evaluate the role of self-regulation behaviors in this process (5).

Our findings may have important implications for educational program planning in diabetes. Patients who take insulin could potentially derive major benefits from participation in relatively inexpensive short-term education programs that teach self-regulation skills. Effective education for patients who do not take insulin may require more expensive programs with frequent contact and long-term follow-up, because targets for improved metabolic control necessarily involve changes in life-style. In the interests of efficient resource allocation, we should provide self-regulation training to all who may benefit from it and reserve more resource-intensive alternatives for those who need to make fundamental changes in life-style.

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Elevated Cholesteryl Ester Transfer Protein Activity in IDDM Men Who Smoke

Possible Factor for Unfavorable Lipoprotein Profile

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Objectives: To determine the effect of cigarette smoking on the activity of cholesteryl ester transfer protein (CETP) and high-density (HDL), low-density (LDL), and very-low-density (VLDL) lipoproteins in insulin-dependent diabetic (IDDM) men with microvascular complications. Research Design and Methods: We perfomed a case-control study in a referral-based diabetes clinic on a sequential sample of 9 cigarette-smoking and 12 nonsmoking IDDM men with microvascular complications and 12 nonsmoking control men. CETP activity was determined in each serum with an isotope assay with exogenous cholesteryl ester–labeled LDL and HDL. The method is independent of the endogenous lipoprotein present in serum. Results: The HDL-cholesterol (VLDL and LDL) ratio was lower in the smoking diabetic men than in the other groups ($P < 0.05$ vs. the nonsmoking diabetic men and $P < 0.01$ vs. the control subjects). CETP activity was 70% higher in the smoking diabetic men than in the control subjects ($P < 0.01$) and 30% higher than in the nonsmoking diabetic men ($P < 0.05$). The HDL-cholesterol (VLDL and LDL) ratio and the apolipoprotein A-I–B ratio were inversely correlated to CETP activity in the diabetic patients ($r = -0.52$, $P < 0.02$ and $r = -0.45$, $P < 0.05$, respectively). Conclusions: CETP activity is increased in cigarette-smoking IDDM men with microvascular complications. High CETP activity may contribute to the unfavorable lipoprotein profile in these patients.
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The excess risk for cardiovascular mortality in cigarette smokers can in part be attributed to an atherogenic lipoprotein profile (1). In insulin-dependent diabetes mellitus (IDDM), the major cause of premature death is cardiovascular disease.(2)

Among all other pathways involved in lipoprotein metabolism, the process of cholesteryl ester transfer, catalyzed by the cholesteryl ester transfer protein (CETP), is instrumental in the distribution of cholesteryl ester between lipoproteins (3). CETP activity, measured independently of endogenous lipoproteins, was higher in hypercholesterolemic and dys-β-lipoproteinemic subjects and in IDDM patients with microvascular and macrovascular complications (4–6). A deficiency of CETP

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TABLE 1
Clinical characteristics of smoking and nonsmoking men with insulin-dependent diabetes mellitus (IDDM) and nonsmoking control men

	IDDM		
	Smoking	Nonsmoking	Control
n	9	12	12
Age (yr)	45 ± 12	44 ± 13	40 ± 13
Duration of diabetes (yr)	23 ± 10	23 ± 11	
Body mass index (kg/m^2)	24.04 ± 1.01	24.77 ± 2.08	23.59 ± 1.90
Cigarettes per day (*n*)	13 ± 5	0	0
HbA_1 (%)	7.41 ± 1.66	7.75 ± 1.10	4.88 ± 0.64*
Fasting blood glucose (mM)	12.38 ± 4.80	12.54 ± 3.49	4.47 ± 0.37*
Urinary albumin excretion rate (μg/min)	24 (3–152)	16 (2–109)	3 (1–6)*
Microalbuminuria (*n*)	6	8	
Retinopathy (A/B/P)†	0/8/1	3/7/2	

Values are means ± SD, except urinary albumin excretion rate, which is given as median with range in parentheses.
*$P < 0.01$ vs. smoking and nonsmoking IDDM.
†Retinopathy format: A, absent; B, background; P, proliferative.

has been documented in some cases of hyper-α-lipoproteinemia (7). Immunologic inhibition of CETP activity in rabbits resulted in an increase in high-density lipoprotein (HDL) cholesteryl ester and in HDL particle size, a decrease in very-low-density lipoprotein (VLDL) cholesteryl ester, and a redistribution of cholesteryl ester from low-density lipoproteins (LDL) toward HDL (8,9). This study was carried out to investigate whether an increased CETP activity is involved in the low proportion of cholesterol in HDL relative to that in VLDL and LDL in cigarette-smoking IDDM men with microvascular complications.

RESEARCH DESIGN AND METHODS

Consent was obtained from all subjects after explanation of the purpose of the study, which was approved by the local medical ethics committee. Three groups of subjects were investigated: 9 cigarette-smoking IDDM men with microvascular complications (either microalbuminuria, retinopathy, or both); 12 nonsmoking IDDM men, also with microvascular complications; and 12 nonsmoking male control subjects (Table 1).

