Adipose tissue-specific CETP expression in mice: impact on plasma lipoprotein metabolism

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Abstract **Adipose tissue appears to be a highly conserved site of cholesteryl ester transfer protein (CETP) expression across species. To investigate the impact of adipose CETP expression on lipid metabolism, we created adipose tissue-specific CETP transgenic (CETPTg) mice. CETP mRNA is predominantly expressed in adipose tissue. Plasma CETP mass and activity are readily detectable in CETPTg mice but not in controls. Plasma lipoprotein analysis shows marked reductions in HDL cholesterol and phospholipids, increases non-HDL lipids, decreases apolipoprotein A-I (apoA-I), and increases apoB. Unexpectedly, CETPTg adipocytes are significantly smaller than those in control mice (44%), triglyceride and cholesterol in adipose tissue were significantly decreased compared with controls (50% and 37%, respectively), and phospholipids showed no significant changes. To study the mechanism, we measured peroxisome proliferator-activated receptor γ, sterol-regulatory element binding protein-1c, LPL, and hormone-sensitive lipase (HSL) in aP2-CETPTg adipose tissue and controls and found that, except for HSL, all mRNA levels are significantly decreased in the transgenic mice compared with controls (26, 33, and 22%). In conclusion, adipose tissue CETP makes a major contribution to CETP in the circulation, reduces HDL, and increases non-HDL cholesterol levels. Moreover, adipose tissue CETP expression changes triglyceride and cholesterol content and the size of adipocytes.**—Zhou, H., Z. Li, M. R. Hojjati, D. Jang, T. P. Beyer, G. Cao, A. R. Tall, and X. C. Jiang. **Adipose tissue-specific CETP expression in mice: impact on plasma lipoprotein metabolism.** ***J. Lipid Res.*** **2006.** 47: **2011–2019.**

Supplementary key words cholesteryl ester transfer protein • adipocyte • transgenic mice

Most prospective epidemiological studies have found an inverse correlation between levels of HDL cholesterol (HDL-C) and the incidence of atherosclerosis (1–3). Cholesteryl ester transfer protein (CETP) is a hydrophobic plasma glycoprotein that mediates the transfer and exchange of cholesteryl esters (CEs) and triglycerides between plasma lipoproteins and plays an important role in HDL metabolism (4). Humans with genetic CETP deficiency have increased HDL-C levels (5). Mice do not express CETP, and human CETP transgenic (CETPTg) mice with predominant liver expression show reduced HDL-C levels (6, 7). In humans, CETP mRNA is expressed predominantly in adipose tissue, liver, and spleen, with lower levels of expression in the small intestine, adrenal gland, kidney, skeletal muscle, and heart (8, 9). In monkeys, a positive correlation has been observed between plasma CETP levels and the abundance of hepatic CETP mRNA, and the liver was shown to be one of the predominant sources of this CETP (10). However, the liver is not the major source of CETP in all mammalian species. In hamsters, for example, adipose tissue, muscle, and small intestine show the highest levels of CETP expression, and CETP mRNA is almost undetectable in the liver (8). Adipose tissue appears to be a highly conserved site of CETP expression across species. However, its function in adipose tissue is still unknown. It has been reported that plasma CETP concentrations are positively correlated with adipose tissue CETP mRNA levels in hamsters (11). In monkeys, a correlation between adipose CETP mRNA and CETP levels was also observed (10). Moreover, human adipose tissue maintained in organ culture synthesizes and secretes CETP (12). Other studies have shown that plasma CETP activity in humans correlates with the degree of adiposity (13, 14) and that weight reduction is associated with a decrease in plasma CETP activity (13). A hamster study also indicated that adipose tissue releases CETP activity during incubation in vitro and is subject to hormonal and nutritional regulation (15). In addition to studies of physiological regulation, the release of CETP activity from cultured hamster adipose tissue increased after a period of fasting (16).

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Despite these correlative findings, there is no direct evidence that adipose-derived CETP enters the circulation or that it influences plasma lipoprotein levels. To investigate these issues, we established adipose tissue-specific CETPTg mouse lines.

MATERIALS AND METHODS

aP2-CETPTg mouse preparation

An 8.2 kb synthetic CETP structural gene was assembled by combining genomic and cDNA fragments. The 5′ genomic region (including a 135 bp flanking sequence, exon 1, intron 1, and part of exon 2; *Bam*HI-*Eco*RV genomic fragment) and the 3′ genomic region (including part of exon 12, exons 13–16, introns 12–15, and 2.0 kb of 3′ flanking sequence; *Eco*RV-*Eco*RI genomic fragment) were linked together through a fragment taken from human CETP cDNA (*Eco*RV-*Eco*RV fragment). This fusion resulted in the complete removal of introns 2–11 and the generation of one synthetic exon. This fragment was then ligated downstream of the 5.4 kb mouse aP2 enhancer/promoter region (17) in the pBluescript SK vector. To generate transgenic mice, the CETP transgene was microinjected into the male pronuclei of fertilized mouse eggs taken from superovulated (C57BL/6J×CBA/J) F1 females. Injected embryos were implanted into the oviducts of surrogate females of the same genetic background.

