



Glycation of Apolipoprotein C1 Impairs Its CETP Inhibitory Property: Pathophysiological Relevance in Patients With Type 1 and Type 2 Diabetes

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OBJECTIVE

Apolipoprotein (apo)C1 is a potent physiological inhibitor of cholesteryl ester transfer protein (CETP). ApoC1 operates through its ability to modify the electrostatic charge at the lipoprotein surface. We aimed to determine whether the inhibitory ability of apoC1 is still effective *in vivo* in patients with diabetes and whether *in vitro* glycation of apoC1 influences its electrostatic charge and its CETP inhibitory effect.

RESEARCH DESIGN AND METHODS

ApoC1 concentrations and CETP activity were measured in 70 type 1 diabetic (T1D) patients, 113 patients with type 2 diabetes, and 83 control subjects. The consequences of *in vitro* glycation by methylglyoxal on the electrostatic properties of apoC1 and on its inhibitory effect on CETP activity were studied. An isoelectric analysis of apoC1 was performed in patients with T1D and in normolipidemic-normoglycemic subjects.

RESULTS

An independent negative correlation was found between CETP activity and apoC1 in control subjects but not in patients with diabetes. HbA_{1c} was independently associated with CETP activity in T1D patients. *In vitro* glycation of apoC1 modified its electrostatic charge and abrogated its ability to inhibit CETP activity in a concentration-dependent manner. The isoelectric point of apoC1 in T1D patients was significantly lower than that in control subjects.

CONCLUSIONS

The ability of apoC1 to inhibit CETP activity is impaired in patients with diabetes. Glycation of apoC1 leads to a change in its electrostatic properties that might account, at least in part, for a loss of constitutive CETP inhibition and an increase in plasma CETP activity in patients with diabetes.

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Apolipoprotein (apo)C1, a small, basic apolipoprotein mainly produced by the liver (1), has been shown to play a significant and complex role in VLDL and HDL metabolism. ApoC1 was found to stimulate hepatic production of VLDL (2), to inhibit the hydrolysis of VLDL (3–5), and to impair the recognition of VLDL by its cellular receptors (6,7). In vivo studies in animal models support the hypothesis that apoC1 plays a complex and significant role in both the accumulation of VLDL particles in the blood stream and the reduction in their cholesteryl ester content relative to triglycerides (TG) (8–11).

In addition, apoC1 has been shown to be a potent inhibitor of cholesteryl ester transfer protein (CETP) activity. In vitro, apoC1 inhibits CETP activity when associated with the plasma HDL fraction (12). The physiological relevance of apoC1 as a CETP inhibitor has been shown in apoC1-knocked out/human CETP transgenic and human apoC1 transgenic/human CETP transgenic mice (13,14). The inhibitory effect of apoC1 was found to rely, at least in part, on its ability to modulate the electrostatic charge at the HDL surface, which is recognized as a major determinant of the CETP-lipoprotein interaction and thus of the exchange of core neutral lipids (15,16). In healthy, normolipidemic subjects, the concentration of plasma apoC1 has been shown to correlate negatively with CETP activity and positively with plasma HDL cholesterol (HDL-C) levels (17,18). In contrast, such relationships were not observed in nondiabetic patients with hypertriglyceridemia or combined hyperlipidemia on secondary prevention (17), suggesting that apoC1 might become dysfunctional as a constitutive/endogenous CETP inhibitor in high-risk populations.

Glycation is known to change side chains of reactive amino acids, essentially lysine and arginine, to form Schiff base and to reduce the isoelectric point of proteins (pI) (19). Glycation is a consequence of hyperglycemia during diabetes. ApoC1 contains nine lysine residues and three arginine residues and is thus likely to be susceptible to glycation.

We hypothesized that glycation of apoC1 might change its electrostatic properties, which are critical in its inhibitory effect, and

might impair its ability to inhibit CETP activity. We also hypothesized that the physicochemical properties of apoC1 might be altered in diabetes and might result in the impairment of its ability to modulate CETP activity.

In the first part of the current study, the association between apoC1 concentration and CETP activity was assessed in patients with type 1 (T1D) and type 2 (T2D) diabetes in comparison with healthy normolipidemic-normoglycemic individuals. In the second part, the effect of in vitro glycation of apoC1 on its electrostatic charge properties was investigated and the consequences of this on its ability to inhibit CETP activity were determined. This study provides evidence of the role of glycation of apoC1 on the loss of the CETP inhibitory effect of apoC1 observed in T1D and T2D patients.

RESEARCH DESIGN AND METHODS

Study Design and Patients

Seventy T1D patients, 113 T2D patients, and 83 normoglycemic-normolipidemic control subjects were studied. All subjects were older than 18 years.

