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Cholesteryl ester transfer protein (CETP) expression enhances HDL cholesteryl ester liver delivery, which is independent of scavenger receptor BI, LDL receptor related protein and possibly LDL receptor

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Abstract

Cholesteryl ester transfer protein (CETP) is a hydrophobic plasma glycoprotein that mediates the transfer and exchange of cholesteryl ester (CE) and triglyceride (TG) between plasma lipoproteins, and also plays an important role in HDL metabolism. Previous studies have indicated that, compared to wild type mice, human CETP transgenic mice had significantly lower plasma HDL CE levels, which was associated with enhancement of HDL CE uptake by the liver. However, the mechanism of this process is still unknown. To evaluate the possibility that this might be directly mediated by CETP, we utilized CETP transgenic (CETPTg) mice with liver scavenger receptor BI (SR-BI) deficiency [i.e., PDZK1 gene knockout (PDZK1O)], and with receptor associated protein (RAP) overexpression, to block LDL receptor-related protein (LRP) and LDL receptor (LDLR). We found that 1) CETPTg/PDZK1O mice have significantly lower HDL-C than that of PDZK1 KO mice (36%, $P < 0.01$); 2) CETPTg and CETPTg/PDZK1O mice have same HDL-C levels; 3) CETPTg/PDZK1O/RAP mice had significant lower plasma HDL-C levels than that of PDZK1O/RAP ones (50%, $p < 0.001$); 4) there is no incremental transfer of HDL CE radioactivity to the apoB-containing lipoprotein fraction in mice expressing CETP; and 5) CETPTg/PDZK1O/RAP mice had significant higher plasma and liver [³H]Cet-HDL turnover rates than that of PDZK1O/RAP ones (50% and 53%, $p < 0.01$, respectively). These results suggest that CETP expression in mouse increases direct removal of HDL CE in the liver and this process is independent of SR-BI, LRP, and possibly LDLR.

Introduction

Most prospective epidemiological studies have found an inverse correlation between levels of HDL and the incidence of atherosclerosis (1-3). Some of these studies have indicated that HDL participates in the reverse cholesterol transport pathway, i.e., the centripetal movement of cholesterol from peripheral tissues, or from macrophage foam cells in the arterial wall to the

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FOOTNOTES

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liver, via the plasma compartment (4). There are two steps in this process. First, excess cholesterol in peripheral tissues is removed by HDL, which in conjunction with LCAT action (converting free cholesterol into cholesteryl ester) provides the driving force for net cholesterol movement (5). Second, HDL-cholesteryl esters (CE) are delivered to the liver. It is believed that the transport of HDL CE from plasma to the liver involves a direct uptake pathway, mediated by hepatic scavenger receptor B-I (SR-BI) (6), as well as an indirect pathway, involving transfer of HDL CE to triglyceride-rich lipoproteins (TRL) by stimulating CE-TG interchange, followed by the uptake of TRL remnants in the liver (7,8).

SR-BI is an HDL receptor that plays an essential role in the hepatic uptake of plasma HDL-derived cholesterol and CE into the liver for excretion into the bile (6,9). SR-BI deficiency results in a significant increase in plasma HDL CE and increased atherosclerotic lesions in multiple mouse models (10). The multi-PDZ domain-containing protein PDZK1 has been shown to interact with SR-BI from rat liver (11). Recently, gene-targeted disruption of PDZK1 was found to result in the near absence of SR-BI in mouse liver, proving that PDZK1 is essential for SR-BI expression in the liver (12,13).

Two members of the LDL receptor (LDLR) gene family are highly expressed in the liver. One is LDLR, which regulates plasma cholesterol by mediating uptake and catabolism of plasma LDL (14,15). The other is LDL receptor-related protein (LRP), which mediates removal of different ligands, including VLDL remnants and chylomicron remnants (16). An important feature shared by all these receptors is the inhibition of ligand interaction by a 39-kDa receptor-associated protein (RAP). Studies have shown that RAP exhibits high-affinity ($K_D \sim 1\text{--}10\text{ nM}$) for LRP (17), and low-affinity binding ($K_D \sim 250\text{ nM}$) for the LDLR (18).

