Cholesteryl ester transfer protein expression is down-regulated in hyperinsulinemic transgenic mice

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Abstract Cholesteryl ester transfer protein (CETP) mediates cholesteryl ester (CE) and triglyceride redistribution among plasma lipoproteins. In this work, we investigated whether varying levels of insulin regulate the CETP expression in vivo. Insulin deficiency [streptozotocin (STZ) injection], and hyperinsulinemia (insulin injections, 14 days) were induced in transgenic mice expressing a human CETP minigene flanked by its natural regulatory sequences. Glucose supplementation was provided to the hyperinsulinemic group (INS+GLUC) and to an extra group of mice (GLUC). In the STZ group, endogenous CE transfer rate, plasma CETP, and hepatic CETP mRNA levels were enhanced 3.0-, 1.5-, and 2.5-fold, respectively, as compared with controls. Insulin replacement in STZ mice normalized their glycemia and liver mRNA levels. Higher plasma CETP levels were observed in GLUC mice, which were decreased in INS+GLUC mice. Hepatic CETP mRNA was not altered in GLUC mice and was reduced by one-third in INS+GLUC mice. These results show that: *1*) STZ treatment increases CETP plasma levels and liver mRNA expression; *2*) diet glucose supplementation increases plasma CETP levels but does not change liver mRNA abundance; and *3*) daily insulin injections blunt the glucose-stimulated CETP expression by reducing its liver mRNA levels. These data suggest that insulin down-regulates CETP gene expression.—Berti, J. A., A. C. Casquero, P. R. Patrício, E. J. B. Bighetti, E. M. Carneiro, A. C. Boschero, and H. C. F. Oliveira. **Cholesteryl ester transfer protein expression is down-regulated in hyperinsulinemic transgenic mice. *J. Lipid Res.* 2003.** 44: **1870–1876.**

Supplementary key words cholesteryl ester transfer protein gene expression • insulin • hyperglycemia • experimental diabetes • lipoprotein lipase • triglycerides • nonesterified fatty acids

Plasma cholesteryl ester transfer protein (CETP) facilitates the transfer of cholesteryl ester (CE) from HDL to the apolipoprotein B (apoB)-containing lipoproteins (LPs) (1). The significance of CETP activity for atherogenesis in human and animal studies has been debated. Plasma CETP may elicit opposing effects on the development of atherosclerosis, depending on the metabolic context [as reviewed in ref. (2)]. Blocking CETP has been considered as a potential antiatherosclerosis therapy for humans (3–5).

Plasma CETP levels can be influenced by diet composition and hormonal and inflammatory stimuli (6). The CETP gene expression is up-regulated by dietary and genetic hypercholesterolemia (7–9). Other metabolic factors related to the postprandial state may also be involved in the regulation of CETP expression. MacLean et al. (10) reported that plasma CETP and liver mRNA increased in fed compared with fasted human CETP transgenic (Tg) mice. In contrast, Jiang et al. (11) showed that heart and adipose tissue CETP mRNA levels in fasted hamsters fell after feeding. A fat load after an overnight fast did not change the plasma CETP levels in diabetic patients compared with healthy subjects (12).

Regardless of species differences, insulin is a plausible modulator of variations in CETP levels during acute changes in the prandial state. Several studies have suggested that insulin may regulate CETP expression. However, no consensus has yet been reached. A hyperinsulinemic euglycemic clamp stimulated plasma CETP activity in rabbits (13) but reduced it in some human studies (14–16). On the other hand, in studies in type 2 diabetic subjects, plasma CETP activity was increased, unaltered, diminished, or suppressed following insulin administration [as reviewed in ref.(17)]. Most of these discrepancies probably reflect differences in the glycemic control of the subjects and in the methods used to measure CETP. In these studies, CETP activity may be altered by secondary causes, such as modifications in the concentration and composition of plasma LPs (17). Alternatively, CETP expression could be directly regulated by insulin action.

