Plasma Cholesteryl Ester Transfer Protein and Its Relationship to Plasma Lipoproteins and Apolipoprotein A-I-Containing Lipoproteins in IDDM Patients With Microalbuminuria and Clinical Nephropathy

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OBJECTIVE — To study the distribution of high-density lipoprotein (HDL) subclasses in insulin-dependent diabetes mellitus (IDDM) patients with nephropathy and factors involved in the regulation of HDL, including plasma cholesteryl ester transfer protein (CETP) and postheparin plasma lipoprotein lipase (LPL) and hepatic lipase (HL) activities.

RESEARCH DESIGN AND METHODS — Participants included 52 microalbuminuric IDDM patients (with a urinary albumin excretion rate [UAER] of 20–200 μg/min), 37 macroalbuminuric IDDM patients (UAER >200 μg/min), and 64 normoalbuminuric IDDM patients (UAER <20 μg/min). Groups were matched for age, body mass index, duration of diabetes, and glycemic control (HbA_1).

RESULTS — Median concentrations of HDL and HDL_2 cholesterol were 11.6 ($P = 0.01$) and 22.7% ($P = 0.01$) less in microalbuminuric patients and 5.1 and 15.5% less in macroalbuminuric patients compared with normoalbuminuric patients. No significant differences were observed in the concentrations of apoA-I, apoA-II (apolipoprotein) or LpA-I or LpA-I:A-II (lipoprotein) particles between the groups. HDL cholesterol: apoA-I + apoA-II ratio was significantly lower in micro- (19.7 ± 4.2 (± SD); $P < 0.01$) and macroalbuminuric patients (20.0 ± 3.7, $P < 0.05$) than in normoalbuminuric patients (22.1 ± 4.4). Postheparin plasma LPL:HL ratio was lower in microalbuminuric patients compared with normoalbuminuric patients (1.65 vs. 1.05 [median], $P < 0.01$). Plasma CETP activity was higher in the macroalbuminuric patients than in micro- ($P < 0.05$) and normoalbuminuric patients ($P < 0.05$) but did not correlate with HDL, HDL_2, or HDL_3 cholesterol. LPL:HL ratio correlated positively with HDL cholesterol ($r = 0.372$, $P < 0.001$), HDL_2 cholesterol ($r = 0.413$, $P < 0.001$) and with LpA-I particles ($r = 0.355$, $P < 0.001$) but not with LpA-I:A-II particles ($r = -0.065$, NS).

CONCLUSIONS — IDDM patients with micro- and macroalbuminuria show only trivial changes in concentrations of different HDL parameters, which cannot explain the excess risk of coronary heart disease in these patients. Data also indicate that elevation of CETP activity in IDDM patients with nephropathy is probably not responsible for the lowering of HDL cholesterol.

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CHD, coronary heart disease; IDDM, insulin-dependent diabetes mellitus; UAER, urinary albumin excretion rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; apo, apolipoprotein; Lp, lipoprotein; CETP, cholesteryl ester transfer protein; BMI, body mass index; LPL, lipoprotein lipase; HL, hepatic lipase; ACE, angiotensin-converting enzyme; AU, arbitrary units; RIA, radioimmunoassay; FFA, free fatty acid.

Recent epidemiological data confirm that coronary heart disease (CHD) mortality is >30-fold higher in proteinuric insulin-dependent diabetes mellitus (IDDM) patients than in those with normal urinary albumin excretion rate (UAER) (1). Changes in plasma lipoproteins have been proposed as one pathogenetic mechanism responsible for the increase of CHD risk in diabetic nephropathy (2–3). In contrast to normoalbuminuric IDDM patients with good to moderate glycemic control (4–5), proteinuric IDDM patients show elevations of serum and low-density lipoprotein (LDL) cholesterol and serum triglycerides but lowering of plasma high-density lipoprotein (HDL) and HDL_2 values (2–3). In the general population, low plasma HDL-cholesterol concentration is a potent risk factor for CHD (6). The heterogeneity of HDL was recognized previously (7): HDL can be subfractionated according to its main apolipoprotein (apo) content into the particles that contain only apoA-I (lipoprotein A-I [LpA-I] particles) and into LpA-I:A-II particles that contain both apoA-I and apoA-II (8). It has been suggested that LpA-I particles would be the "anti-atherogenic HDL" (9). So far, there are no reports on distribution of LpA-I and LpA-I:A-II particles in diabetic nephropathy.

The metabolism of HDL is regulated by several factors. Plasma cholesteryl ester transfer protein (CETP), which catalyzes the transfer of esterified cholesterol from HDL to very-low-density lipoprotein (VLDL) and LDL (10), is one of the major factors that remodels the concentration and composition of HDL in circulation (11–12). Available data also suggest that plasma CETP activity correlates positively with the extent of atherosclerosis (13). In IDDM patients with micro- or macrovascular lesions, plasma CETP activity has been reported to be increased (14). In line, plasma CETP concentration has been demonstrated to be high in nondiabetic patients with nephrotic syndrome (15). So far, no studies

have focused on plasma CETP activity in different stages of diabetic kidney disease (i.e., normo-, micro-, and macroalbuminuria).