CETP is a hydrophobic plasma glycoprotein that promotes the transfer of CE from antiatherogenic HDLs to proatherogenic apoB-containing lipoproteins, including VLDLs, VLDL remnants, IDLs, and LDLs (7,8). Mice lack CETP activity in plasma (19), and when a human CETP transgene is introduced, there is a lowering of plasma HDL levels associated with increased catabolism of HDL CE due to enhanced clearance by the liver (20,21). However, the precise mechanism of this process is unknown.

To investigate the role of CETP in mediating HDL CE delivery to the liver, we utilized CETP transgenic mice with a liver deficiency of SR-BI (i.e., PDZK1 deficient), as well as RAP overexpression (blocking LRP and blocking or partially blocking LDLR pathways). We found that blocking SR-BI, LRP, and LDLR did not influence CETP-mediated HDL CE delivery to the liver. Moreover, our findings suggest that humans deficient in SR-BI would not exhibit high plasma HDL, as seen in SR-BI deficient mice, because of the actions of CETP in mediating hepatic clearance of HDL CE.

Materials and Methods

Mice

All animals were between 8 and 12 weeks of age. Generation of PDZK1O mice has been described previously (13). These PDZK1O animals were crossed with natural flanking region (NFR)-CETPTg transgenic mice (22) with a C57BL/6 genetic background. Fifty percent of the progeny had heterozygous PDZK1O and CETPTg genotypes, and these animals were further crossed with homozygous PDZK1O mice to generate CETPTg/PDZK1O mice and their littermates, PDZK1O. All were fed a chow diet.

RAP Expression

Recombinant adenovirus containing either the RAP cDNA (AdCMV-RAP, 2×10^{11} viral particles) (a gift from Dr. Joachim Herz) or LacZ cDNA (AdCMV-LacZ, 2×10^{11} viral

particles) (ViraQuest, Inc) were injected into mice. At 6 days after virus administration, samples (0.5 μ l) were separated by SDS PAGE, and the proteins were transferred to nitrocellulose filters. Western blot analysis for RAP was performed, using polyclonal antimouse RAP antibody (also a gift from Dr. Joachim Herz). Horseradish peroxidase-conjugated rabbit polyclonal antibody to mouse IgG (Novus Biologicals) was used as a secondary antibody for RAP. The SuperSignal West detection kit (Pierce) was used for detection.

Plasma Lipid and Lipoprotein Assays

Fasting 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 (FPLC), using Sepharose 6B column.

In Vivo Turnover Studies

HDL was isolated by ultracentrifugation ($1.063 < d < 1.21$ g/ml). Mice were injected intravenously with their own HDL that had been labeled with [3 H]cholesteryl oleyl ether ([3 H]CEt) (2×10^6 cpm). After injection, blood (70 μ l) 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 according to the Matthews' method (23). Finally, the animals were anesthetized and euthanized, the organs were harvested, the tissue content of [3 H]CEt was analyzed, and organ-clearance rates were determined as previously described (24). To determine the CETP-mediated transfer of HDL CE to apoB-containing lipoproteins, VLDL + LDL fraction was isolated by ultracentrifugation of pooled plasma (70 μ l) at $d < 1.063$ and the amount of [3 H]CEt present in the fraction was determined at 0.25, 0.5, 1, 2, 5, and 9h, after tracer injection in the different groups of mice.

Statistical Analysis

Each experiment was conducted at least three times. Data are typically expressed as mean $\pm$ SD. Differences between groups were tested by Mann-Whitney U test (non-parametric test) and among multiple groups by ANOVA followed by the Post-Hoc test.