Control subjects were not taking any treatment that could interfere with lipoprotein metabolism (lipid-lowering agents, anti-HIV agents, combined oral contraceptive pill, corticoids, or retinoic acid), none were pregnant, the fasting glycemia level was <6.1 mmol/L, the TG level was <1.7 mmol/L, and the LDL cholesterol (LDL-C) level was <4.128 mmol/L.

In patients with diabetes, the presence of microvascular complications (retinopathy and nephropathy), was recorded. Three stages were used to define nephropathy: 0 for no nephropathy, 1 for microalbuminuria between 30 and 300 mg per day, and 2 for proteinuria >300 mg per day. The estimated glomerular filtration rate was calculated with the MDRD equation.

This prospective single-center study was approved by our regional ethics committee. Written informed consent was obtained from all patients before inclusion in this study.

Plasma Preparation

Blood samples were collected at inclusion and were centrifuged for

serum separation within 2 h after collection. The plasma was divided into aliquots and stored at -80°C until biological analysis.

Biochemical Assay

Fasting plasma glucose, plasma creatinine, fasting total and HDL-C, and TG were measured on a Vista analyzer with dedicated reagents (Siemens Healthcare Diagnostics, Deerfield, IL). LDL-C was calculated by the Friedewald equation when TG levels were <3.87 mmol/L (20). When TG levels were >3.87 mmol/L, LDL-C was measured directly on the Vista analyzer with dedicated reagents (Siemens Healthcare Diagnostics). HbA_{1c} was measured by high-performance liquid chromatography on a Variant II device (Bio-Rad, Richmond, CA) (normal range 4–6% [20–60 mmol/mol]).

ApoC1 Concentration

ApoC1 was measured in total plasma by a specific ELISA using an anti-human apoC1 antiserum from rabbit as previously described (21).

Measurement of Cholesteryl Ester Transfer Activity Using a Radioactivity Method

Cholesteryl ester transfer activity in individual plasma samples (25 μL per assay) was the measured transfer from [³H]cholesteryl ester-containing HDL (2.5 nmol per assay) toward endogenous apoB-containing lipoproteins. [³H]cholesteryl ester-containing HDL was obtained with HDL from one subject with no lipid and no glucose metabolism abnormalities.

The cholesteryl ester transfer rate was calculated from the known specific radioactivity of the HDL donors and the accumulation of radiolabeled cholesteryl esters in the LDL acceptors after the deduction of blank values from control mixtures that were incubated at 37°C without a plasma sample (12,22).

CETP mass concentration was measured by a specific ELISA with TP1 anti-CETP antibodies as previously described (23). CETP activity values were calculated as the ratio of the plasma cholesteryl ester transfer rate to the plasma CETP mass concentration and were expressed as

nanomoles of ^3H -CE transferred per milligram of CETP per hour.

Purification of ApoC1 by Chromatofocusing

ApoC1 was purified from delipidated HDL apos by using the chromatofocusing method of Tournier et al. (24).

Preparation of Lipoproteins

HDLs were isolated from plasma by sequential flotation ultracentrifugation, according to their density, as previously described (12).

In Vitro Glycation of ApoC1, HDL, and CETP With Methylglyoxal

Methylglyoxal (MG) was used to glycate in vitro apoC1, HDL, and CETP.

MG reacts with the free amino groups of lysine and arginine (19). Thirteen reactive groups are contained in 1 mol apoC1, 112 reactive groups are contained in 1 mol HDL, and 41 reactive groups are contained in 1 mol CETP. The amount of MG used is represented as the ratio of the reactive groups in apoC1, HDL, and CETP that can interact with MG. For example, 50 μg apoC1 was treated with 0, 10, 25, 50, 100, and 500 nmol MG corresponding to a ratio of 0:1, 0.1:1, 0.25:1, 0.5:1, 1:1, and 5:1 of apoC1 reactive groups.

The mixtures with apoC1 and HDL were incubated for 24 h at 37°C and dialyzed twice for 16 h against TBS. The mixtures with CETP were incubated for 1 h at room temperature.

Isoelectric Analysis

Native apoC1, MG-treated apoC1, and HDL apoproteins from control subjects ($n = 6$) or patients with T1D ($n = 6$) and protein pl standards (SERVA) were diluted in 125 μL hydration buffer (8 mol/L urea, 4% CHAPS, 20 mmol/L dithiothreitol, 0.2% Bio-Lyte 3–10). After overnight hydration of 7-cm-long ReadyStrip, pH 3–10 (Bio-Rad), at 50 V in a Protean IEF cell (Bio-Rad), isoelectric focusing was conducted for 12 kV/h. Either the strips were stained with Coomassie Brilliant Blue G-250 (for native or MG-treated apoC1) or proteins were transferred to a polyvinylidene fluoride membrane for Western blotting with a rabbit anti-human apoC1-immunoglobulin and an HRP-conjugated anti-rabbit antibody (Dako) (for HDL apoproteins

from control subjects and patients with T1D).