Animals and diet used in this study

All phenotypic characterizations were performed with wild-type (WT) and CETPTg littermates of the F2 generation, 10–12 weeks old (~25 g). One diet was used: Purina Rodent Chow (No. 5001).

Northern blot analysis

Total RNA was isolated from livers with Trizol (Invitrogen). For CETP mRNA Northern blotting, total RNA (20 μg) from different tissues was analyzed, using a human CETP 444 bp cDNA (nucleotides 726–1170) as a probe. The procedure was as described previously (8).

Real-time PCR analysis

CETP, peroxisome proliferator-activated receptor γ (PPARγ), sterol-regulatory element binding protein-1c (SREBP-1c), LPL, and hormone-sensitive lipase (HSL) mRNA levels were quantitated by real-time PCR on the ABI Prism 7000HT Sequence Detection System (Applied Biosystems). The following primers and probe sets were used: CETP forward primer, cctggtgttgaaccacgaga; reverse primer, ctggatgttgacttgacttgg; probe, cagatatcacgggcgagaaggcc. PPARγ forward primer, ggcccgagaagacctcctt; reverse primer, tcaatggtgcctctggagat; probe, ccaggctttgggcatcaccacg. SREBP1c forward primer, gcacagcaaccagaagctca; reverse primer, tttcatgccccatctgtgcc; probe, gacctggtgtcagcttgtggca. LPL forward primer, tccagccaggatgcaaca; reverse primer, ccacgtctccgagtcctctct; probe, tggagaagccatccgtgtgattgc. HSL forward primer, ggagcactacaaacgcaacga; reverse primer, tcggccaccggtaaagag; probe, caggcctcagtgtgaccgccagt.

CETP activity and mass determination

CETP activity was measured by a fluorescence method (Roar Biomedical, Inc., New York, NY) that is comparable to the radiolabeled method (18). A human CETP ELISA kit (Wako Diagnostics, Richmond, VA) was used to measure CETP protein concentration in mouse plasma. This method is comparable to our RIA (19). The plasma samples were serially diluted with PBS, and the assay was performed as recommended by the manufacturer.

In vivo turnover studies

HDL was isolated by ultracentrifugation ($1.063 < d < 1.21$ g/ml). For double labeling, HDL protein was initially labeled by covalent attachment of the intracellularly trapped ^{125}I-*N*-methyl tyramine cellobiose (^{125}I-NMTC) ligand. (20) Thereafter, ^{125}I-NMTC-HDL was labeled with [^{3}H]cholesteryl oleyl ether ([^{3}H]CEt; Amersham) to trace the CE moiety (21). [^{3}H]CEt was introduced in a liposome preparation and exchanged (6 h, 37°C) into ^{125}I-NMTC-HDL using highly purified recombinant human plasma CETP. The donor liposomes were separated from labeled HDL by ultracentrifugation at $d = 1.063$ g/ml, and then HDL was reisolated by another spin at $d = 1.21$ g/ml to remove CETP. Thereafter, the HDL preparation was exhaustively dialyzed against PBS (pH 7.4) containing EDTA (0.3 mmol/l) and sterile-filtered (0.45 μm). WT and aP2-CETPTg mice were injected intravenously with their own HDL that was labeled with [^{3}H]CEt (2×10^{6} cpm) or ^{125}I (1×10^{6} cpm). After injection, blood (70 μl) was taken from the tail vein at 0.25, 0.5, 1, 2, 5, 9, and 24 h for determination of radioactivity. The clearance rate was calculated from the decay curves of [^{3}H]CEt and ^{125}I according to the Matthews method (22). Finally, the animals were anesthetized and euthanized, the organs were harvested, tissue contents of [^{3}H]CEt and ^{125}I were analyzed, and organ clearance rates were determined as described previously (23). For single labeling, [^{3}H]CEt was introduced in a liposome preparation and exchanged (6 h, 37°C) into HDL as described above.

Plasma lipid and lipoprotein assays

Fasting (from 9:00 AM to 2:00 PM) plasma was collected for lipoprotein isolation and lipid measurement. The total cholesterol, phospholipids, and triglyceride in plasma were assayed by enzymatic methods (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Lipoprotein profiles were obtained by fast-protein liquid chromatography using a Sepharose 6B column (24). Apolipoprotein analysis by SDS-PAGE was also done as described previously (24).

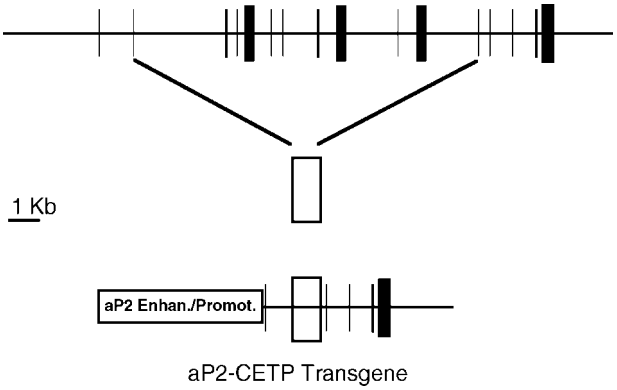

Fig. 1. Structure of the aP2-cholesteryl ester transfer protein (CETP) transgene. The organization of the human CETP gene is shown (top). The vertical bars represent exons. A minigene derivative was constructed by combining genomic and cDNA fragments (middle). The fusion of these fragments produced a synthetic exon (open box, bottom). The fully assembled transgene (bottom) was placed under the control of the mouse aP2 enhancer/promoter and then used to develop transgenic mice.