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Abbreviations: CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FPLC, fast-protein liquid chromatography; GLUC, glucose; HL, hepatic lipase; INS, insulin; LP, lipoprotein; LPL, lipoprotein lipase; STZ, streptozotocin; Tg, transgenic; TG, triglyceride.

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The aim of the present study was to investigate whether varying levels of insulin modulate CETP expression. For this purpose, we used Tg mice that express a human CETP minigene (all 16 exons plus introns 1 and 13–15) flanked by its natural regulatory up- and downstream sequences (7). This mouse model has been very useful for studying the regulation of CETP gene expression (8, 13, 18–20).

MATERIALS AND METHODS

Animals and treatments

All experimental protocols were approved by the university's Committee for Ethics in Animal Experimentation and followed the "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985). The mice used in this study have been described elsewhere (7) and were derived from a colony maintained by Dr. A. R. Tall (Columbia University, New York, NY). The mice were housed in a temperature-controlled room on a 12 h light/dark cycle and had free access to food (rodent chow diet; Nuvital CR1, Colombo, Brazil) and water. Chow diet composition was (% by weight): 22% protein, 4% soybean oil, and 41.5% carbohydrates, in a total of 290 Kcal/100 g, and AIN93 mineral and vitamin mixes. Male and female heterozygous mice expressing a human CETP minigene flanked by its natural up- and downstream sequences (line 5203), 6–10 months old, were used. Hypoinsulinemic mice were obtained with an acute intraperitoneal injection of streptozotocin (STZ) (Calbiochem, Darmstadt, Germany), 160 mg/kg body weight (BW) in 0.05 M sodium citrate buffer, pH 4.5 (21). Control mice (CONT) received an injection of citrate buffer. Seven days after STZ injection, only mice with plasma glucose concentrations >200 mg/dl were used in the experiments. A subgroup of STZ mice received daily insulin injections (subcutaneous injections of 3–4 units of pork insulin; Monotard®MC, Novo Nordisk A/S, Bagsvaerd, Denmark) during the second week after the STZ injection (STZ+INS mice) in order to replace insulin and normalize glycemia levels. Anesthetized, overnight-fasted mice were killed by total exsanguination between 8:00 and 11:00 AM. In another set of experiments, CETP Tg mice were made hyperinsulinemic through daily subcutaneous injections of increasing doses of NPH insulin (0.14–1.63 U/30 g BW; IOLIN®, Eli Lilly, Indianapolis, IN) for 14 days. Two-thirds of the dose was given at 8 PM and one-third at 8 AM. To prevent hypoglycemia, these mice had free access to sugar cubes in addition to the chow diet, and a 5% glucose solution was the only drinking solution offered (22) (INS+GLUC mice). Control glucose-fed mice received identical daily subcutaneous injections of saline and also had free access to sugar cubes and 5% glucose solution (GLUC mice). Control intact mice received chow diet and plain water (CONT mice). Anesthetized nonfasting mice were killed by total exsanguination between 8:00 and 11:00 AM.

Biochemical analysis

Mouse plasma was isolated from blood obtained from the retroorbital plexus in heparinized hematocrit tubes. Total cholesterol, triglycerides (TGs) and NEFAs were determined using enzymatic colorimetric assays (Wako Chemicals, Neuss, Germany). Ten microliters of whole blood precipitated with 5% trichloroacetic acid was used for glucose analysis by the glucose oxidase method (Merck Diagnostic, Chennevières-les-Louvres, France). Plasma insulin levels were measured by radioimmunoassay (23) using rat insulin as the standard.

Fast protein liquid chromatography

Plasma pools (200 μl) from treated and control CETP Tg mice were fractionated by fast-protein liquid chromatography (FPLC) using an HR10/30 Superose 6 column (Amersham-Pharmacia Biotech, Uppsala, Sweden), equilibrated with Tris-buffered saline, pH 7.2, as previously described (24). Total cholesterol was determined enzymatically in each FPLC fraction.