Measurement of Cholesteryl Ester Transfer Activity by the Fluorescent Method

For the in vitro analyses, CETP activity was assessed by the fluorescent method as previously described (15). For the measurement of cholesteryl ester transfer activity with apoC1, donor liposomes (5 μL), acceptor VLDL (5 μL), and purified CETP were incubated in the presence of 250 pmol native apoC1 or MG-treated apoC1. For the measurement of cholesteryl ester transfer activity with HDL, donor liposomes (5 μL) and purified CETP (5 μL) were incubated in the presence (125 μL) of control HDL or HDL treated with MG (100 nmol cholesterol).

Agarose Gel Electrophoresis

The electrophoretic mobility of native or MG-treated HDL was determined by electrophoresis on 0.5% agarose gels (Paragon Lipo kit; Beckman Instruments) according to the method of Sparks and Phillips (25).

Statistics

Data are shown as means \pm SD or percentages as indicated. Differences between two groups were evaluated by the Student *t* test for continuous variables. Differences between more than two groups for continuous variables were analyzed by ANOVA.

The Pearson correlation coefficients were determined by linear regression analysis. Statistical significance of the correlation coefficients was determined by the method of Fisher and Yates.

Multivariate analyses were done with CETP activity as a dependent variable in each group (control subjects, T1D, and T2D). The variables introduced into the model were age, BMI, HDL-C, LDL-C, TG, and apoC1. In addition, HbA_{1c} and statin treatment, which has been shown to potentially modify CETP activity, were introduced into the model in patients with diabetes. Multivariate analyses were done by stepwise multivariate linear regression.

The statistical analysis was done using SPSS software (Chicago, IL). Differences

with *P* values < 0.05 were considered statistically significant.

RESULTS

CETP Activity Correlates With ApoC1 Concentrations in Healthy Normolipidemic-Normoglycemic Control Subjects but Not in Patients With T1D and T2D

Table 1 presents the characteristics of the subjects divided into three groups: healthy normolipidemic-normoglycemic subjects, T1D patients, and T2D patients. Plasma TG were higher in T2D patients than in control subjects (2.34 ± 1.79 vs. 0.86 ± 0.24 mmol/L, $P < 0.001$). TG levels in T1D patients were significantly higher than those in control subjects (1.16 ± 0.77 vs. 0.86 ± 0.24 mmol/L, $P < 0.0001$), and significantly lower than in T2D patients (1.16 ± 0.77 vs. 2.34 ± 1.79 mmol/L, $P < 0.0001$).

Plasma apoC1 concentrations were significantly higher in T1D and T2D patients than in control subjects (100.87 ± 27.58 , 96.7 ± 28.28 , and 86.53 ± 18.01 mg/L, respectively; $P < 0.005$). Plasma apoC1 levels in T1D and T2D patients were similar.

CETP activity was significantly higher in T1D and T2D patients than in control subjects (0.32 ± 0.14 , 0.43 ± 0.13 , and 0.25 ± 0.075 nmol ^3H -CE/mg/h, respectively; $P < 0.0001$). In addition, CETP activity was higher in T2D patients than in T1D patients ($P < 0.0001$) (Table 1).

As shown in Fig. 1A and in Table 2, the plasma apoC1 concentration correlated negatively with CETP activity in normolipidemic-normoglycemic control subjects ($r = -0.309$, $P = 0.005$). In multivariate analysis, apoC1 concentration still correlated negatively and significantly with CETP activity ($\beta = -0.364$, $P = 0.001$). In addition, in this population, the TG level correlated positively and significantly with CETP activity ($\beta = 0.255$, $P = 0.018$).

In contrast to control subjects, no significant relationship between CETP activity and apoC1 concentration was observed in T1D and T2D patients (Fig. 1B–C).

In the T1D group, univariate analysis revealed that CETP activity correlated positively with HbA_{1c} ($P = 0.001$), LDL-C