Quantitation of lipoprotein size

Fasting plasma was collected and submitted to LipoScience, Inc. (Raleigh, NC), for lipoprotein subclass analysis by nuclear magnetic resonance spectroscopy (25) on a fee-for-service basis.

Adipose tissue lipid extraction and assays

Epididymal fat pat (0.1 g) was homogenized in 2 ml of PBS, then 15 ml of $CHCl_3$/methanol (2:1) and 6 ml of 0.05% H_2SO_4 were added to two separate phases. Two milliliters of organic phase was taken and dried down under N_2. Fifty microliters of DMSO was added to dissolve the lipids; 6 μl of the solution was used for cholesterol, triglyceride, and phospholipid measurements using enzymatic methods (Wako Pure Chemical Industries).

Histology and image analysis

Samples of adipose tissue were obtained from epididymal fat pads of 14 week old male littermates. The same region of the fat pad was used for all animals to minimize cell size variation attributable to differences in anatomical location (26). The samples were fixed in paraformaldehyde, embedded in paraffin, cut into 10 μm sections, and stained with hematoxylin and eosin. The histology sections were viewed at 10× magnification, and images were obtained with a SPOT digital camera (Diagnostic Instruments, Sterling Heights, MI). These images were analyzed to determine the cross-sectional surface area of each adipocyte (27) using Image-Pro Plus (Media Cybernetics, Inc.).

Statistical analysis

Differences between groups were tested by Mann-Whitney *U*-test (nonparametric test). Data are presented as means ± SD. $P < 0.05$ was considered significant.

RESULTS

Creation of the aP2-CETPTg mouse

aP2-CETPTg mice were prepared using a CETP minigene (7) linked to a 5.4 kb aP2 enhancer/promoter (17) (**Fig. 1**). The resulting five founders were crossed with WT mice to yield the F1 generation. Two lines of aP2-CETPTg mice, 1-3 and 1-9, were established and used for further studies. A Northern blot of RNA prepared from different mouse tissues was probed with a human CETP 444 bp cDNA probe. CETP mRNA was predominantly expressed in adipose tissue in lines 1-3 and 1-9 (**Fig. 2A**). We also observed that in line 1-3, there were very low levels of CETP expression in muscle and lung (<5% of that in adipose

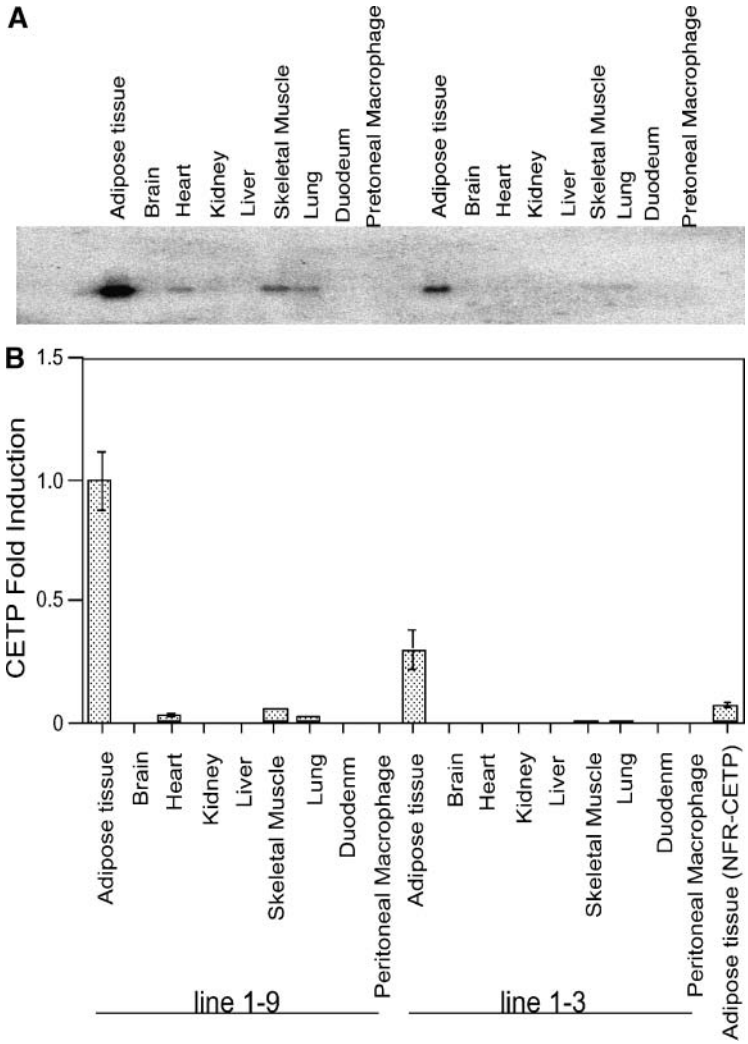

Fig. 2. CETP mRNA analysis in various mouse tissues. A: Northern blot analysis. Total RNA from various mouse tissues (20 μg/lane) was probed with a 444 bp fragment (nucleotides 726–1170) that was random primer-labeled. B: Real-time PCR analysis. Values shown are means ± SD. For details, see Materials and Methods.