CETP levels and transfer rate (exogenous substrate assay)

A mixture of human VLDL and LDL (100 μg protein) was incubated with 10,000 dpm of human HDL_3 labeled with cholesteryl [1-^{14}C]oleate (Amersham Life Sciences, Buckinghamshire, England) (25) and 5 μl of mouse plasma as source of CETP in a final volume of 100 μl. Blanks were prepared with Tris-saline-EDTA buffer (10 mM Tris, 140 mM NaCl, 1 mM EDTA, pH 7.4), and negative controls with plasma from non-Tg mice. The mixtures were incubated for 1 h at 40°C. After incubation, 400 μl of TSE was added and the apo B-containing LPs were precipitated with 50 μl of 1.6% dextran sulfate-1 M $MgCl_2$ solution (1:1; v/v). The tubes were kept at room temperature for 10 min and then centrifuged (10,000 rpm) at 4°C. Radioactivity was measured in the supernatant using Triton scintillation solution (SARDI, São Paulo, Brazil) in an LS6000 Beckman Beta Counter. The percent CE transferred from [^{14}C]HDL to VLDL+LDL was calculated as: [(dpm in blank tube − dpm in sample tube)/dpm in blank tube] × 100.

Endogenous CE transfer rate

One hundred thirty microliters of fresh mouse plasma was incubated with trace amounts of human HDL_3 labeled with [1α, 1α(n) ^{3}H]CE (Amersham Life Sciences) (10^5 dpm, 0.5 μg HDL-cholesterol/tube). After 1 h incubation at 40°C, the tubes were transferred to an ice bath, human LDL (100 μg) was added as carrier, and the apoB-LPs were precipitated with dextran sulfate-$MgCl_2$ solution. The [^{3}H]CE transfer was determined as described above.

Lipoprotein lipase activities

Total lipase activity was determined according to Ehnholm and Kuusi (26). Briefly, plasma was obtained 10 min after a subcutaneous injection of heparin (100 IU/kg BW) and incubated with [^{3}H]triolein/arabic-gum substrate (glycerol tri [9,10(n)-^{3}H]oleate, Amersham Life Sciences) in 0.2 M Tris-HCl buffer, pH 8.5, at 37°C for 1 h. Hepatic lipase (HL) activity was determined in tubes where the lipoprotein lipase (LPL) was inhibited by 2 M NaCl. The hydrolyzed labeled free fatty acids were extracted with methanol-chloroform-heptane (1.4:1.25:1; v/v/v), 0.14 M K_2CO_3-H_3CO_3, pH 10.5, dried under a stream of N_2, and their radioactivity determined in a liquid scintillation solution in an LS6000 Beckman Beta Counter. Samples were assayed in triplicate. Rat and human pre- and postheparin plasma were used as interassay controls. The LPL activity was calculated as the difference between the total lipase and the HL activities.

RT-PCR for liver CETP mRNA

Total liver RNA was extracted from ∼200 mg of tissue using Trizol reagent (Invitrogen, Grand Island, NY). The integrity of the RNA was checked in Tris-borate 1.2% agarose gels stained with ethidium bromide. The amount and purity of the RNA were determined by optical density readings at 260 and 280 nm (Gene Quant, Amersham-Pharmacia Biotech). Genomic DNA contamination was excluded by running a PCR on the RNA samples. cDNA was obtained from 1 μg of total RNA by reverse transcription using 150 ng of random primers, 10 mM of deoxy ribonucleotides triphosphate (dNTPs), and 200 U of Moloney murine leukemia virus reverse transcriptase (Superscript II; Invitrogen) in a final volume of 20 μl. The tubes were incubated for 60 min at 42°C fol-