Table 1—Characteristics of study population

	Control subjects	T1D patients	T2D patients
<i>n</i>	83	70	113
Age (years)	43.07 ± 14.22	42.1 ± 15.9	59.34 ± 9.48 ^{b,c}
Male sex, <i>n</i> (%)	47 (57)	39 (56)	61 (54)
Weight (kg)	68.3 ± 11.37	74.59 ± 16.49 ^a	96.67 ± 20.38 ^{b,c}
BMI (kg/m ²)	23.58 ± 3.67	25.66 ± 5.34 ^a	34.81 ± 7.01 ^{b,c}
Statin, <i>n</i> (%)	0 (0)	18 (25.7)	57 (50.4)
Duration of diabetes (years)		20 ± 12.5	12.7 ± 9.5
Retinopathy, <i>n</i> (%)		36 (51.4)	34 (30)
Nephropathy, <i>n</i> (%)			
Stage 0		53/68 (77.9)	51/109 (46.8)
Stage 1		10/68 (14.7)	41/109 (37.6)
Stage 2		5/68 (7.4)	17/109 (15.6)
Treatment of diabetes, <i>n</i> (%)			
OAD		2 (2.9)	97 (85.8)
Metformin		2 (2.9)	69 (61.1)
Sulfonylureas		0 (0)	69 (61.1)
α-Glucosidase inhibitors		0 (0)	2 (1.8)
DPP4 inhibitors		0 (0)	12 (10.6)
GLP-1 agonists		0 (0)	8 (7.1)
Insulin therapy		70 (100)	65 (53.7)
Long acting		0 (0)	37 (32.7)
Long acting plus short acting		61 (87.1)	28 (24.8)
Pump therapy		9 (12.9)	0 (0)
Insulin-OAD combination		2 (2.9)	51 (45.1)
Glycemia (mmol/L)	5.24 ± 0.44	9.69 ± 4.41 ^b	10.25 ± 4.2 ^b
HbA _{1c}			
%	ND	8.7 ± 1.76	8.77 ± 1.76
mmol/mol		72 ± 0.21	72 ± 0.21
Creatinine (μmol/L)	86.42 ± 13.63	78.3 ± 37.37	90.07 ± 29.28
eGFR (mL/min)	81.23 ± 13.4	101.09 ± 30.83	77.49 ± 24.92
>60	79	62	83
30–60	4	6	30
15–30	0	1	0
<15	0	0	0
Total cholesterol (mmol/L)	4.72 ± 0.89	4.94 ± 1.26	4.74 ± 1.18
LDL-C (mmol/L)	3.06 ± 0.71	2.84 ± 1.05	2.74 ± 0.94 ^a
HDL-C (mmol/L)	1.48 ± 0.41	1.62 ± 0.46	1.08 ± 0.25 ^{b,c}
TG (mmol/L)	0.86 ± 0.24	1.16 ± 0.77 ^a	2.34 ± 1.79 ^{b,c}
ApoC1 mass (mg/L)	86.53 ± 18.01	100.87 ± 27.58 ^b	96.7 ± 28.28 ^a
CETP mass (mg/L)	4.55 ± 0.94	3.71 ± 1 ^b	2.82 ± 0.76 ^{b,c}
CETP activity (nmol ³ H-CE/mg/h)	0.25 ± 0.075	0.32 ± 0.142 ^b	0.43 ± 0.135 ^{b,c}

Data are means ± SD, unless otherwise indicated. DPP4, dipeptidyl-peptidase 4; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide 1; OAD, oral antidiabetic drug. ^{a,b}Significantly different from control, $P < 0.01$, $P < 0.0001$, respectively. ^cSignificantly different from T1D, $P < 0.0001$.

($P = 0.02$), and TG ($P < 0.0001$) and negatively with HDL-C ($P < 0.0001$) and age ($P = 0.019$) (Table 2). In multivariate analysis, CETP activity was independently and positively associated with the TG level ($\beta = 0.411$, $P < 0.0001$) and HbA_{1c} ($\beta = 0.240$, $P = 0.022$) and negatively with statin therapy ($\beta = -0.295$, $P = 0.004$).

As plasma TG were higher in T1D patients than in control subjects and TG

is known to influence CETP activity (17,26), CETP activity in one subgroup of T1D patients with plasma TG <1.7 mmol/L was compared with CETP activity in control subjects. Whereas there was no significant difference between control subjects and T1D patients with TG <1.7 mmol/L in TG levels (0.86 ± 0.24 mmol/L, $n = 83$, vs. 0.89 ± 0.27, $n = 58$, respectively; ns), the ability of CETP to exchange

cholesteryl esters was again significantly higher in T1D patients with TG <1.7 mmol/L than in control subjects (0.29 ± 0.12 and 0.25 ± 0.07 nmol ³H-CE/mg/h, respectively; $P = 0.022$). In the subgroup of T1D patients with plasma TG <1.7 mmol/L, univariate analysis revealed that CETP activity correlated positively with HbA_{1c} ($P = 0.03$) and negatively with age ($P = 0.037$). In multivariate analysis, CETP activity was independently and positively associated with HbA_{1c} ($\beta = 0.274$, $P = 0.028$) and negatively with BMI ($\beta = 0.286$, $P = 0.021$) and statin therapy ($\beta = -0.250$, $P = 0.044$). In contrast, no relationship between CETP activity and both apoC1 ($\beta = 0.08$, $P = 0.516$) and TG ($\beta = 0.153$, $P = 0.245$) was found in this subgroup. Finally, in order to assess the influence of HbA_{1c} on CETP activity, we divided T1D patients into quartiles according to HbA_{1c}. CETP activity was significantly higher in the fourth quartile (0.42 ± nmol ³H-CE/mg/h, HbA_{1c} >9.6% [81 mmol/mol]) than in the first quartile (0.26 ± 0.07 nmol ³H-CE/mg/h, HbA_{1c} <7.4% [57 mmol/mol], $P < 0.0001$). No significant difference in TG levels was observed between the first and the fourth quartile. The ANOVA analysis for CETP activity between quartiles was strongly significant ($F = 6.913$, $P < 0.0001$). ApoC1 was significantly higher in the fourth quartile (107.6 mg/L) than in the first quartile (90.9 mg/L) ($P = 0.045$).