The aim of this study was to examine the distribution of serum LpA-I and LpA-I:A-II particles in normo-, micro-, and macroalbuminuric patients. We also investigated the regulation of HDL metabolism in diabetic nephropathy by measuring plasma CETP activity and postheparin plasma lipoprotein lipase (LPL) and hepatic lipase (HL) activities.

RESEARCH DESIGN AND METHODS

— For this study, 152 patients with IDDM were recruited from the diabetic outpatient clinics of Helsinki University Hospital, Finland, and Guy's Hospital, London, England. All patients had a history of ketone bodies in the urine and/or ketoacidosis at the time of diagnosis and had been insulin-treated from the time of diagnosis. Their C-peptide values were <0.05 nM. The overnight UAER is annually screened in each diabetic patient in these clinics, and the IDDM subjects invited to the study were selected to represent a wide range of UAER distribution. At entry into the study, the patients were subdivided into three subgroups according to their median UAER value of three consecutive timed overnight urine collections. The microalbuminuric group (35 men, 17 women) consisted of IDDM patients with UAER from 20 to 200 μg/min who were available for the study. Patients in the macroalbuminuric group (17 men, 20 women) had UAER >200 μg/min. The normoalbuminuric group comprised 64 (31 men, 33 women) IDDM patients with UAER <20 μg/min. This group represents a subpopulation of a larger study group of normoalbuminuric IDDM patients (n = 86) (16), who were matched with the micro- and macroalbuminuric groups with respect to age, duration of diabetes, and degree of glycemic control. IDDM patients taking diuretics or beta blockers were excluded when the case records were screened. Seven proteinuric IDDM patients were treated with angiotensin-converting enzyme (ACE) inhibitors; one was taking a Ca-channel blocker; and three were on a combination of ACE inhibitor and Ca-channel blocker for treatment of hypertension. Three microalbuminuric patients received antihypertensive therapy with ACE inhibitors and one with Ca-channel blocker. Five normoalbuminuric and three proteinuric patients were taking oral contraceptives. Fourteen normoalbuminuric patients, 21 microalbuminuric, and 10 macroalbuminuric patients were smokers.

The majority of the patients were on a multiple insulin injection regimen (n = 73), whereas 63 patients had regimens of one to three times daily insulin injections. Sixteen patients received insulin subcutaneously via a pump. Ninety-four patients had background retinopathy; 81 patients had symptoms or signs of diabetic neuropathy; and 18 patients had a clinical history of macroangiopathy. None of the patients were taking drugs known to affect lipid metabolism. They were asked to refrain from alcohol during the 3 days before the study. Case histories of excessive drinking and laboratory signs of alcoholism (mean red cell volume >96 fL, gammaglutamyltransferase activity >50 U/L) were exclusion criteria. The study protocol was approved by the ethical committees of the both clinics.

Serum lipids and lipoprotein analysis

All the participants were studied as outpatients. Venous blood samples for the lipoprotein analysis were drawn after a 12-h fast, and the serum was immediately separated. Serum samples from England were mailed on the same day in boxes cooled to 4°C, and they were handled immediately. All the lipid and lipoprotein determinations were made in the same lab in Helsinki. Serum lipoproteins (VLDL, IDL [intermediate-density lipoprotein], LDL, HDL, HDL_2, and HDL_3) were isolated by sequential ultracentrifugation (17) in a Beckman L8–70 ultracentrifuge (Palo Alto, CA) in a Kontron TFT 45.8 rotor as previously described (18).

Postheparin plasma LPL and HL activities

At the same visit, blood was drawn before and 15 min after a bolus injection of heparin (100 IU/kg body weight) into chilled, heparinized tubes kept on ice to measure postheparin plasma LPL and HL activities. Plasma was immediately isolated in a cooled centrifuge and stored at −20°C. LPL activities were measured by an immunochemical assay using a specific antiserum raised against HL in rabbits (19). Assay conditions to measure HL activity comprised ^{14}C-labeled triolein substrate containing 1 M NaCl to inactivate the LPL. Interassay variations for LPL and HL activity measurements were 5.1 and 8.4%, respectively.