In all diabetic men, the disease was present before 35 yr of age, and insulin therapy was started immediately after diagnosis. The participants were studied after a 12-h fast. No subject had arterial hypertension. No medication other than insulin was used. Serum creatinine was <120 μM in all subjects. No subject suffered from thyroid or liver disease. Microalbuminuria was defined as a mean urinary albumin excretion rate between 10 and 200 μg/min, determined in three consecutive overnight urine collections.

Venous blood was collected on ice, and the erythrocytes were removed by centrifugation at 3000 rpm for 15 min. Serum samples were frozen at −20°C and analyzed within 8 wk. The assays were performed in duplicate in one run.

The activity of CETP was determined in serum from each control and IDDM subject with an isotope assay detecting the transfer/exchange of radioactive cholesteryl ester between exogenous [oleate 1-^{14}C]cholesteryl ester–labeled LDL (Amersham, Aylesbury, UK) and an excess of unlabeled HDL (10). The exogenous LDL and HDL fractions were isolated from one pool of freshly isolated normolipidemic human plasma by repeated ultracentrifugation. In short, CETP activity was determined in each serum after removal of endogenous VLDL and LDL by polyethylene glycol precipitation. The resulting supernatant that contained CETP was mixed with the exogenous radiolabeled LDL and unlabeled HDL and incubated for 16 h at 37°C. Phosphatidyl choline–sterol acyltransferase was inhibited. The amount of endogenous HDL-cholesterol was <15% of the total amount of HDL-cholesterol in the assay system and did not affect the measurement (10). Therefore, the method is independent of the endogenous lipoproteins present in serum. In addition, no effect of ambient serum free–fatty acid levels on the measurement of CETP activity could be demonstrated. CETP activity was calculated as the bidirectional transfer of cholesteryl ester between radiolabeled LDL and HDL (11). The within-assay coefficient of variation was 2.7%.

Lipids were measured in serum and in the HDL-containing supernatant after precipitation of apolipoprotein (apo) B–containing lipoproteins with sodium phosphotungstate and $MgCl_2$ (Merck, Darmstadt, Germany). Cholesterol and triglyceride were assayed enzymatically. Apo AI and apo B were measured by immunoturbidimetry with commercially available kits (Boehringer Mannheim, nos. 726478 and 726494, respectively). Urinary albumin was measured with a commercially available radioimmunoassay ($KHAD_2$; Diagnostic Products, Apeldoorn, Netherlands). HbA_1 was determined

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TABLE 2
Serum lipid parameters in smoking and nonsmoking men with insulin-dependent diabetes mellitus (IDDM) and nonsmoking control men

	IDDM		
	Smoking	Nonsmoking	Control
n	9	12	12
Cholesterol (mM)	5.88 ± 0.90	5.75 ± 0.83	5.36 ± 1.11
Triglyceride (mM)	1.00 ± 0.42	0.75 ± 0.25*	1.19 ± 0.41
HDL-cholesterol (mM)	1.10 ± 0.37	1.32 ± 0.33	1.31 ± 0.21
Cholesterol (VLDL and LDL) (mM)	4.78 ± 0.72	4.43 ± 0.87	4.05 ± 1.07
HDL-cholesterol (VLDL and LDL)	0.23 ± 0.07†‡	0.31 ± 0.12	0.34 ± 0.11
Apolipoprotein A-I (g/L)	1.17 ± 0.26‡	1.35 ± 0.19	1.25 ± 0.16
Apolipoprotein B (g/L)	0.91 ± 0.18	0.86 ± 0.18	0.83 ± 0.18
Apolipoprotein A-I/B	1.33 ± 0.39	1.64 ± 0.44	1.56 ± 0.37

Values are means ± SD. HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein.
*$P < 0.05$, †$P < 0.01$, vs. control
‡$P < 0.05$ vs. nonsmoking IDDM.

by colorimetry. Blood glucose was measured on a YSI glucose analyzer (model 23A, Yellow Springs, OH).

Data are given as means ± SD. Urinary albumin excretion levels are given as median. Data were compared by analysis of variance with adjustment for multiple comparisons with Duncan's method. Relationships were assessed with Spearman's rank correlation. $P < 0.05$ was considered significant.

RESULTS

The levels of the serum lipids and apolipoproteins are given in Table 2. No significant differences could be demonstrated with respect to serum cholesterol and VLDL-, LDL-, and HDL-cholesterol. The HDL-cholesterol (VLDL and LDL) ratio was significantly lower in smoking IDDM men than in the other groups. ApoA-I was lower in smoking IDDM men than in nonsmoking IDDM men. CETP activity was 170 ± 43, 129 ± 30, and 98 ± 20 nmol cholesteryl ester · ml^{-1} serum · h^{-1} in smoking IDDM men, nonsmoking IDDM men, and control men, respectively (Fig. 1). The HDL-cholesterol (VLDL and LDL) ratio and the apoAI–B ratio were inversely correlated to CETP activity in the diabetic patients ($r = -0.52$, $n = 21$, $P < 0.02$ and $r = -0.45$, $n = 21$, $P < 0.05$, respectively).