In T2D patients, CETP activity correlated positively with total cholesterol ($P = 0.004$), LDL-C ($P = 0.04$), and TG ($P < 0.0001$) and negatively with HDL-C ($P < 0.0001$) (Table 2). Multiple linear regression analyses showed in T2D that CETP activity was independently associated with LDL-C ($\beta = 0.204$, $P = 0.019$), TG ($\beta = 0.249$, $P = 0.015$), and HDL-C ($\beta = -0.345$, $P = 0.001$). Thus, we hypothesize that glycation of apoC1 might change its electrostatic properties and impair its ability to inhibit CETP activity.

Glycation of ApoC1 by MG Changes Its Electrostatic Charge and Impairs Its Ability to Inhibit CETP Activity

Glycation of apoC1 in the presence of MG led to the emergence of new acidic isoforms. A gradual shift of apparent pI values from 8.3 for native apoC1 down to 4.0 was observed when apoC1 was treated with the highest amount of MG.

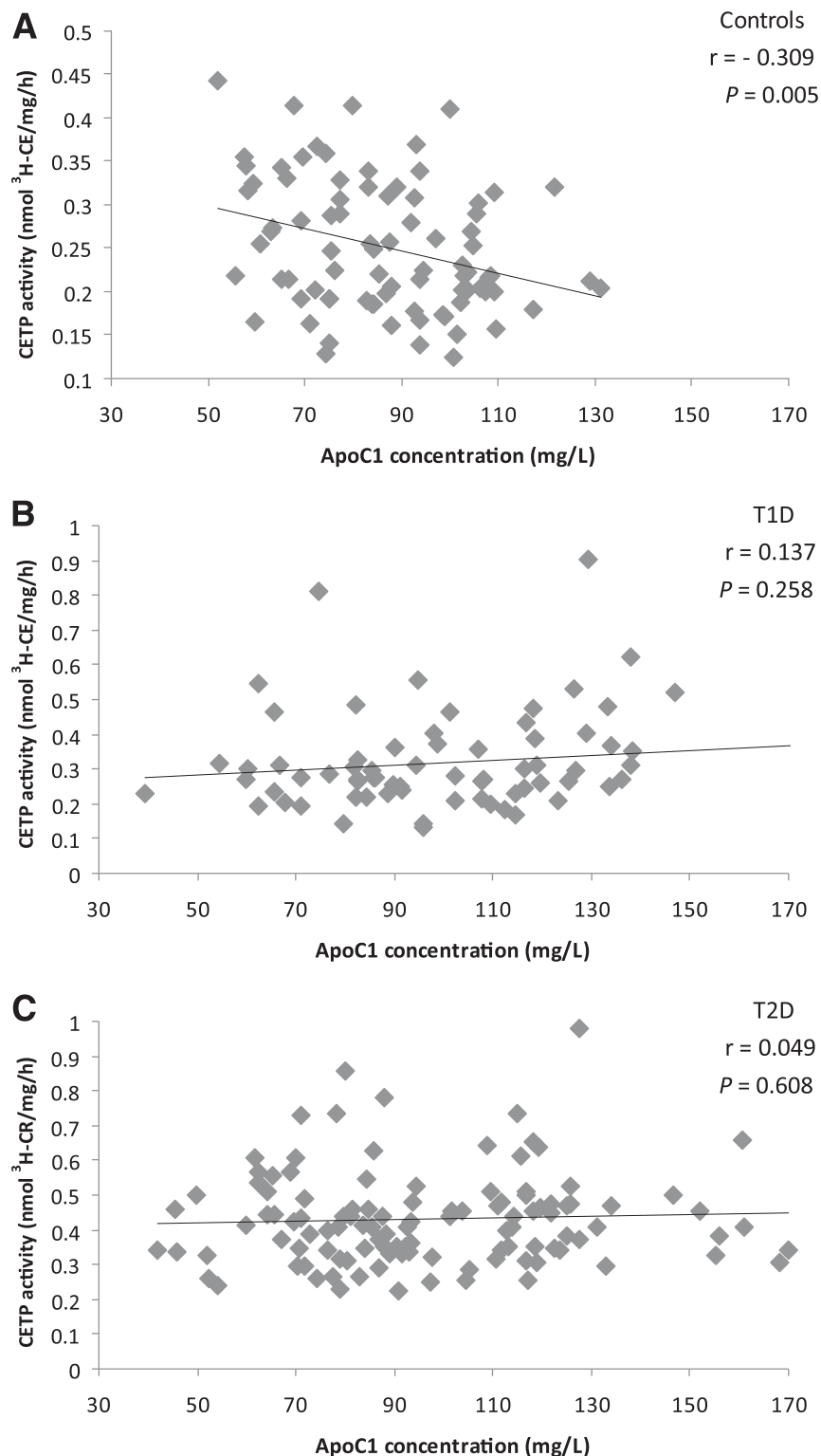


Figure 1—Relationship between apoC1 concentration and CETP activity in control subjects (A), T1D patients (B), and T2D patients (C). Correlation coefficients were calculated by Pearson test.

In accordance with previous studies, the addition of purified apoC1 to reconstituted mixtures containing isolated lipoprotein substrates and CETP

was accompanied by a marked inhibition of the lipid transfer reaction (Fig. 2). The inhibitory potential of human apoC1 was markedly affected by

pretreatment of apoC1 with MG. MG-induced glycation of CETP produced a significant decrease in CETP activity in a dose-dependent manner ($P = 0.004$).

Table 2—Correlations between CETP activity and age, BMI, glycemia, HbA_{1c}, total cholesterol, HDL-C, LDL-C, TG, and ApoC1 in control subjects and T1D and T2D patients

	CETP activity (nmol ³ H-CE/mg/h)	
	<i>r</i>	<i>P</i>
Control subjects (<i>n</i> = 83)		
Age	0.110	0.323
BMI	0.062	0.577
Total cholesterol	0.042	0.705
LDL-C	0.135	0.225
HDL-C	−0.163	0.141
TG	0.176	0.112
ApoC1	−0.309	0.005
T1D patients (<i>n</i> = 70)		
Age	−0.279	0.019
BMI	0.199	0.098
HbA _{1c}	0.392	0.001
Total cholesterol	0.192	0.114
LDL-C	0.279	0.02
HDL-C	−0.418	<0.0001
TG	0.460	<0.0001
ApoC1	0.137	0.258
T1D patients with TG <1.7 mmol/L (<i>n</i> = 58)		
Age	−0.275	0.037
BMI	0.259	0.05
HbA _{1c}	0.285	0.03
Total cholesterol	0.174	0.192
LDL-C	0.245	0.064
HDL-C	−0.181	0.174
TG	0.121	0.366
ApoC1	0.128	0.339
T2D patients (<i>n</i> = 113)		
Age	−0.072	0.446