TABLE 1. CETP activity in aP2-CETPTg mice

Lines	CETP Mass	CETP Activity
	μg/ml	*pmol/μl/h*
WT (1-3) (n = 5)	0	0
aP2-CETPTg (1-3) (n = 3)	3.9 ± 1.1	87 ± 11
WT (1-9) (n = 5)	0	0
aP2-CETPTg (1-9) (n = 5)	7.9 ± 1.8	110 ± 15
Human plasma (n = 3)	2.2 ± 0.3	36 ± 9
NFR-CETPTg[a] (n = 3)	2.9 ± 0.6	52 ± 7

CETP, cholesteryl ester transfer protein; CETPTg, CETP transgenic; WT, wild-type.
[a] CETP expression in mouse driven by the natural flanking region.

tissue), and that in line 1-9, there were low levels of CETP expression in lung, heart, and skeletal muscle (<5% of that in adipose tissue). We confirmed these results by real-time PCR (Fig. 2B). Importantly, there was no expression in other tissues, including the liver, small intestine, and peritoneal macrophages (Fig. 2B). Moreover, we compared adipose tissue CETP expression levels in natural flanking region (NFR)-CETPTg mice (line 5203) (7) and aP2-CETPTg mice (line 1-9) and found that the latter expresses 11 times more CETP mRNA than the former (Fig. 2B).

Plasma CETP mass and activity

CETP mass, evaluated by ELISA, was moderately higher than that of human plasma or NFR-CETPTg mice, with predominant expression in liver (7) (**Table 1**). Human plasma CETP levels of up to 4–5 μg/ml are observed in some dyslipidemic patients (28). Plasma CETP activities were proportional to the mass measurements (Table 1).

Plasma lipoprotein analysis

As indicated in **Table 2**, both lines of aP2-CETPTg mice showed significant reductions in total cholesterol (29% and 43%, $P < 0.05$ and $P < 0.01$, respectively) and phospholipids (29% and 39%, $P < 0.05$ and $P < 0.01$, respectively). There were no significant changes in plasma triglyceride levels. The distribution of cholesterol was determined by fast protein liquid chromatography of pooled plasma samples (**Fig. 3**). This showed that HDL-C was significantly decreased and non-HDL-C was significantly increased in both lines of aP2-CETPTg mice compared with WT mice. Moreover, a dose-related effect was observed, and the higher expresser (line 1-9) had a more profound effect on plasma lipoproteins than the lower expresser (line 1-3) (Table 2, Fig. 3). Assessment of apolipoprotein composition of centrifugally isolated lipoproteins by

TABLE 2. Plasma lipid analysis in WT and ap2-CETPTg mice (n = 5)

Mice	Cholesterol	Phospholipid	Triglyceride
WT (1-3)	98 ± 10	143 ± 18	34 ± 12
aP2-CETPTg (1-3)	70 ± 9[a]	101 ± 15[a]	26 ± 13
WT (1-9)	105 ± 7	162 ± 13	62 ± 17
aP2-CETPTg (1-9)	60 ± 3[b]	98 ± 3[b]	41 ± 20

[a] $P < 0.05$.
[b] $P < 0.01$.

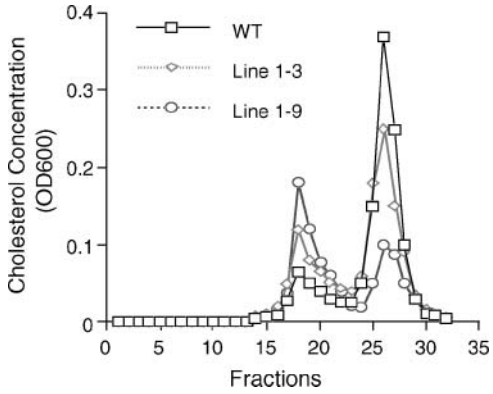

Fig. 3. Plasma cholesterol distribution analysis by fast protein liquid chromatography in wild-type (WT) mice and two lines of CETP transgenic (aP2-CETPTg) mice. A 200 μl aliquot of pooled fasting (from 9:00 AM to 2:00 PM) plasma was loaded onto a Sepharose 6B column and eluted with 50 mM Tris and 0.15 M NaCl (pH 7.5). An aliquot of each fraction was used for the determination of cholesterol. OD600, optical diffraction at 600 nm.

reducing SDS-PAGE revealed a statistically significant decrease of apolipoprotein A-I (apoA-I) and a statistically significant increase of apoB-100/apoB-48 in aP2-CETPTg mice compared with WT mice (**Fig. 4**). Gel scans based on three separate analyses of different pooled plasma samples indicated *1*) 52% and 73% decreases of apoA-I in line 1-3 and line 1-9 mice compared with WT animals ($P < 0.01$) and *2*) 192% and 209% increases of apoB-100/apoB-48

