, Liu G, Fielding PE. Increased free cholesterol in plasma low and very low density lipoproteins in non-insulin-dependent diabetes mellitus: its role in the inhibition of cholesteryl ester transfer. Proc Natl Acad Sci USA 1984;81:2512–6.
[19] Bagdade JD, Lane JT, Subbaiah PV, Otto ME, Ritter MC. Accelerated cholesteryl ester transfer in non-insulin-dependent diabetes mellitus. Atherosclerosis 1993;104:69–77.
[20] Sutherland WH, Walker RJ, Lewis-Barned NJ, Pratt H, Tillman HC. Plasma cholesteryl ester transfer in patients with non-insulin dependent diabetes mellitus. Clin Chim Acta 1994;231:29–38.
[21] Elchebly M, Porokhov B, Pulcini T, Berthezène F, Ponsin G. Alterations in composition and concentration of lipoproteins and elevated cholesteryl ester transfer in non-insulin-dependent diabetes mellitus (NIDDM). Atherosclerosis 1996;123:93–101.
[22] Bhatnagar D, Durrington PN, Kumar S, Mackness MI, Boulton AJM. Plasma lipoprotein composition and cholesteryl ester transfer from high density lipoproteins to very low density and low density lipoproteins in patients with non-insulin-dependent diabetes mellitus. Diabetic Med 1996;13:139–44.
[23] Bagdade JD, Kelley DE, Henry RR, Eckel RH, Ritter MC. Effects of multiple daily insulin injections and intraperitoneal insulin therapy on cholesteryl ester transfer and lipoprotein lipase activities in NIDDM. Diabetes 1997;46:414–20.
[24] Lottenberg SA, Lottenberg AMP, Nunes VS, McPherson R, Quintao ECR. Plasma cholesteryl ester transfer protein concentration, high-density lipoprotein cholesterol esterification and transfer rates to lighter density lipoproteins in the fasting state and after a test meal are similar in Type II diabetics and normal controls. Atherosclerosis 1996;127:81–90.
[25] Ahnadi CE, Masmoudi T, Berthezène F, Ponsin G. Decreased ability of high density lipoproteins to transfer cholesterol esters in non-insulin-dependent diabetes mellitus. Eur J Clin Invest 1993;23:459–65.
[26] Kahri J, Syvänne M, Taskinen MR. Plasma cholesteryl ester transfer protein activity in non-insulin-dependent diabetic patients with and without coronary artery disease. Metabolism 1994;43:1498–502.
[27] van Tol A. CETP-catalysed transfer of cholesterylesters from HDL to apo B-containing lipoproteins in plasma from diabetic patients (letter). Eur J Clin Invest 1993;23:856.
[28] Dullaart RPF, Sluiter WJ, Dikkeschei LD, Hoogenberg K, van Tol A. Effect of adiposity on plasma lipid transfer protein activities: a possible link between insulin resistance and high density lipoprotein metabolism. Eur J Clin Invest 1994;24:188–94.
[29] Dullaart RPF, Hoogenberg K, Dikkeschei BD, van Tol A. Higher plasma lipid transfer protein activities and unfavorable lipoprotein changes in cigarette-smoking men. Arterioscler Thromb 1994;14:1581–5.
[30] van Tol A, Ligtenberg JJM, Riemens SC, van Haeften TW, Reitsma WD, Dullaart RPF. Lowering of plasma phospholipid transfer protein activity by acute hyperglycaemia-induced hyperinsulinaemia in healthy men. Scan J Clin Lab Invest 1997;57:147–58.