BMI	−0.020	0.831
HbA _{1c}	0.143	0.134
Total cholesterol	0.271	0.004
LDL-C	0.194	0.04
HDL-C	−0.428	<0.0001
TG	0.386	<0.0001
ApoC1	0.049	0.608

Data are in boldface type when *P* < 0.05.

Glycation of HDLs by MG Made Them More Electronegative and Made Them Better Substrates for CETP

Because apoC1 is an inhibitor of CETP activity when associated with HDL, the electrostatic properties of HDL and the ability of HDL to inhibit CETP activity were assessed after glycation with MG. MG-induced glycation of HDL produced a significant change in the electrostatic properties of plasma HDL, which gradually became more electronegative as the MG concentration increased (data not shown). The ability of HDL treated with MG to block the lipid transfer reaction was significantly weaker than that achieved by native HDL (data not shown).

Importantly, *in vitro* glycation of apoC1 by MG was shown here to abrogate its CETP inhibitory effect and to change its electrostatic charge properties. Because glycation is a well-known consequence of hyperglycemia in diabetes, we set out to determine whether the electrostatic charge of apoC1 in patients with diabetes was different from that in healthy normolipidemic-normoglycemic subjects.

Electrostatic Charge of Plasma ApoC1 Is Abnormal in Patients With T1D

ApoC1 is known to have a pI value of 8.3. Accordingly, the pI of apoC1 in most of the healthy control subjects ranged between 7.4 and 9.5. In T1D patients,

the proportion of apoC1 located between 7.4 and 9.5 of the pI range was significantly lower than that in control subjects (18.6 vs. 43.2%, respectively; *P* = 0.0065), whereas the proportion between 3.5 and 4.2 of pI range was significantly higher (18.1 vs. 5.9%, respectively; *P* = 0.0067). These results indicate that the electrostatic properties of apoC1 are modified in T1D.

CONCLUSIONS

In the current study, the negative correlation between apoC1 and CETP activity found in control subjects is shown here for the first time to be absent in T1D and T2D patients. As glycation, a consequence of hyperglycemia in diabetes, is known to change isoelectrophoretic point of proteins, we hypothesized and demonstrated that *in vitro* glycation of apoC1 modified its electrostatic properties and impaired its ability to inhibit CETP activity and that the electrostatic properties of apoC1 are changed in patients with diabetes.