Assay of CETP activity

Plasma for the measurement of CETP activity was available from the last 103 patients (48 normoalbuminuric, 37 microalbuminuric, and 28 macroalbuminuric patients). LDL and HDL substrates used in the assay were isolated from plasma of healthy donors by ultracentrifugation at $1.019 < d < 1.063$ g/ml and $1.063 < d < 1.210$ g/ml, respectively (17). The isolated lipoproteins were reisolated at the same density and dialyzed extensively against 10 mM Tris HCl, pH 7.4 containing 150 mM NaCl, 1 mM EDTA, and 0.1 g/l NaN_3. LDL was labeled with cholesteryl(1-^{14}C)oleate (Amersham, U.K.) with the lipid dispersion technique of Morton and Zilversmit (20). The labeled LDL was isolated by density gradient ultracentrifugation (21). CETP activity was analyzed by the method of Groener et al. (22). In this method, HDL fraction containing CETP was isolated from each subject by precipitation of the apoB-containing lipoproteins with polyethylene glycol. Briefly, 100 μl of plasma and 200 μl of polyethylene glycol (95 g/L, molecular weight 20,000) were mixed and centrifuged at 2,000 g for 15 min at 4°C. Of the resulting supernate, 30 μl was used in the incubation, which included ^{14}C-cholesteryl ester–labeled LDL (250 nmol total cholesterol), cold HDL (100

nmol total cholesterol), 0.7 μM 5–5′-dithiobis, and 17.5 μM of phosphate buffer at pH 7.4 in a total incubation volume of 355 μl. The incubation was performed at 37°C for 16 h. After incubation, the tubes were placed in ice and 150 μl of 80 g/L bovine serum albumin and 50 μl of cold LDL (total cholesterol 1 μM) were added. Thereafter, 55 μl of an equivolume mixture of dextran sulfate (20 g/L) and 2 M magnesium chloride was added, and the tubes were kept on ice for 20 min, after which the precipitated lipoproteins were pelleted by centrifugation at 2,000 *g* for 20 min at 4°C. The radioactivity of the supernate, which represented the counts transferred from exogenously labeled LDL to HDL by CETP present in the HDL + VHDL plasma fraction from each patient, was counted. All the determinations were performed in duplicate. Results were first calculated as nmol · ml^{-1} · h^{-1} as described previously (23) and adjusted to the value of a pooled serum standard stored at −80°C measured in each assay series. Final results, therefore, are expressed as arbitrary units (AU). Intra-assay variation of CETP activity, measured in 6 dilutions of (1:6–1:1) standard sample, was 4.4%. Interassay variation using different substrate batches is 5.0 ± 3.0%.

ApoA-I, apoA-II, LpA-I, and LpA-I:A-II concentrations

Concentrations of apoA-I and apoA-II were measured from serum stored at −20°C by an immunoturbidometric method with commercially available kits (726478 and 726486, Boehringer Mannheim, Mannheim, Germany). The concentration of LpA-I particles was measured by using immunoelectrophoresis (24) with commercially available kits (Sebia, France). The concentration of LpA-I:A-II particles was determined by subtracting LpA-I concentration from the turbidometrically analyzed apoA-I concentration. Interassay variations for apoA-I, apoA-II, and LpA-I particle measurements were 3.5, 2.4, and 7.3%.

Analytical methods

Serum lipoprotein cholesterol and triglyceride concentrations were measured enzymatically (25) (Hoffmann-La Roche, Basel, Switzerland, kits 0722138 and 0715166) with an automated Cobas Mira® analyzer (Hoffmann-La Roche). Interassay coefficients of variation for both measurements were <3%. Glycosylated hemoglobin (HbA_1) (normal range 6–8.5%) was measured by microcolumn chromatography (Isolab, Akron, OH) (26). Blood glucose was determined with the glucose oxidase method (Autoanalyzer, Technicon, Tarrytown, NY). Urinary albumin excretion was measured by a radioimmunoassay (RIA) (27). Serum C-peptide was determined by RIA using the RIA-mat C-peptide II kit (BYK-Sangtec Diagnostica, Frankfurt, Germany). Plasma-free insulin concentrations were determined by RIA with Phasedeph insulin RIA kits (Pharmacia, Uppsala, Sweden) after precipitation with polyethylene glycol (28).

Statistical analysis

BMDP statistical software (University of California Press) was used. Differences between the study groups were tested by the Kruskal-Wallis test (BMDP3S), and, thereafter, the Mann-Whitney *U* test was performed to test differences between two groups (BMDP3D). For the correlation analyses, Pearson's correlation coefficients were calculated (BMDP6D).

RESULTS

Patient characteristics

The groups of patients were well matched for age, body mass index (BMI), glycemic control, insulin dose, and duration of diabetes (Table 1). The concentration of serum total and LDL cholesterol were clearly higher in the micro- and macroalbuminuric patients, especially so in men (Table 2).