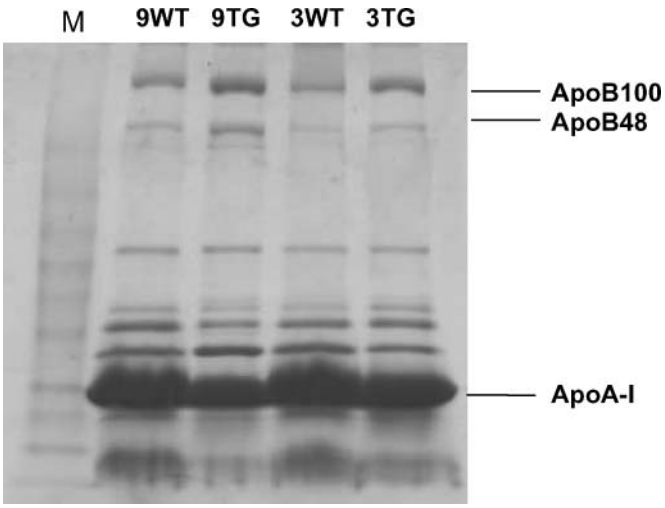

Fig. 4. SDS-PAGE analysis of apolipoproteins from ultracentrifugally isolated plasma lipoproteins. Plasma was adjusted to d = 1.210g/ml. All of the lipoproteins, including HDL (d = 1.063–1.21 g/ml) and VLDL + LDL (d = 1.006–1.063 g/ml), were separated by preparative ultracentrifugation as described previously (24). SDS-PAGE was performed on 3–20% SDS–polyacrylamide gradient gels, and the apolipoproteins were stained with Coomassie brilliant blue and scanned as described previously (24). 3TG, line 1-3 aP2-CETPTg mice; 9TG, line 1-9 aP2-CETPTg mice; 3WT, line 1-3 WT mice; 9WT, line 1-9 WT mice. These results are representative of three independent experiments.

TABLE 3. Lipoprotein size determination (n = 3)

Mice	LDL	HDL
	nm	
WT (1-3)	21.27 ± 0.75	10.67 ± 0.15
aP2-CETPTg (1-3)	19.3 ± 0.20[a]	9.57 ± 0.15[a]
WT (1-9)	22.03 ± 0.06	10.63 ± 0.35
aP2-CETPTg (1-9)	18.83 ± 0.15[b]	9.13 ± 0.06[b]

Lipoprotein size was determined by LipoScience, Inc., using nuclear magnetic resonance spectroscopy (21).
[a] $P < 0.01$.
[b] $P < 0.001$.

in line 1-3 and line 1-9 mice compared with WT animals ($P < 0.01$).

We also measured lipoprotein particle size and the concentration of each particle. The transgenic mice showed significantly decreased average particle sizes of LDL and HDL ($P < 0.01$ for line 1-3 and $P < 0.001$ for line 1-9) (**Table 3**). Moreover, the major LDL in both lines of aP2-CETPTg mice were small LDLs that constituted ~85% and 81% of total LDL particles, whereas there were much lower levels of small particles in WT mice (**Table 4**). In both lines of aP2-CETPTg mice, the medium and small HDLs were ~36% and 45% in total HDL particles, respectively, whereas similar sized particles were not detectable in WT animals (Table 4). VLDL particle concentration was too low to draw conclusions from this study.

Plasma and organ clearance rate determination

To understand the mechanism for the significant changes of lipoprotein metabolism in aP2-CETPTg, we carried out HDL turnover studies in aP2-CETPTg and WT mice using autologous HDL. aP2-CETPTg and WT mice were injected with their own HDL labeled with [^{3}H]CEt. The plasma clearance rate of [^{3}H]CEt-HDL in line 1-3 mice was 2.4-fold greater than that in WT mice (**Fig. 5A**, **Table 5**), whereas the clearance rate in line 1-9 mice was 3.6-fold greater than that in WT mice (Fig. 5B, Table 5). To investigate the catabolism of HDL particles, we injected ^{125}I-HDL into line 1-9 mice and their littermate controls and found that there was no difference in plasma clearance rate between WT and aP2-CETPTg mice (Fig. 5C, **Table 6**). This result suggests that CETP may cause an inhibition of apoA-I synthesis, because