As previously reported (16,17), we found a significant negative correlation between apoC1 concentration and CETP activity in normolipidemic-normoglycemic control subjects. These data confirm that apoC1 is a potent endogenous inhibitor of CETP activity. One of our major results is that the inhibitory effect of apoC1 on CETP activity is abrogated in both T1D and T2D patients, as reflected by the loss of the negative correlation between specific CETP activity and apoC1 concentration in these populations. The major common abnormality in T1D and T2D is hyperglycemia. We hypothesized that hyperglycemia might be responsible for this loss of inhibitory potential. The independent association between CETP activity and HbA_{1c} observed in T1D patients is in keeping with this hypothesis. On top of that, CETP activity in the quartile of T1D patients with the highest HbA_{1c} level was significantly greater than that in the quartile of T1D patients with the lowest HbA_{1c} level. The higher the degree of hyperglycemia, the greater the loss of inhibitory potential of apoC1 seemed to be. We did not find a

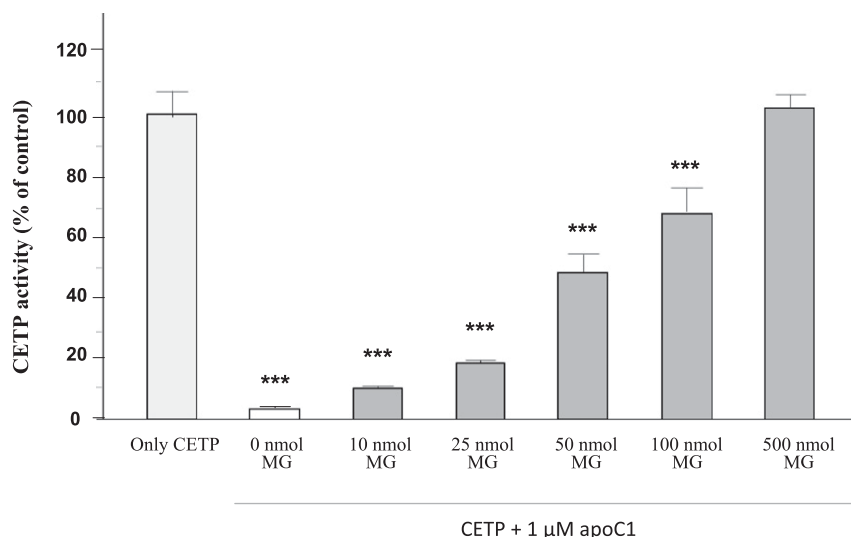


Figure 2—Effect of MG on isoelectrophoretic charge of apoC1 and on its ability to inhibit CETP. Effect of glycation of CETP with MG on CETP activity. Purified human CETP (5 μ L) was incubated with apoC1 (1 μ mol/L) treated with 0, 10, 25, 50, 100, and 500 nmol MG. CETP activity was expressed as compared with CETP activity measured without apoC1, which represents 100%. ***Significantly different from control, $P < 0.0001$.

significant correlation between HbA_{1c} and CETP activity in T2D patients, but it seems likely that hypertriglyceridemia, which is highly present in this population, has a major effect on CETP activity and, thus, the effect of hyperglycemia on CETP activity may have been overwhelmed by that of hypertriglyceridemia.

ApoC1 is a highly basic protein containing nine lysine residues and three arginine residues. It has been shown that the inhibitory effect of human apoC1 is a direct consequence of its unique electrostatic properties, which impair HDL-CETP interactions (15). Glycation, a consequence of hyperglycemia in diabetes, is known to affect reactive amino acids of proteins, essentially lysine and arginine, and to reduce the isoelectric point of proteins. For the first time, we investigated whether glycation could change the electrostatic properties of apoC1, and thus its ability to inhibit CETP activity, and we studied the effect of *in vitro* glycation of human apoC1 with MG (a protein glycosylating agent) on the electrostatic and inhibitory properties of apoC1. MG interacts with lysine and arginine residues to create Schiff bases and adducts that decrease the pI of the protein (19). As expected, the electrostatic properties of apoC1 were markedly changed by pretreatment with MG and in a concentration-dependent

manner. Importantly, the decreasing electropositivity of apoC1 with increasing amounts of MG was associated with a progressive loss of the CETP-inhibitory properties of apoC1. These findings are in accordance with earlier *in vitro* studies with acetylated apoC1, which linked the reduction in the positive electrostatic charge of apoC1 with its impaired inhibitory activity on CETP (15).