Plasma HDL subfractions

Concentrations of HDL and HDL_2 cholesterol showed a trend toward lower values in micro- and macroalbuminuric patients than in the normoalbuminuric group, but a significant difference was observed only between micro- and normoalbuminuric patients. A higher concentration of HDL cholesterol and HDL_2 cholesterol in women compared with men was found in all patient groups. No significant differences were found either in the concentrations of apoA-I or LpA-I particles or in apoA-II and LpA-I:A-II particles between normo-, micro-, or macroalbuminuric patients (Fig. 1). The HDL:apoA-I + apoA-II ratio was generally lower in micro- (19.7 ± 4.2, $P < 0.01$) and macroalbuminuric patients (20.0 ± 3.7, $P < 0.05$) than in normoalbuminuric patients (22.1 ± 4.4). LpA-I particles correlated with HDL cholesterol and HDL_2 cholesterol but not with HDL_3 cholesterol in all patient groups (Table 3). In normo- and microalbuminuric patients, no consistent correlation existed between LpA-I:A-II particles and HDL cholesterol. In the macroalbuminuric patients, however, we found a positive relationship between LpA-I:A-II particles and HDL cholesterol ($r = 0.420$, $P < 0.01$) and with HDL_3 cholesterol ($r = 0.573$, $P < 0.001$). Overall, the concentration of LpA-I particles was related positively to the concentration of apoA-I. The concentration of LpA-I:A-II exhibited a highly significantly positive correlation with both apoA-I and apoA-II in all three groups.

Plasma CETP activity

In plasma of patients in the macroalbuminuric group, CETP activity was significantly higher than in microalbuminuric or normoalbuminuric patients (Fig. 2). Notably, microalbuminuric men exhibited significantly lower plasma CETP activity than microalbuminuric women. No definite relationship existed between plasma CETP activity and the concentrations of HDL, HDL_2, or HDL_3 cholesterol in any of the patient groups (data not shown). No significant correlations were found between plasma CETP activity and HbA_1, insulin dose, plasma-free insulin,

Table 1—*Patient characteristics*

	Men			Women			All		
	Normo	Micro	Macro	Normo	Micro	Macro	Normo	Micro	Macro
n	31	35	17	33	17	20	64	52	37
Age (years)	35.8 (25.9–61.4)	39.0 (23.2–61.4)	41.5 (30.0–56.0)	34.0 (18.9–61.1)	35.2 (21.6–60.6)	32.8 (22.9–50.8)	35.5 (18.9–61.4)	38.3 (21.6–61.4)	37.0 (22.9–56.0)
BMI (kg/m^2)	24.3 (19.3–34.0)	24.7 (19.1–31.6)	24.1 (20.6–30.4)	22.8 (19.1–27.8)	24.1 (21.0–28.3)	24.6 (20.8–31.5)	23.6 (19.1–34.0)	24.6 (19.1–31.6)	24.4 (20.6–31.5)
HbA_1 (%)	9.0 (6.4–12.4)	9.9* (7.6–14.2)	9.8 (7.6–13.3)	9.6 (6.6–13.5)	9.4 (6.4–12.7)	9.8 (7.9–13.1)	9.1 (6.4–13.5)	9.9 (6.4–14.2)	9.8 (7.6–13.3)
Duration of diabetes (years)	24.0 (7.7–38.3)	23.0 (7.7–46.5)	26.5 (14.5–41.0)	20.1 (8.1–43.2)	22.8 (10.7–42.8)	23.3 (13.5–36)	22.0 (7.7–43.2)	22.9 (7.7–46.5)	24.0 (13.5–41.0)
Insulin dose (IU/day)	51 (23–100)	54 (20–96)	50 (33–72)	44 (22–104)	40 (26–76)	43 (20–122)	46 (22–104)	48 (20–96)	46.0 (20–122)
Plasma-free insulin (mU/L)	4.5 (2.0–198.0)	5.7 (1.0–28.9)	7.2 (2.0–47.2)	5.1 (2.3–35.8)	4.9 (2.0–68.8)	8.1 (2.9–25.4)	4.9 (2.0–198.0)	5.2 (1.0–68.8)	7.8 (2.0–47.2)
Mean UAER (μg/min)	8 (1–18)	55 (20–192)	512 (223–1756)	7 (1–19)	49 (23–131)	428 (201–1490)	7 (1–19)	53 (20–192)	433 (201–1756)

Data are median (range). *$P < 0.01$ for difference from normoalbuminuric men.

or UAER in any of the patient groups (data not shown).

The relationship of postheparin lipolytic enzymes to HDL parameters

Postheparin plasma LPL was similar in the patient groups (Table 4). Postheparin plasma HL activity was slightly higher in the micro- and macroalbuminuric patients compared with normoalbuminuric patients (Table 4). Consequently, LPL:HL ratio was lower in micro- and macroalbuminuric groups than in normoalbuminuric patients. Overall, female subjects had lower HL activities than did men in all three groups (Table 4). As expected, LPL activity had a positive relationship with HDL and HDL_2 cholesterol, whereas HL activity showed borderline inverse correlations with HDL_2 cholesterol in all three groups (data not shown). Consequently, postheparin plasma LPL:HL ratio showed a positive relationship with plasma HDL

Table 2—*Concentrations of serum lipids and lipoproteins in patients with normo-, micro-, and macroalbuminuria*