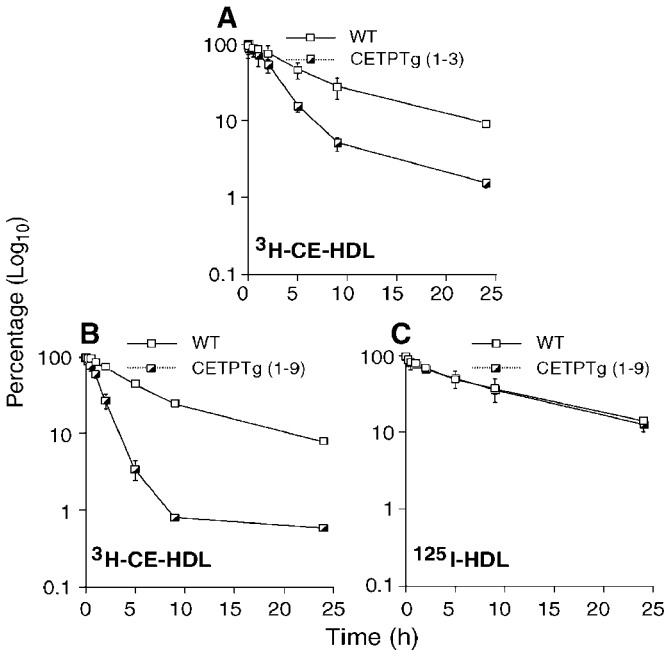

Fig. 5. Plasma fractional catabolic rates for radiolabeled HDL in mice (autologous HDL). A: WT mice (n = 4) and line 1-3 aP2-CETPTg mice (n = 4) were injected intravenously (femoral vein) with their own HDL labeled with [^{3}H]CEt. B: WT mice (n = 4) and line 1-9 aP2-CETPTg mice (n = 4) were injected with their own HDL labeled with [^{3}H]CEt. C: WT mice (n = 4) and line 1-9 aP2-CETPTg mice (n = 4) were injected with their own HDL labeled with ^{125}I-HDL. Blood (70 μl) was collected from the tail vein at 10 min, 0.5, 1, 2, 4, 8, and 24 h for determination of radioactivity. Values shown are means ± SD.

aP2-CETPTg mice have significantly less apoA-I in the circulation (Fig. 4).

To investigate tissue sites of HDL uptake, the mice were euthanized 24 h after [^{3}H]CEt-HDL and ^{125}I-HDL injection (23) and tissues were harvested for analysis of the radiotracer content. Based on plasma clearance rates and fractional tracer recovery in tissues, organ clearance rates for each HDL tracer were calculated (23). These organ clearance rates represent the fraction of the plasma pool of the traced HDL component cleared per hour by a tissue. The liver is the major organ for [^{3}H]CEt-HDL uptake. The hepatic organ clearance rates for [^{3}H]CEt in lines 1-3 and 1-9

TABLE 4. Lipoprotein particle concentration (n = 3)

Lipid	aP2-CETPTg (1-3)	WT (1-3)	aP2-CETPTg (1-9)	WT (1-9)
LDL (nmol/l)	419.7 ± 113.9[a]	209.7 ± 16.1	430.1 ± 12.5[a]	256.3 ± 37.9
Large	34.3 ± 7.2[b]	120.0 ± 7.6	26.7 ± 6.67[b]	138.3 ± 14.6
Medium	27.0 ± 5.3[b]	89.7 ± 10.0	52.0 ± 9.0[a]	118.0 ± 23.3
Small	358.3 ± 119.7[b]	0	350.0 ± 26.2[b]	0
HDL (μmol/l)	16.9 ± 1.9[a]	25.9 ± 3.3	12.0 ± 1.0[b]	26.1 ± 4.6
Large	10.7 ± 1.9[a]	25.9 ± 3.3	6.6 ± 1.1[a]	26.1 ± 4.6
Medium	3.9 ± 1.2	0	3.3 ± 0.7	0
Small	2.3 ± 1.1	0	2.1 ± 0.2	0

Lipoprotein particle concentration was determined by LipoScience, Inc., using nuclear magnetic resonance spectroscopy (21).
[a] $P < 0.05$.
[b] $P < 0.01$.

TABLE 5. Plasma decay of [^{3}H]CEt-HDL in WT and aP2-CETPTg mice and tracer uptake rates by tissues

FCR Type	WT (1-3)	aP2-CETPTg (1-3)	WT (1-9)	aP2-CETPTg (1-9)
Plasma FCR (pools/h)	0.095 ± 0.03	0.256 ± 0.037[a]	0.101 ± 0.03	0.399 ± 0.05[a]
Organ FCR ($\times h^{-1} g^{-1} \times 10^3$)				
Epididymal fat pad	9.7 ± 1.6	7.2 ± 0.9[b]	10.0 ± 1.5	6.2 ± 1.7[b]
Liver	51.3 ± 5.5	172.7 ± 15.1[a]	67.2 ± 12.1	312.7 ± 17.9[a]
Heart	3.9 ± 0.6	4.5 ± 1.1	3.0 ± 0.3	3.4 ± 0.5
Spleen	12.9 ± 1.4	20.8 ± 2.9[b]	11.4 ± 3.7	17.5 ± 4.5[b]
Kidney	5.5 ± 0.1	5.3 ± 0.5	5.5 ± 0.2	4.9 ± 1.2

FCR, fractional catabolic rate. Autologous [^{3}H]CEt-HDL was injected into WT or aP2-CETPTg mice. During the subsequent 24 h interval, blood was harvested periodically to determine the plasma FCR. Twenty-four hours after tracer injection, the animals were humanely euthanized and tissues were analyzed for radioactivity. Plasma and organ FCRs for [^{3}H]CEt were calculated as described in Materials and Methods. Values shown are means ± SD of five mice in each group.

[a] $P < 0.001$.

[b] $P < 0.05$.

of aP2-CETPTg mice were increased dramatically compared with that of WT (3.4-fold and 4.7-fold, respectively) (Table 5). Although epididymal fat pads made <2% contribution to [^{3}H]CEt-HDL uptake, both lines of aP2-CETPTg mice showed significant decreases of organ clearance rates (26% and 38%, respectively; $P < 0.05$). We also noticed that there were no differences in other organ clearance rates between WT and aP2-CETPTg mice (Table 6), indicating that CETP might be involved in a process that independently delivers HDL-CE into the liver.