The electrostatic properties of apoC1 were shown *in vitro* to be able to produce a significant change in HDL electronegativity (15), which is recognized today as a leading factor that determines both the strength of CETP-HDL interactions and the velocity of CETP-mediated lipid transfers (27–29). The inhibition of CETP activity by apoC1 is dependent, at least in part, on its peculiar electrostatic properties, and human apoC1 was found in earlier studies to contribute significantly to the overall surface charge potential of HDL (15,16). In the current study, we showed that HDLs treated with MG were more electronegative than native HDLs. The glycation of positively charged amino acid residues of apoC1 may explain in part why glycated HDLs are more electronegative and therefore show a weaker ability to inhibit CETP activity.

We hypothesized that the electrostatic properties of apoC1 from patients with

diabetes might be different from those of apoC1 from normolipidemic-normoglycemic subjects. Isoelectric analysis, conducted *in vivo* in the current study, showed that the electrostatic properties of apoC1 from T1D patients were different (reduced electropositivity) from those in control subjects. Indeed, apoC1 from T1D patients was significantly less basic than control apoC1. Pretreatment of apoC1 with MG was found to modify its pI value in a way similar to what is shown in the current study to occur in T1D patients.

In the current study, CETP activity in both T1D and T2D patients was greater than that in control subjects as previously reported (30,31). CETP activity was significantly higher in T2D than in T1D patients. This difference may be partly explained by a significantly higher TG level in T2D, which directly stimulates CETP activity. The strong and independent association between CETP activity and TG in T2D patients stresses the fact that hypertriglyceridemia is a major determinant of CETP activity as previously reported (26). Although T1D patients have plasma TG levels in the normal range, plasma TG levels were higher than those in control subjects, and plasma TG correlates significantly with CETP activity. However, T1D patients with TG levels <1.7 mmol

showed significantly higher CETP activity than control subjects when their plasma TG levels were not different from those in control subjects. On top of that, in this population, no correlation was observed between CETP activity and TG. Thus, the greater ability of CETP to exchange cholesteryl esters between lipoproteins in T1D patients is probably due to mechanisms other than increased TG levels, such as hyperglycemia. Indeed, in T1D patients, hyperglycemia correlated with CETP activity in both univariate and multivariate analyses.

In this study, whereas CETP activity was shown to increase in patients with diabetes, CETP activity was significantly decreased by glycation of CETP in a dose-dependent manner. Thus, the contribution of direct CETP glycation to the observed changes is unlikely, and this reinforced the potential role of glycation of apoC1 and its implication in increasing CETP activity in diabetic patients.

In T1D patients, we would expect to find low plasma HDL-C, as CETP activity is increased, but this was not the case. A slight increase in the level of HDL-C has been observed in T1D patients (32). Some studies have shown an increase in HDL₂ (33,34) or in HDL₃ (35). This increase in plasma HDL-C could be the consequence of the elevated lipoprotein lipase (LPL)-to-hepatic lipase (HL) ratio (increased LPL activity and normal HL activity) (34). The increased LPL activity observed in these patients is probably due to peripheral hyperinsulinemia as a consequence of the subcutaneous route of insulin administration (34).

The plasma concentration of apoC1 was found to be slightly though significantly higher in T1D and T2D patients than in control subjects. High apoC1 concentrations were reported in dyslipidemic patients in one clinical study (36) but not in another one (17). The increase in apoC1 concentrations in patients with diabetes may be explained by increased hepatic production or decreased clearance of apoC1. Kinetic studies of apoC1 are needed to understand this rise in apoC1 concentration. Although the

mechanism underlying this increase remains unclear, it shows that increased CETP activity in diabetic patients occurs despite elevated apoC1 concentrations, stressing once again the fact that apoC1 is dysfunctional in these patients.

High CETP activity is frequently observed in subjects with metabolic disorders. These observations support the view that a reduction in CETP activity in this population might be antiatherogenic. However, despite a clear favorable impact on the lipid profile, with a large increase in HDL-C levels and a specific reduction in dense LDL particles (37), CETP inhibition therapy failed to demonstrate any significant efficacy to reduce the progression of atherosclerosis or the occurrence of cardiovascular events (38). This suggests that residual optimal CETP activity should be maintained in order to preserve the antiatherogenic properties associated with the physiological action of CETP (39).

As glycation is a consequence of hyperglycemia, we hypothesize that lowering hyperglycemia may solve, at least in part, the loss of constitutive CETP inhibition, thus maintaining optimal CETP activity in patients with diabetes.

We showed that *in vitro* glycation of apoC1 impairs its ability to inhibit CETP activity. We suggest that the glycation of apoC1, which modifies its electrostatic properties and thus the electrostatic properties of HDL particles, leads to increased binding of CETP to HDL and thus to increased CETP activity and cholesteryl ester transfer. We also showed *in vivo* that the electrostatic properties of apoC1 are modified in patients with T1D and that apoC1 from diabetic patients has lost its ability to inhibit CETP activity. Hyperglycemia plays a major role in the loss of the inhibitory potential of apoC1 on CETP activity.

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