	Men			Women			All		
	Normo	Micro	Macro	Normo	Micro	Macro	Normo	Micro	Macro
Triglycerides (mM)	0.85 (0.61–1.40)	1.10 (0.56–2.92)	1.01 (0.35–2.20)	0.81 (0.49–4.94)	0.91 (0.60–1.35)	0.90 (0.52–1.84)	0.84 (0.49–4.94)	1.03† (0.56–2.92)	0.97 (0.35–2.20)
Cholesterol (mM)	4.54	5.29*	5.36†	4.68	5.05	5.33	4.60	5.24*	5.36*
Total	(3.08–8.30)	(3.45–8.54)	(2.43–6.96)	(3.86–7.15)	(3.68–6.11)	(3.90–8.22)	(3.08–8.30)	(3.45–8.54)	(2.43–8.22)
LDL	2.65 (1.33–5.67)	3.11† (1.82–5.74)	3.51† (1.16–4.56)	2.53 (1.97–4.72)	2.83 (1.80–4.31)	2.87 (1.93–5.40)	2.57 (1.33–5.67)	2.94* (1.80–5.74)	3.23* (1.16–5.40)
HDL	1.50 (0.89–2.36)	1.43 (0.96–1.86)	1.25 (0.95–2.02)	1.71‡ (1.28–2.38)	1.60 (1.10–2.74)	1.74§ (1.05–2.81)	1.64 (0.89–2.38)	1.45* (0.96–2.74)	1.56 (0.95–2.81)
HDL_2	0.80 (0.34–1.64)	0.71 (0.34–1.25)	0.66 (0.35–1.27)	1.01‡ (0.46–1.64)	0.98‡ (0.53–1.91)	1.05‡ (0.31–2.13)	0.97 (0.34–1.64)	0.75* (0.34–1.91)	0.82 (0.31–2.13)
HDL_3	0.69 (0.47–1.02)	0.74 (0.44–1.00)	0.67 (0.41–0.87)	0.71 (0.46–0.89)	0.62§ (0.45–0.86)	0.67 (0.47–0.90)	0.70 (0.46–1.02)	0.70 (0.44–1.00)	0.67 (0.41–0.90)

Data are median (range). *$P \leq 0.01$ for difference from respective values of normoalbuminuric patients. †$P \leq 0.05$ for difference from respective values of normoalbuminuric patients. ‡$P < 0.01$ for difference from opposite sex in the same group. §$P < 0.05$ for difference from opposite sex in the same group. Mann-Whitney *U* test was used.

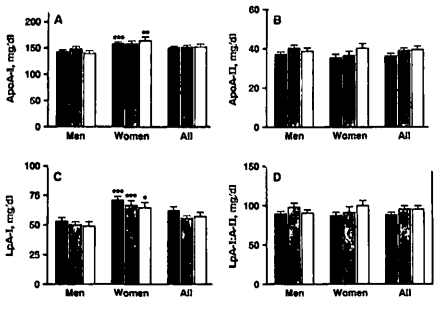

Figure 1—*Concentrations (mg/dl) of A) apoA-I, B) apoA-II, C) LpA-I, and D) LpA-I:A-II particles in normo- (■), micro- (▨), and macroalbuminuric (□) men, women, and combined groups. ***P < 0.001, **P < 0.01, and *P < 0.05 for difference from the opposite sex in the same study group.*

and HDL_2 cholesterol ($r = 0.372$, $P < 0.001$; $r = 0.413$, $P < 0.001$, respectively, for the combined group). LPL:HL ratio correlated positively with LpA-I particles in all three patient groups ($r = 0.355$, $P < 0.001$ for the combined group). By contrast, we found no correlations between postheparin plasma lipolytic activity and LpA-I:A-II particles in any of the groups (Table 4). Postheparin plasma HL activity showed a significant negative relationship with HDL cholesterol:apoA-I + apoA-II ratio ($r = -0.312$, $P < 0.001$), both in women ($r = -0.262$, $P < 0.05$) and in men ($r = -0.243$, $P < 0.05$).

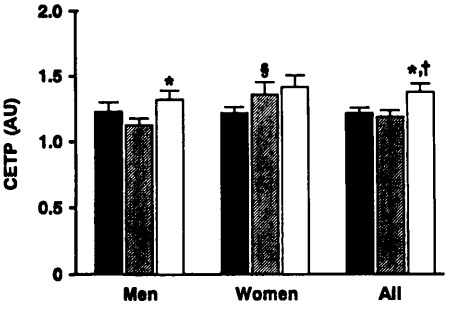

Figure 2—*Plasma CETP activity in normo- (■, n = 48), micro- (▨, n = 37), and macroalbuminuric (□, n = 28) men, women, and combined groups. *P < 0.05 for respective values of microalbuminuric patients. §P < 0.05 for difference from the values of men in the same group. †P < 0.05 for difference from respective values of normoalbuminuric patients.*

CONCLUSIONS— The major finding of this study is that diabetic nephropathy is accompanied by only trivial changes of HDL parameters. We observed a trend toward a lower concentration of HDL and HDL_2 cholesterol in IDDM patients with kidney disease but not a concomitant decrease of apoA-I and apoA-II levels. Our data are in accord with previous reports, which did not distinguish for gender, that demonstrated a lowering of plasma HDL cholesterol and especially HDL_2 cholesterol in proteinuric IDDM patients (2–3,29). However, the ambient values in both micro- and macroalbuminuric patients are comparable to those observed in a nondiabetic cohort (16). We observed a slight tendency for the level of apoA-II to increase with progression of diabetic kidney disease. Consequently, the composition of HDL in micro- and macroalbuminuric patients was abnormal, as shown by the decrease of HDL cholesterol:apoA-I + apoA-II ratio, which suggests a disruption between the response of HDL lipids and proteins to diabetic nephropathy.