lowed by 15 min at 70°C to inactivate the enzyme. For PCR, a pair of primers was designed to amplify the region spanning part of exon 7 and exons 8 to 14 of the human CETP cDNA, which generated a 668 bp fragment. PCR mixtures consisted of 1 μl of cDNA, 15 pmol of primers, 2.5 U Taq polymerase, and 200 μM of dNTPs in a final volume of 25 μl. An initial denaturation at 94°C for 4 min was followed by 28 cycles of 1 min at 94°C, 1.5 min at 58°C, and 2 min at 72°C in a Gene Amp PCR System 9700 (Perkin-Elmer, Norwalk, CT). In parallel tubes, the same cDNA was amplified with primers (10 pmol) for rat β-actin cDNA, which generated a 533 bp fragment as an internal standard for the samples. The PCR conditions for β-actin were 2 min at 94°C followed by 23 cycles of 30 s at 94°C, 30 s at 57°C, and 45 s at 72°C. The PCR products were separated by electrophoresis on 1.8% agarose gels, and the DNA was visualized by ethidium bromide staining. The band intensities were determined by digital scanning and quantitation using Scion Image analysis software (Scion Corp., Frederick, MD).

Statistical Analysis

Two-group comparisons were performed using Student's *t*-test for absolute values or Mann-Whitney U test for relative values. For three-group comparisons, one-way analysis of variance was employed for absolute values or Kruskall-Wallis test for relative values using the software Graph Pad Instat®, version 3.00.

RESULTS

CETP Tg mice made hypoinsulinemic with an acute dose of STZ showed increased food and water consumption and reduced body and perigonadal adipose tissue weights and an increase in the relative weight of the liver (**Table 1**). Changes in tissue weights probably represented alterations in fat fuel fluxes, i.e., increased adipose tissue lipolysis and increased liver uptake of free fatty acids as a consequence of low plasma levels of insulin. After seven days of STZ treatment, the blood glucose levels were approximately 3-fold higher and plasma TGs and NEFAs were significantly increased, but total cholesterol levels decreased (**Table 2**). As expected, peripheral LPL was significantly reduced in hypoinsulinemic STZ mice, whereas HL was unchanged by this treatment (Table 2).

Plasma CETP level was measured using an exogenous acceptor and donor LP assay that indicates the amount of CETP in the plasma sample (27). The CETP-mediated endogenous transfer rate was determined by an endogenous substrate assay (whole-mouse plasma) that reflects both the amount of CETP and the substrate-dependent effects of LP concentration. As shown in Table 2, insulin-deficient hyperglycemic mice (STZ) showed a 1.5-fold increase in plasma CETP levels and a 3-fold increase in endogenous transfer rate. Thus, the latter can be explained by an elevation in the amount of CETP plus acceptor particles (VLDL). Plasma LP analysis (**Fig. 1A**) revealed a 3-fold increase in VLDL-cholesterol and a 38% reduction in HDL-cholesterol in STZ-treated mice compared with control mice. Because CETP activity is inversely related to the

TABLE 1. Body and tissue weights and food and water ingestion in control and streptozotocin-treated cholesteryl ester transfer protein transgenic mice

	CONT	STZ-Treated
BW (g)	23.2 ± 0.3	21.0 ± 0.7[a]
Perigonadal adipose tissue (% BW)	1.0 ± 0.14	0.07 ± 0.05[a]
Liver (% BW)	4.0 ± 0.1	5.0 ± 0.1[a]
Food (g/mouse/day)	3.8 ± 0.45	5.4 ± 0.05[a]
Water (ml/mouse/day)	5.2 ± 1.1	18 ± 0.04[a]

BW, body weight; CONT, control; STZ, streptozotocin. Mean ± SE, n = 7–10.
[a] $P < 0.05$ versus CONT.