[31] National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28:1039–57.
[32] Ostlund RE, Staten M, Kohrt WM, Schultz J, Malley M. The ratio of waist-to-hip circumference, plasma insulin level, and glucose intolerance as independent predictors of the HDL_2 cholesterol level in older adults. New Engl J Med 1990;322:229–34.
[33] Demacker PN, Hijmans AG, Vos-Janssen HE, van't Laar A, Jansen AP. A study of the use of polyethylene glycol in estimating cholesterol in high-density lipoprotein. Clin Chem 1980;26:1775–9.
[34] Hindriks FR, Wolthers BG, Groen A. The determination of total cholesterol in serum by gas-liquid chromatography compared with two other methods. Clin Chim Acta 1977;74:207–15.
[35] Channon KM, Clegg RJ, Bhatnagar D, Ishola M, Arrol S, Durrington PN. Investigation of lipid transfer in human serum leading to the development of an isotopic method for the determination of endogenous cholesterol esterification and transfer. Atherosclerosis 1990;80:217–26.
[36] Glomset JA, Wright JL. Some properties of a cholesterol esterifying enzyme in human plasma. Biochim Biophys Acta 1964;89:266–7.
[37] Groener JEM, Pelton RW, Kostner GM. Improved estimation of cholesteryl ester transfer/exchange activity in serum or plasma. Clin Chem 1986;32:283–6.
[38] Hannuksela M, Marcel YL, Kesäniemi YA, Savolainen MJ. Reduction in the concentration and activity of plasma cholesteryl ester transfer protein by alcohol. J Lipid Res 1992;33:737–44.
[39] Floren CH, Chen CH, Franzen J, Albers JJ. Lecithin: cholesterol acyltransferase in liver disease. Scand J Clin Lab Invest 1987;47:613–7.
[40] Speijer H, Groener JEM, van Ramshorst E, van Tol A. Different locations of cholesteryl ester transfer protein and phospholipid transfer protein activities in plasma. Atherosclerosis 1991;90:159–68.
[41] Jansen H, Hop W, van Tol A, Bruschke AVG, Birkenhager JC. Hepatic lipase and lipoprotein lipase are not major determinants of the low density lipoprotein subclass pattern in human subjects with coronary heart disease. Atherosclerosis 1994;107:45–54.
[42] Mann CJ, Yen FT, Grant AM, Bihain BE. Mechanism of plasma cholesteryl ester transfer in hypertriglyceridemia. J Clin Invest 1991;88:2059–66.
[43] Eisenberg S. Preferential enrichment of large-sized very low density lipoprotein with transferred cholesteryl esters. J Lipid Res 1985;26:487–94.
[44] Lagrost L, Florentin E, Guyard-Dangremont V, Athias A, Gandjini H, Lallemant C, Gambert P. Evidence for non-esterified fatty acids as modulators of neutral lipid transfers in normolipidemic human plasma. Arterioscler Thromb Vasc Biol 1995;15:1388–96.
[45] Passarelli M, Catanozi S, Nakandakare ER, Rocha JC, Morton RE, Shimabukuro AFM, Quintao ECR. Plasma lipoproteins from patients with poorly controlled diabetes mellitus and in vitro glycation of lipoproteins enhance the transfer rate of cholesteryl ester from HDL to apo B-containing lipoproteins. Diabetologia 1997;40:1085–93.