We also measured apoA-I–containing HDL subfractions in diabetic nephropathy. In line with the data on apoA-I levels, our patients exhibited no significant differences in LpA-I particles between the three groups. Note that we have recently reported an elevation of LpA-I particles in normoalbuminuric IDDM patients compared with healthy control subjects (16). In line with our previous data (16), the actual LpA-I values in female micro- and macroalbuminuric patients were even higher than those of the healthy population. In turn, LpA-I:A-II particles showed a slight tendency toward higher values as nephropathy ensued. The increase of LpA-I:A-II particles also paralleled that of apoA-II. The values remained closely similar to those of the healthy population (16).

What relevance do these findings

Table 3—*Pearson's correlation coefficients of LpA-I and LpA-I:A-II particles and HDL parameters in patients with normo-, micro-, and macroalbuminuria*

	Men	Women	All
LpA-I particles vs.			
HDL cholesterol	0.692*	0.680*	0.727*
HDL_2 cholesterol	0.698*	0.722*	0.764*
HDL_3 cholesterol	0.214	0.034	0.060
ApoA-I	0.436*	0.373†	0.489*
ApoA-II	−0.064	−0.275‡	−0.215†
LPL	0.286‡	0.207	0.261‡
HL	−0.098	−0.249	−0.308*
LPL:HL ratio	0.301‡	0.198	0.355*
LpA-I:A-II particles vs.			
HDL cholesterol	0.067	0.043	0.041
HDL_2 cholesterol	0.050	−0.088	−0.053
HDL_3 cholesterol	0.161	0.397*	0.273*
ApoA-I	0.794*	0.744*	0.717*
ApoA-II	0.486*	0.734*	0.627*
LPL	0.087	−0.051	0.018
HL	0.055	0.119	0.094
LPL:HL ratio	−0.014	−0.084	−0.065

*$P < 0.001$. †$P < 0.01$. ‡$P < 0.05$.

Table 4—*Postheparin plasma LPL and HL activities in normo-, micro-, and macroalbuminuric patients*

	Men			Women			All		
	Normo	Micro	Macro	Normo	Micro	Macro	Normo	Micro	Macro
LPL (μmol FFA · $ml^{-1} \cdot min^{-1}$)	36.9 (21.6–63.0)	32.6 (19.2–60.4)	36.6 (14.7–46.3)	39.0 (17.7–61.3)	37.6 (18.4–52.7)	36.3 (21.7–61.0)	38.5 (17.7–63.0)	35.7 (18.4–60.4)	36.6 (14.7–61.0)
HL (μmol FFA · $ml^{-1} \cdot min^{-1}$)	27.9 (13.1–50.5)	32.8 (15.1–49.6)	36.1 (10.1–47.0)	21.3* (7.53–43.4)	23.5 (12.8–48.9)	23.3† (12.3–63.0)	24.3 (7.53–50.5)	28.5‡ (12.8–49.6)	26.3 (10.1–63.0)
LPL:HL ratio	1.36 (0.57–1.95)	0.96 (0.54–3.09)	1.09 (0.40–2.59)	1.95* (0.66–5.94)	1.47† (0.61–3.22)	1.54† (0.43–2.82)	1.65 (0.57–5.94)	1.05§ (0.54–3.22)	1.30 (0.39–2.82)

Data are median (range). *$P < 0.001$ for difference from the respective values of the opposite sex in the same group. †$P < 0.05$ for difference from the respective values of the opposite sex in the same group. ‡$P < 0.05$ for respective values of normoalbuminuric patients. §$P < 0.01$ for respective values of normoalbuminuric patients.

have to atherogenesis? Patients with IDDM are more susceptible to coronary artery disease and other vascular complications than the normoglycemic population (30). An increase in early cardiovascular morbidity and mortality occurs in those diabetic patients who develop proteinuria (1,31). Low plasma HDL is a recognized risk factor for atherosclerosis (6). However, the trivial reduction in serum HDL cholesterol in albuminuric groups cannot explain the excess CHD risk because the values remained comparable to those in nondiabetic cohorts (16).