TABLE 2. Fasting plasma levels of glucose, triglycerides, nonesterified fatty acids, cholesterol, insulin, lipoprotein lipase, hepatic lipase, and CETP in control and STZ-treated CETP transgenic mice

	CONT	STZ-Treated
Glucose (mg/dl)	92 ± 5	298 ± 14[a]
TG (mg/dl)	56 ± 6	78 ± 8[b]
NEFA (mmol/l)	0.88 ± 0.06	1.36 ± 0.14[b]
Cholesterol (mg/dl)	56 ± 2	44 ± 3[b]
Insulin (ng/ml)	0.44 ± 0.05	0.06 ± 0.01[a]
LPL (mmol/ml/h)	1,104 ± 239	254 ± 142[c]
HL (mmol/ml/h)	3,777 ± 201	4,286 ± 240
CETP levels (%, exogenous assay)	22 ± 1	33 ± 2.3[b]
CETP transfer rate (%, endogenous assay)	4.5 ± 1.3	15 ± 3[b]

CEPT, cholesteryl ester transfer protein; HL, hepatic lipase; LPL, lipoprotein lipase; TG, triglyceride. Mean ± SE, n = 7–10.
[a] $P < 0.0001$.
[b] $P < 0.05$.
[c] $P < 0.003$.

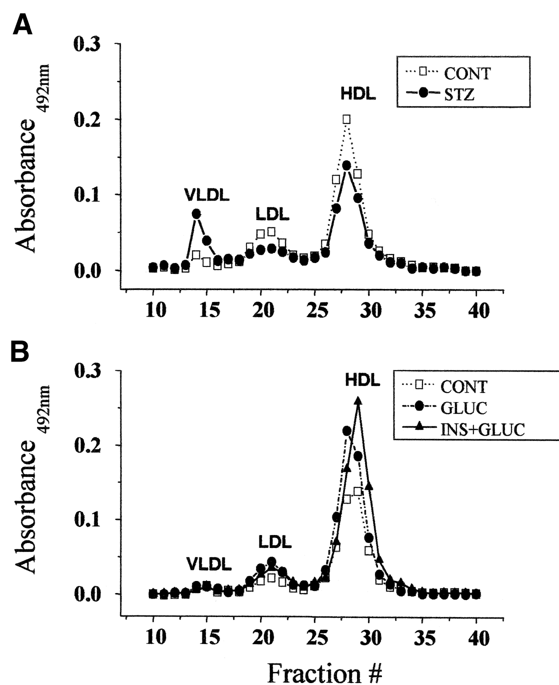

Fig. 1. Cholesterol distribution in plasma lipoproteins (LPs). A: Control (CONT) and streptozotocin-treated (STZ) cholesteryl ester transfer protein (CETP) transgenic (Tg) mice. B: CONT, glucose-fed (GLUC), and hyperinsulinemic (INS+GLUC) CETP Tg mice. Each point represents the mean of two plasma pools from two mice. VLDL-, LDL- and HDL-cholesterol concentrations (mg/dl) calculated as the area under each LP peak were, respectively, 3, 13, and 40 for CONT mice; 10, 9, and 25 for STZ mice in panel A; and 2, 7, and 41 for the CONT group; 2, 12, and 51 for the GLUC group; and 2, 10, and 56 for the INS+GLUC group in panel B.

concentration of HDL-cholesterol, the higher CETP transfer rate could explain the observed decrease in this LP fraction, and hence the lower total cholesterol levels in STZ-treated CETP Tg mice.

Hyperglycemic-hypoinsulinemic STZ mice exhibited a marked (2.5-fold) elevation in liver CETP mRNA abundance compared with control mice (**Fig. 2A**). Replacement of insulin in STZ mice reduced their glycemia (108 ± 32 mg/dl) and brought the mRNA back to the control levels, reinforcing the role of insulin instead of some other effect of STZ (Fig. 2A).

In another set of experiments, CETP Tg mice treated with increasing doses of insulin for 14 days and with a glucose-supplemented diet (INS+GLUC) were compared with glucose-fed (GLUC) and chow-fed (CONT) control mice. There were no changes in body weight among the three groups at the end of the experimental period. However, INS+GLUC mice had higher perigonadal adipose tissue and lower liver relative weight compared with control mice (**Table 3**).