Adipose tissue lipid level and adipocyte size determination

To evaluate the impact of CETP expression on adipose tissue lipid levels, we extracted total lipids from both WT and aP2-CETPTg mouse adipose tissues and measured triglyceride, cholesterol, and phospholipids. We found that both triglyceride and cholesterol levels were significantly decreased (50% and 37%; $P < 0.001$ and $P < 0.01$, respectively) compared with WT levels (**Fig. 6**), whereas there was no significant change in phospholipid levels (Fig. 6). It has been reported that when triglyceride and cholesterol stores are depleted, adipocytes reduce their size (27, 29). To determine whether this is the case in aP2-CETPTg adipocytes, we dissected the adipose tissue and stained with hematoxylin and eosin. The images were analyzed to determine the cross-sectional surface area of each adipocyte. aP2-CETPTg mice had a greater number of small adipocytes than WT mice. This difference in frequency distribution was reflected in a 44% decrease in mean surface area of adipocytes from aP2-CETPTg mice. We published this set of data in our previous review article (30).

To further evaluate the mechanism of adipocyte changes, we measured adipose tissue PPARγ, SREBP-1c, and LPL mRNA levels, which are involved in lipid storage or adipogenesis (31–33), and HSL mRNA levels, which is involved in lipid mobilization (33). We found that PPARγ, SREBP-1c, and LPL mRNA levels were significantly decreased in aP2-CETPTg mice compared with WT mice (**Fig. 7A–C**), whereas HSL mRNA levels did not change (Fig. 7D).

DISCUSSION

In this study, we created adipose tissue-specific CETPTg mice using an aP2 promoter and enhancer and demonstrated for the first time that adipose tissue-specific CETP expression *1*) made a major contribution to the CETP mass and activity in the circulation; *2*) caused a dose-dependent reduction in HDL-C as well as phospholipids

TABLE 6. Plasma decay of ^{125}I-HDL in WT and aP2-CETPTg mice and tracer uptake rates by tissues

FCR Type	WT (1-9)	aP2-CETPTg (1-9)
Plasma FCR (pools/h)	0.079 ± 0.05	0.081 ± 0.07
Organ FCR ($\times h^{-1} g^{-1} \times 10^3$)		
Epididymal fat pad	1.3 ± 0.3	1.2 ± 0.2
Liver	22.3 ± 7.5	21.7 ± 4.1
Heart	1.3 ± 0.2	1.5 ± 0.2
Spleen	1.9 ± 0.2	2.2 ± 0.5
Kidney	8.3 ± 1.1	9.2 ± 1.5

FCR, fractional catabolic rate. Autologous ^{125}I-HDL was injected into WT or aP2-CETPTg mice. During the subsequent 24 h interval, blood was harvested periodically to determine the plasma FCR. Twenty-four hours after tracer injection, the animals were humanely euthanized and tissues were analyzed for radioactivity. Plasma and organ FCRs for ^{125}I were calculated as described in Materials and Methods. Values shown are means ± SD of five mice in each group.

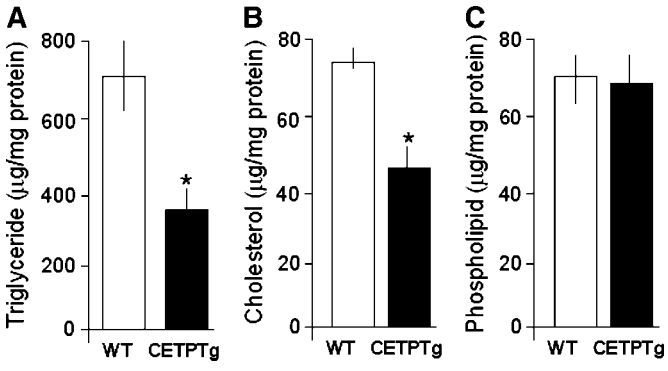

Fig. 6. Adipose tissue lipid content determination in aP2-CETPTg and WT mice. Adipose tissue lipid extraction and triglyceride, cholesterol, and phospholipid measurements are described in Materials and Methods. Values shown are means ± SD (n = 4/group). * $P < 0.01$.

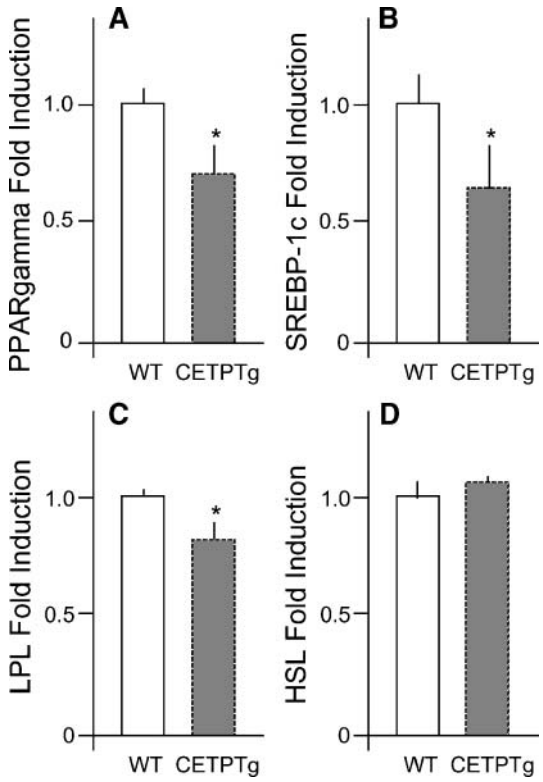

Fig. 7. Peroxisome proliferator-activated receptor γ (PPARγ) (A), sterol-regulatory element binding protein-1c (SREBP-1c) (B), LPL (C), and hormone-sensitive lipase (HSL) (D) mRNA level determinations. PPARγ, SREBP-1c, LPL, and HSL mRNAs were quantified by real-time PCR. Values shown are means ± SD (n = 5). * $P < 0.05$.