Which mechanisms lie behind the changes of HDL composition in diabetic nephropathy? One regulator of plasma HDL concentration and composition is CETP, which catalyzes the transfer of esterified cholesterol from HDL to VLDL/LDL and reciprocal transfer of triglycerides (10). The increase of CETP activity in our macroalbuminuric patients is consistent with previous data showing the elevation of plasma CETP concentration in nondiabetic patients with nephrotic syndrome (15). Since plasma CETP concentration is the main determinant of plasma CETP activity (32), the increased activity could be because of an increased synthesis of CETP. The fact that the cholesterol content of HDL and HDL_2 was not related to CETP activity in any of our groups disputes a major role of CETP activity as a determinant of HDL cholesterol levels in type I diabetic patients with and without nephropathy. The concept is consistent with previous data from cross-sectional studies showing that variation of CETP within normal range is not necessarily negatively related to plasma HDL cholesterol concentration (32–33). On the other hand, extreme alterations of CETP are usually reflected in HDL cholesterol levels (11–12). Because the concentration and the composition of cholesteryl ester donor and acceptor lipoproteins are important determinants of cholesteryl ester transfer (34), it may be possible that, despite normal CETP activity in microalbuminuric patients, these patients may exhibit increase of cholesteryl ester transfer from HDL to apoB-containing lipoproteins, which reduces serum HDL levels. The positive correlation between plasma CETP levels and the extent of atherosclerosis in cynomolgus monkeys (13) suggest that CETP activity may act as a cardiovascular risk factor. Interestingly, increased plasma CETP activities also have been observed in IDDM patients who smoke and in IDDM patients with micro- and macrovascular lesions (14,35). In our data, however, smoking or higher frequency of macroangiopathy could not explain the higher CETP activities in macroalbuminuric patients, because smoking-adjusted or macroangiopathy-adjusted group means were virtually the same (data not shown).

The opposite effects of LPL and HL activities on plasma HDL cholesterol concentration have been reported previously (36), and they were also operative in our IDDM patients. Notably, HL also had a negative relationship with HDL cholesterol:apoA-I + apoA-II ratio. Whether this correlation means a causal relationship remains an open question.

We conclude that micro- and macroalbuminuric IDDM patients display qualitatively multiple changes in HDL cholesterol/apolipoprotein relationships. However, the changes are small, and these changes cannot explain the excess of CHD risk in these patients.