Nonfasting glucose levels were mildly but significantly elevated in GLUC and INS+GLUC mice compared with control mice. However, these glycemia levels were still within the normal range for fed mice (**Table 4**). Cholesterol levels were higher, whereas TG and NEFA levels were lower in GLUC and INS+GLUC groups than in the control group (Table 4). Postheparin plasma LPL activity did not differ significantly among the three groups of mice, whereas HL activity was significantly reduced in the hyperinsulinemic INS+GLUC mice.

Feeding glucose induced a 60% increase in the plasma levels of CETP in GLUC mice, while an excess of exogenous insulin (INS+GLUC) blunted this glucose-induced effect (Table 4). On the other hand, endogenous CE transfer rate was similar in CONT and GLUC groups and 40% lower in INS+GLUC mice (Table 4). Both GLUC and INS+GLUC mice had lower TG plasma concentrations than did control mice. This would be expected to diminish the efficiency of the CETP reaction, as observed in the INS+GLUC group. Despite the lower TG levels in the GLUC group, there was an increase in the plasma levels of CETP, which resulted in endogenous CE transfer similar to that in the control mice.

FPLC fractionation of plasma (Fig. 1B) showed that VLDL-cholesterol levels were similar among the three groups. LDL-cholesterol tended to increase in both GLUC and INS+GLUC mice compared with the controls. HDL-cholesterol was increased in GLUC and INS+GLUC mice by 24% and 37%, respectively. The high levels of HDL-cholesterol in INS+GLUC mice may have resulted from the decreased endogenous CE transfer and reduced HL activity in these mice. In GLUC mice, CE transfer rate and HL activity were unaltered. This situation had less impact on the level of HDL-cholesterol.

Liver CETP mRNA abundance was similar in control and glucose-fed animals, but was markedly reduced to one-third of control levels in hyperinsulinemic INS+GLUC mice (Fig. 2B).

TABLE 3. Body and tissue weights, and food, glucose, and drink solution consumption (g/mouse/day) in CONT, GLUC, and INS+GLUC CETP transgenic mice

	CONT	GLUC	INS+GLUC
BW (g)	28.8 ± 0.7	28.1 ± 0.7	29.7 ± 0.8
Perigonadal adipose tissue (% BW)	1.3 ± 0.1	1.5 ± 0.2	2.0 ± 0.2[a]
Liver (% BW)	4.3 ± 0.3	3.9 ± 0.5	3.7 ± 0.2[b]
Food (g/mouse/day)	4.0 ± 0.09	1.9 ± 0.03	1.8 ± 0.1
Sugar cubes (g/mouse/day)	—	1.5 ± 0.05	1.6 ± 0.2
5% Glucose (ml/mouse/day)	—	8.6 ± 0.1	7.9 ± 0.1
Water (ml/mouse/day)	4.9 ± 0.2	—	—

Mean ± SE (n = 8–10).
[a] $P = 0.055$.
[b] $P < 0.05$ versus CONT.

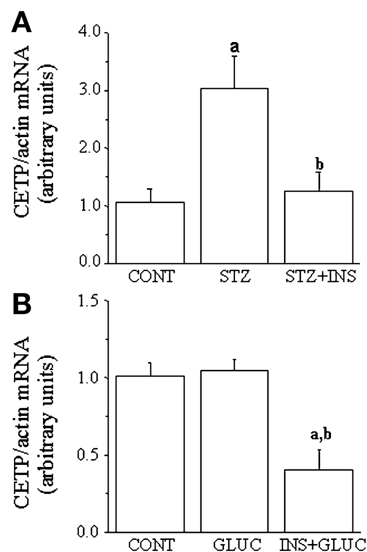

Fig. 2. Hepatic CETP mRNA corrected by β-actin mRNA. A: CONT, STZ, and STZ+INS mice; n = 8 for the STZ and CONT groups, and n = 4 for STZ+INS. [a] $P = 0.02$ versus CONT; [b] $P = 0.02$ versus STZ. B: CONT, GLUC, and INS+GLUC mice; n = 5–8; [a] $P < 0.05$ versus CONT; [b] $P < 0.05$ versus GLUC. Error bars indicate SE.