[46] Dullaart RPF, Groener JE, Dikkeschei BD, Erkelens DW, Doorenbos H. Increased cholesterylester transfer activity in complicated type 1 insulin-dependent diabetes mellitus: its relationship with serum lipids. Diabetologia 1989;32:14–9.

[47] Elchebly M, Pulcini T, Porokhov B, Berthezène F, Ponsin G. Multiple abnormalities in the transfer of phospholipids from VLDL and LDL to HDL in non-insulin-dependent diabetes. Eur J Clin Invest 1996;26:216–23.

[48] Syvänne M, Castro G, Dengremont C, Geitere De C, Jauhiainen M, Ehnholm C, Michelagnoli S, Franceschini G, Kahri J, Taskinen MR. Cholesterol efflux from Fu5AH hepatoma cells induced by plasma of subjects with or without coronary artery disease and non-insulin-dependent diabetes: importance of LpA-I:A-II particles and phospholipid transfer assay. Atherosclerosis 1996;127:245–53.

[49] Schernthaner G, Kostner GM, Dieplinger H, Prager R, Mühlhauser I. Apolipoproteins (A-I, A-II, B), Lp(a) lipoprotein and lecithin:cholesterol acyltransferase activity in diabetes mellitus. Atherosclerosis 1983;49:277–93.

[50] Durlach V, Attia N, Zahouani A, Leutenegger M, Girard-Globa A. Postprandial cholesteryl ester transfer and high density lipoprotein composition in normotriglyceridemic non-insulin-dependent diabetic patients. Atherosclerosis 1996;120:155–65.

[51] Murakami T, Michelagnoli S, Longhi R, Gianfranceschi G, Pazzucconi F, Calabresi L, Sirtori CR, Franceschini G. Triglycerides are major determinants of cholesterol esterification/transfer and HDL remodeling in human plasma. Arterioscler Thromb Vasc Biol 1995;15:1819–28.

[52] Clay MA, Newnham HH, Forte TM, Barter PJ. Cholesteryl ester transfer protein and hepatic lipase activity promote shedding of apo AI from HDL and subsequent formation of discoidal HDL. Biochim Biophys Acta 1992;1124:52–8.

[53] Jiang X, Francone OL, Bruce C, Milne R, Mar J, Walsh A, Breslow JL, Tall AR. Increased preβ-high density lipoprotein, apolipoprotein AI, and phospholipid in mice expressing the human phospholipid transfer protein and human apolipoprotein AI transgenes. J Clin Invest 1996;96:2373–80.

[54] Albers JJ, Tu AY, Paigen B, Chen H, Cheung MC, Marcovina SM. Transgenic mice expressing human phospholipid transfer protein have increased HDL/non-HDL cholesterol ratio. Int J Clin Lab Res 1996;26:262–7.

[55] Ishida BY, Frolich J, Fielding CJ. Pre-beta-migrating high density lipoprotein: quantitation in normal and hyperlipidemic plasma by solid phase radioimmunoassay following electrophoretic transfer. J Lipid Res 1987;28:778–86.

[56] Syvänne M, Ahola M, Lahdenperä S, Kahri J, Kuusi T, Virtanen KS, Taskinen MR. High density lipoprotein subfractions in non-insulin-dependent diabetes mellitus and coronary artery disease. J Lipid Res 1995;36:573–82.

[57] Frénais R, Ouguerram K, Maugeais C, Mahot P, Maugère P, Krempf M, Magot T. High density lipoprotein apolipoprotein AI kinetics in NIDDM: a stable isotope study. Diabetologia 1997;40:578–83.

[58] Tall AR, Granot E, Brocia R, Tabas I, Hesler C, Williams K, Denko M. Accelerated transfer of cholesteryl esters in dyslipidemic plasma. Role of cholesteryl ester transfer protein. J Clin Invest 1987;79:1217–25.

[59] Marotti KR, Castle CK, Boyle TP, Lin AH, Murray RW, Melchior GW. Severe atherosclerosis in transgenic mice expressing simian cholesteryl ester transfer protein. Nature 1993;364:73–5.

[60] Inazu A, Brown ML, Hesler CB, Agellon LB, Koizumi J, Takata K, Maruhama Y, Mabuchi H, Tall AR. Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. New Engl J Med 1990;323:1234–8.

[61] Hayek T, Masucci-Magoulas L, Jiang X, Walsh A, Rubin E, Breslow JL, Tall AR. Decreased early atherosclerotic lesions in hypertriglyceridemic mice expressing cholesteryl ester transfer protein transgene. J Clin Invest 1995;96:2071–4.

[62] Zhong S, Sharp DS, Grove JS, Bruce C, Yano K, Curb JD, Tall AR. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. J Clin Invest 1996;97:2917–23.