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References

1. Borch-Jensen K, Kremer S: Proteinuria: value as predictor of cardiovascular mor-

tality in insulin-dependent diabetes mellitus. *Br Med J* 294:1651–1654, 1987
2. Winocour PH, Durrington PN, Ishola M, Anderson DC, Cohen H: Influence of proteinuria on vascular disease, blood pressure, and lipoproteins in insulin-dependent diabetes mellitus. *Br Med J* 294: 1648–1650, 1987
3. Jones SL, Close CF, Mattock MB, Jarrett RJ, Keen H, Viberti GC: Plasma lipid and coagulation factor concentrations in insulin-dependent diabetics with microalbuminuria. *Br Med J* 298:487–490, 1989
4. Nikkilä EA, Hormila P: Serum lipids and lipoproteins in insulin-treated diabetes: demonstration of increased high-density lipoprotein concentrations. *Diabetes* 27: 1078–1086, 1978
5. Mattock MB, Salter AM, Fuller JH, Omer T, Gohari REL, Redmond SD, Keen H: High-density lipoprotein subfractions in insulin-dependent diabetic and normal subjects. *Atherosclerosis* 45:67–79, 1982
6. Miller GJ, Miller NE: Plasma high-density lipoprotein concentration and development of ischaemic heart disease. *Lancet* 1:16–19, 1975
7. Eisenberg S: High-density lipoprotein metabolism. *J Lipid Res* 25:1017–1058, 1984
8. Cheung MC, Albers JJ: Distribution of high-density lipoprotein particles with different apoprotein composition: particles with A-I and A-II and particles with A-I but no A-II. *J Lipid Res* 23:747–753, 1982
9. Fievet C, Fruchart JC: HDL heterogeneity and coronary heart disease. *Diabetes Metab Rev* 7:155–162, 1991
10. Tall AR: Plasma high-density lipoproteins: metabolism and relation to atherogenesis. *J Clin Invest* 86:379–384, 1990
11. Inazú A, Brown ML, Hesler CB, Agellon LB, Koizumi J, Takata K, Matruhama Y, Mabushi H, Tall AR: Increased high-density lipoprotein levels caused by a common cholesteryl ester transfer protein gene mutation. *N Engl J Med* 323:1234–1238, 1990
12. Agellon LB, Walsh A, Hayek T, Moulin P, Jiang XC, Shelanski SA, Breslow JL, Tall AR: Reduced high-density lipoprotein cholesterol in human cholesteryl ester transfer protein transgenic mice. *J Biol Chem* 266:10796–10801, 1991
13. Quinet E, Tall A, Ramakrishnan R, Rudel L: Plasma lipid transfer protein as a determinant of the atherogenicity of monkey plasma lipoproteins. *J Clin Invest* 87: 1559–1566, 1991
14. Dullaart RPF, Groener JEM, Dikkeschei LD, Erkelens DW, Doorenbos H: Increased cholesteryl ester transfer activity in complicated type 1 (insulin-dependent) diabetes mellitus: its relationship with serum lipids. *Diabetologia* 32:14–19, 1989
15. Moulin P, Appel GB, Ginsberg HN, Tall AR: Increased concentration of plasma cholesteryl ester transfer protein in nephrotic syndrome: role in dyslipidemia. *J Lipid Res* 33:1817–1822, 1992
16. Kahri J, Groop P-H, Viberti GC, Elliott T, Taskinen M-R: Regulation of apolipoprotein A-I containing lipoproteins in insulin-dependent diabetes mellitus. *Diabetes* 42:1281–1288, 1993
17. Havel RJ, Eder HA, Brigdon JH: The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* 34:1345–1353, 1955
18. Taskinen M-R, Kuusi T, Helve E, Nikkilä EA, Yki-Järvinen H: Insulin therapy induces antiatherogenic changes of serum lipoproteins in non-insulin-dependent diabetes. *Arteriosclerosis* 8:168–177, 1988
19. Huttunen JK, Ehnholm C, Kinnunen PJ, Nikkilä EA: An immunochemical method for selective measurement of two triglyceride lipases in human postheparin plasma. *Clin Chim Acta* 63:335–347, 1975
20. Morton RE, Zilversmit DB: A plasma inhibitor of triglyceride and cholesteryl ester transfer activities. *J Biol Chem* 256: 11992–11995, 1981
21. Kelley JL, Kruski AW: Density gradient ultracentrifugation of serum lipoproteins in a swinging bucket rotor. *Methods Enzymol* 128:170–181, 1986
22. Groener JEM, Pelton RW, Kostner GM: Improved estimation of cholesterylester transfer/exchange activity in serum or plasma. *Clin Chem* 32:283–286, 1986
23. Barter PJ, Jones ME: Rate of exchange of esterified cholesterol between human plasma low and high-density lipoproteins. *Atherosclerosis* 34:67–74, 1979
24. Parra HJ, Mezdour H, Ghalim N, Bard JM, Fruchart JC: Differential electroimmunoassay of human LpA-I lipoprotein particles on ready-to-use plates. *Clin Chem* 36: 1431–1435, 1990
25. Wahlefeld AW: Triglyceride determination after enzymatic hydrolysis. In *Methods of Enzymatic Analysis*. 2nd ed. Bergmeyer HV, Ed. New York, Academic Press, 1974, p. 1831–1835
26. Welch SG, Boucher DJ: A rapid microscale method for the measurement of haemoglobin $A_{1\ (a+b+c)}$. *Diabetologia* 14: 209–211, 1978
27. Keen H, Chlouverakis G: An immunoassay for urinary albumin at low concentrations. *Lancet* 2:913–916, 1963
28. Desbuquios B, Aurbach GD: Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. *J Clin Endocrinol Metab* 33:732–738, 1971
29. Watts GF, Naumova R, Slavin BM, Morris RW, Houlston R, Kubal C, Shaw KM: Serum lipids and lipoproteins in insulin-dependent diabetic patients with persistent microalbuminuria. *Diabetic Med* 6:25–30, 1989
30. Pyörälä K, Laakso M, Uusitupa M: Diabetes and atherosclerosis: an epidemiologic review. *Diabetes Metab Rev* 3:463–524, 1987
31. Jensen T, Borch-Johnsen K, Enevoldsen AK, Deckert T: Coronary heart disease in young diabetic patients with and without diabetic nephropathy: incidence and risk factors. *Diabetologia* 30:144–148, 1987
32. McPherson R, Mann CJ, Tall AR, Hogue M, Martin L, Milne RW, Marcel YL: Plasma concentrations of cholesteryl ester transfer protein in hyperlipoproteinemia: relation to cholesteryl ester transfer protein activity and other lipoprotein variables. *Arterioscler Thromb* 11:797–804, 1991
33. Marcel YL, McPherson R, Hogue M, Czarnecka H, Zanadzki Z, Weech PK, Whitlock ME, Tall AR, Milne MW: Distribution and concentration of cholesteryl ester transfer protein in plasma of normoli-

pemic subjects. *J Clin Invest* 85:10–17, 1990
34. Mann CJ, Yen FT, Grant AM, Bihain BE: Mechanism of plasma cholesteryl ester transfer in hypertriglyceridemia. *J Clin Invest* 88:2059–2066, 1991
35. Dullaart RPF, Groener JEM, Dikkeschei BD, Erkelens DW, Doorenbos H: Elevated cholesteryl ester transfer protein activity in IDDM men who smoke: possible factor for unfavorable lipoprotein profile. *Diabetes Care* 14:338–341, 1991
36. Taskinen M-R, Kuusi T, Nikkilä EA: Regulation of HDL and its subfractions in chronically insulin-treated patients with type I diabetes. In *Diabetes, Obesity, and Hyperlipidemias*. Vol. III. Crepaldi G, Tiengo A, Baggio G, Eds. Amsterdam, Elsevier, 1985, p. 251–259