TABLE 4. Nonfasting plasma levels of glucose, TGs, NEFAs, cholesterol, LPL, HL, and CETP in CONT, GLUC, and INS+GLUC CETP transgenic mice

	CONT	GLUC	INS+GLUC
Glucose (mg/dl)	122 ± 4	147 ± 6[a]	147 ± 4[a]
TGs (mg/dl)	84 ± 13	50 ± 6[b]	47 ± 2[b]
NEFA (mmol/l)	0.8 ± 0.07	0.5 ± 0.06[a]	0.6 ± 0.04[b]
Cholesterol (mg/dl)	50 ± 2	65 ± 6[b]	68 ± 1[b]
LPL (mmol/ml/h)	2,585 ± 412	2,543 ± 585	1,848 ± 410
HL (mmol/ml/h)	5,952 ± 306	5,405 ± 226	4,369 ± 116[a,c]
CETP levels (%, exogenous assay)	22 ± 3	37 ± 3[b]	21 ± 2[d]
CETP transfer rate (%, endogenous assay)	6.8 ± 1.1	7.5 ± 1.3	4.4 ± 0.9[e]

Mean ± SE, n = 8–10.
[a] $P < 0.01$ versus CONT.
[b] $P < 0.05$ versus CONT.
[c] $P < 0.05$ versus GLUC.
[d] $P < 0.01$ versus GLUC.
[e] $P < 0.10$ versus GLUC.

DISCUSSION

In this work, we investigated the effect of experimental procedures that induce large variations in insulin concentrations in vivo on the CETP expression. Liver CETP mRNA abundance was shown to be elevated in insulin-deficient STZ-treated mice, not altered in insulin-replaced STZ mice and in glucose-fed mice, and reduced in hyperinsulinemic glucose-fed mice. In agreement with our results, hypoinsulinemic fructose-fed hamsters showed higher levels of plasma CETP and adipose tissue mRNA (28). However, the higher plasma cholesterol level in these animals (not seen in our STZ mice) was a confounding effect, inasmuch as elevated plasma cholesterol is known to up-regulate CETP expression. This well-known stimulatory effect of plasma cholesterol was overcome by high insulin levels in INS+GLUC mice, which exhibited a moderate increase of 36% in plasma cholesterol levels and marked reduction in liver CETP mRNA levels. Another study in hamsters (29) reported reduced levels of CE transfer activity in the plasma and adipose tissue of fed (high-insulin) compared with 24 h-fasted (low-insulin) animals. Our results also agree with those of Jiang et al. (11), who reported reduced CETP mRNA levels after feeding a high-carbohydrate meal to 12 h-fasted hamsters. However, the effects of prandial variations on the expression of CETP are complex, because a 44 h fast also reduced CETP mRNA compared with a 12 h fast period (11). In contrast to our results and the above studies, MacLean et al. (10) described higher levels of plasma CETP and liver mRNA in fed than in 12 h-fasted CETP Tg mice. The reason for this discrepancy is unknown, but the chronic conditions reported here differ in several aspects from the acute postprandial variations in nutrients and hormones.