and an increase in non-HDL lipids; *3*) revealed a dose-dependent increase of plasma and liver clearance rates for [^{3}H]CEt-HDL but not for ^{125}I-HDL; *4*) decreased the particle size of LDL and HDL; *5*) decreased adipose tissue [^{3}H]CEt uptake; *6*) reduced adipose tissue triglyceride and cholesterol levels; and *7*) diminished the size of adipocytes.

The expression of CETP in adipose tissue contributes to CETP levels and activity in the circulation. We previously reported that adipose tissue is one of the major sources of CETP mRNA in human, monkey, rabbit, and hamster (8); however, the contribution of adipose tissue CETP to the plasma CETP has been uncertain, because other tissues, including liver, muscle, heart, small intestine, and kidney, all express CETP mRNA (8, 10, 34). CETP mRNA was predominantly expressed in adipose tissue in our two lines of aP2-CETPTg mice. There were also low levels of expression in heart and muscle (Fig. 2). This might be attributable to the contamination of adipose tissue in both organs. For instance, the epicardium contains substantial adipose tissue, which could contribute to the total RNA prepared from heart. It has been reported that the aP2 promoter/enhancer also functions in macrophages (35). However, there was no detectable CETP mRNA in the macrophages from aP2-CETPTg mice (Fig. 2). Although the expression levels are negligible compared with adipose tissue, it is still not known why lung also can express low levels of aP2-CETP transgene (Fig. 2). It is very likely that adipose tissue CETP is the major source for the CETP in the plasma of aP2-CETPTg mice.

Increased plasma CETP levels lead to apoB-containing particle accumulation (Fig. 3, 4). Among monkeys fed an atherogenic diet, there was a strong relationship between plasma CETP levels and LDL-cholesterol (LDL-C) and apoB levels (10). In families with genetic CETP deficiency, plasma apoB, VLDL, intermediate density lipoprotein, and LDL-C are all reduced as a result of the deficiency (5). In NFR-CETPTg mice, CETP expression causes the downregulation of LDL receptor, which in turn causes apoB-containing particle accumulation in the circulation (36). Recent studies show that CETP inhibitors not only increase HDL levels but also decrease non-HDL levels (37, 38). Moreover, CETP activity also is related to LDL size. The presence of heterogeneous small, dense LDLs is a key feature in patients presenting CETP deficiency (39, 40). In vitro studies also indicate that CETP influences LDL particle size by favoring a shift in LDL distribution toward a profile composed mainly of CE-rich, buoyant LDL particles of large size (41, 42).

The findings of decreased cholesterol and triglyceride contents and reduced adipocyte size in aP2-CETPTg mice are interesting. Radeau et al. (12) reported that adipocyte size, estimated as mg triglyceride/mg soluble protein, is inversely correlated with CETP mRNA abundance in fresh human adipose tissue. They also demonstrated that adipose tissues containing small lipid-poor adipocytes express higher amounts of CETP mRNA compared with tissue consisting of large lipid-rich cells (12). Our result further confirmed their observation, although the mechanism is still unknown. One mechanism relevant to this phenomenon might be the decrease of adipose tissue CE uptake (Table 5); however, it has been reported that exogenous CETP promotes CE selective uptake in human adipose tissue culture (43). These contradictory observations might reflect the difference between in vivo and in vitro systems. Reduced cholesterol content in adipose tissue could also indicate enhanced cellular cholesterol efflux. One possibility is that secreted CETP is locally activated in the vicinity of adipocytes, promoting HDL remodeling and the release of lipid-poor apoA-I from HDL particles. The apoA-I might, in turn, interact with ABCA1, known to be highly expressed in adipocytes (44), promoting cholesterol efflux. To investigate the possible mechanism of the reduction of triglyceride in aP2-CETPTg adipose tissue, we measured PPARγ, SREBP-1c, LPL, and HSL mRNA levels, because PPARγ, SREBP-1c, and LPL are involved in lipid storage or adipogenesis (31–33) and HSL is involved in lipid mobilization (33). The finding that PPARγ, SREBP-1c, and LPL mRNA levels were decreased significantly in aP2-CETPTg adipose tissue compared with WT tissue (Fig. 7) may be mechanistically related to the reduced size and triglyceride content of adipocytes.

In conclusion, we have established adipose tissue-specific CETPTg mice. Adipose tissue CETP can be se-

creted into the circulation and makes a contribution to plasma lipoprotein metabolism. Thus, CETP joins the growing list of molecules secreted by adipose tissue that have more widespread systemic effects. CETP expression in adipose tissue affected lipid contents and the size of adipocytes, and the consequences of this deserve further investigation.

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