CONCLUSIONS

HDL has a crucial role in the transport of cholesterol from peripheral tissue back to the liver, the so-called reverse cholesterol transport. HDL can deliver cholesteryl ester directly to the liver (3). The generation of an indirect route for hepatic HDL cholesteryl ester removal via VLDL and LDL by CETP has been suggested to represent an antiatherogenic mechanism (12). However, the process of cholesteryl ester transfer may contribute to the development of atherosclerosis by promoting cholesteryl ester transfer toward VLDL, LDL, and remnant

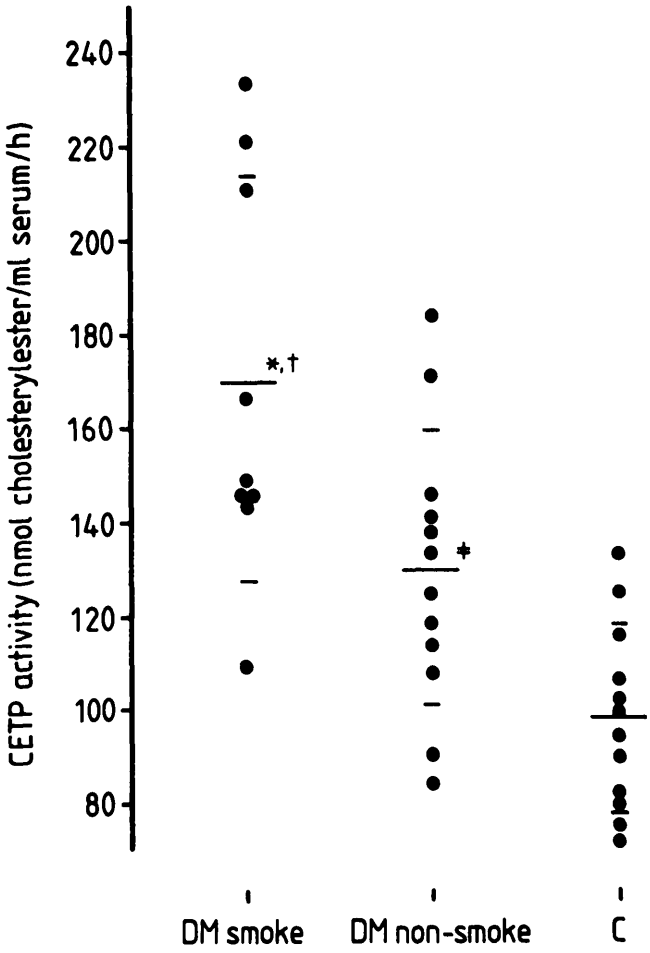

FIG. 1. Cholesteryl ester transfer protein (CETP) activity in 33 men. DM smoke, smoking men with insulin-dependent diabetes mellitus (IDDM); DM non-smoke, nonsmoking men with IDDM; C, control men. *Long bars* indicate means; *short bars* indicate SD. *$P < 0.05$ vs. DM non-smoke; †$P < 0.01$, ‡$P < 0.05$, vs. C.

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particles, which are capable of depositing cholesterol in the arterial wall (3,4). The resistance to the development of atherosclerosis in species that lack CETP activity, such as rats and pigs, might indicate that the ultimate effect of the pathways generated through the action of CETP is atherogenic (3).

This study shows that atherogenic alterations in serum lipids and apolipoproteins, comparable to those found in the nondiabetic population (1), are present in cigarette-smoking IDDM men with microvascular complications. The activity of CETP was higher in the cigarette-smoking IDDM men than in the other groups. CETP activity was also higher in the nonsmoking IDDM men with microvascular complications than in the control subjects. Previous studies have shown that the activity of CETP affects lipoprotein cholesterol levels and lipoprotein composition in vivo (7–9). Therefore, it might be postulated that a high activity of CETP contributes to the low proportion of cholesterol in HDL relative to that in VLDL and LDL. Smoking probably also has an effect on lipoprotein composition. In smokers, HDL-cholesterol is decreased to a greater extent than HDL-apoAI (1,13), and VLDL particles are enriched in cholesterol (1). Such changes are in accordance with the presumed consequences of increased activity of CETP. Further studies are needed to investigate the effect of smoking on lipoprotein composition in IDDM subjects and to determine whether smoking also increases CETP activity in nondiabetic subjects. The mechanisms responsible for an elevated CETP activity are unknown. High CETP activity could be due not only to an increased CETP mass but also to a decreased activity of a putative inhibitor (14).

Several other mechanisms have been proposed to explain the link between smoking and alterations in serum lipids (1). Cigarette smoking stimulates adrenal norepinephrine release, which raises free–fatty acid levels. Free fatty acids enhance hepatic triglyceride and cholesterol synthesis (1). Low dietary intake of polyunsaturated fatty acids in smokers could contribute to the unfavorable lipid profile (15). In this regard, it is important that CETP activity can vary in parallel with diet-induced changes in VLDL and LDL cholesterol (16). This study supports a role for CETP in the unfavorable lipid levels and the increased cardiovascular risk in cigarette-smoking men with IDDM.

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