In insulin-deficient STZ mice, the increase in the plasma levels of CETP paralleled the increase in liver mRNA. However, this was not the case for GLUC mice. Considering that the liver is the main site of CETP expression in this model (7) and in most mammals (30, 31), increased plasma levels of CETP in GLUC mice could have been the result of glucose-induced posttranscriptional mechanisms in the liver, and possibly in other GLUT-4-dependent tissues, such as muscle and adipose tissue. Higher translation efficiency and secretion of intracellular pools of CETP are putative mechanisms. Alternatively, extrahepatic CETP-expressing tissue could be differentially regulated and could contribute to plasma CETP levels in GLUC mice. In INS+GLUC mice, these same glucose-induced mechanisms, combined with the partial repression of the liver CETP mRNA, would lead to no change in plasma CETP levels, as observed when comparing INS+GLUC and CONT mice.

The endogenous plasma CETP-mediated CE transfer rate depends on the amount of CETP and its substrates, e.g., TG-rich LP, and also on the CETP affinity for the substrates. Elevated NEFA levels are reported to increase the CETP reaction rate (25, 32). Thus, higher CETP, TG, and NEFA levels in STZ mice led to a 3-fold elevation in endogenous CE transfer rate. On the other hand, increased CETP levels but decreased TG and NEFA in GLUC mice resulted in no change in endogenous CE transfer rate. In hyperinsulinemic hyperglycemic mice, endogenous CE transfer was lower, probably because TG and NEFA were reduced while CETP levels were unchanged.

Insulin represses the expression of several genes that regulate lipid metabolism. The opposite variations in the plasma TG and NEFA levels observed in the STZ and INS+GLUC groups can be explained by the stimulation (under low insulin) and repression (under high insulin) of four proteins simultaneously, namely, hormone-sensitive lipase (33), microsomal TG transfer protein (34), apoB (35) and apoC-III (36). STZ-induced insulin deficiency may have increased the hepatic expression of LDL receptors, as previously reported for hypercholesterolemic rats (37). This could partly explain the decrease in total cholesterol levels in STZ-treated mice and the moderate increase in LDL-cholesterol levels in hyperinsulinemic mice. Under the present experimental conditions, the changes in HDL-cholesterol could be explained by variations in plasma CETP transfer rates in hypoinsulinemic STZ mice and by CETP plus HL activity in the hyperinsulinemic INS+GLUC mice.

Foretz et al. (38) have recently shown that the sterol response element binding protein 1c (SREBP-1c) is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. SREBP-1c is transcriptionally stimulated by insulin and repressed by glucagon (39). Interestingly, inhibitory effects of insulin on genes encoding gluconeogenic enzymes such as PEPCK can also be mediated by SREBP-1c (40). Overexpression of SREBP-1c in the liver of STZ diabetic mice resulted in increases in glucokinase and lipogenic enzymes and a dramatic decrease in PEPCK expression (41). SREBP-1c has a dual DNA binding specificity, the sterol regulatory element-1 (SRE) or palindromic sequences termed E-boxes (42, 43). The CETP promoter region contains both SRE (44) and E box sequences. Furthermore, a search for the known negative insulin response element consensus sequence T(G/A)TTT(TG)/(GT) (45) in the CETP 5′ natural flanking sequences (−3.4 Kb) revealed 4 sequences identical to this core motif at positions −338, −1,786, −2,258, and −3,311. These findings support the hypothesis that insulin negatively regulates the CETP gene but do not exclude a role for the counter-regulatory hormones. Considering the complex in vivo counter-regulatory responses that occur following depletion of β cell function or glucose-insulin treatments, it is difficult to discern what metabolite or hormone is mediating the regulation of CETP expression and whether insulin effects are direct or indirect in the present experimental conditions.

In conclusion, the present results suggest that in the STZ-induced insulin deficiency state, there is a stimulation of CETP expression that involves a rise in liver CETP mRNA abundance; that diet glucose supplementation up-regulates CETP expression by mechanisms independent of changes in liver mRNA abundance; and that the hyperinsulinemic state blunts the glucose-induced CETP expression by reducing liver CETP mRNA abundance. These

findings may be relevant for understanding the complex metabolic lipid disturbances that occur in diabetes and insulin-resistance syndromes.

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