Results

CETP expression masks the effect of SR-BI deficiency on HDL in mice

To evaluate the effect of liver SR-BI deficiency on plasma HDL levels under a "human-like" scenario with regards to HDL metabolism, we created a mouse model with human CETP expression (CETPTg) and PDZK1 deficient (PDZK1O). We utilized natural flanking region (NFR)-CETPTg mice, since they have human-like CETP concentration in the circulation (22). We use PDZK1O mice but not SR-BI deficient (SR-BIO) mice for our studies because PDZK1 deficiency results in the near absence of SR-BI in mouse liver. More importantly, PDZK1O mice do not have the abnormal HDL particles enriched in unesterified cholesterol that is found in SR-BIO mice (12,13). As indicated in Table I, plasma lipoprotein analysis by precipitation showed that PDZK1O mice had significantly higher total cholesterol (C), phospholipids (PL), HDL-C and HDL-PL levels (34%, 31%, 38% and 32%, $p < 0.01$ respectively) than did wild type (WT) animals, confirming a previous report (12). This increased HDL was shown to be due to the near absence of hepatic SR-BI (12,13). Triglyceride and Non-HDL lipids were not significantly altered.

We next sought to determine whether CETP could reduce the high HDL-C levels found in PDZK1O mice. CETP expression in PDZK1O (CETPTg/PDZK1O) mice resulted in

significantly lower total cholesterol, PL, HDL-C and HDL-PL levels than in PDZK1O mice (36%, 34%, 36% and 39%, $p < 0.01$, respectively) (Table I). Moreover, CETP expression with or without PDZK1 deficiency (CETPTg vs. CETPTg/PDZK1O) resulted in a similar level of total cholesterol, PL, HDL-, HDL-PL, and CETP mass (Table I).

The distribution of lipids was determined by FPLC of pooled plasma samples. This confirmed that HDL-C levels were increased in PDZK1O mice compared with WT animals (Fig.1A). HDL-C levels were decreased in CETPTg/PDZK1O compared with PDZK1O mice (Fig.1B). There were no lipid level changes in the CETPTg mice with or without PDZK1 deficiency (Fig. 1C). Assessment of apolipoprotein composition of centrifugally isolated lipoproteins by reducing SDS-PAGE revealed that CETPTg/PDZK1O and CETPTg mice had significantly lower apoA-I levels than that in PDZK1O animals (45%, and 50%, $p < 0.01$, respectively) (Fig. 2A and 2B), however, there was no significant difference in apoB levels in these mice (Fig. 2A and 2B). All these results indicated that the specific effect of CETP on HDL lowering was independent of hepatic SR-BI pathway.

CETP expression enhances HDL-CE liver delivery

To determine whether CETP could still reduce the HDL-C under the condition when SR-BI, LRP and LDLR are blocked. We injected Adenovirus-CMV-RAP (AdV-RAP), which can block LRP (17) and LDLR (18), in both PDZK1O and CETPTg/PDZK1O mice, and found that CETPTg/PDZK1O/RAP had significantly lower HDL-C levels than that of PDZK1O/RAP (Table 2) (Fig.3), suggesting that CETP might mediate a SR-BI, LRP and possibly LDLR-independent HDL CE delivery pathway. It should be noted that we monitored RAP overexpression by Western blot as previous report (16) and found that RAP was evidently existed in the circulation 6 days after AdCMV-RAP administration, while no RAP was detected after AdV-LacZ injection (Fig.4).

To investigate whether there is a CETP-dependent pathway for HDL CE deliver to the liver, we carried out HDL turnover studies in CETPTg/PDZK1O/RAP and PDZK1O/RAP, using [^3H]Cet labeled HDL. The plasma clearance rate of [^3H]Cet-HDL in CETPTg/PDZK1O/RAP mice was significantly higher than that of PDZK1O/RAP mice (50%, $p < 0.01$, Fig.5, Table 3), which was similar to the previous report, i.e. CETPTg vs. WT mice (20).

To investigate tissue sites of HDL CE uptake, the mice were euthanized 24 hours after [^3H]Cet-HDL injection and tissues were harvested for analysis of the radio-tracer content. Based on plasma-clearance rates and fractional tracer recovery in tissues, organ-clearance rates for each HDL tracer were calculated (24). 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 [^3H]Cet-HDL uptake. The hepatic organ-clearance rates for [^3H]Cet in CETPTg/PDZK1O/RAP mice were significantly increased, compared to that of PDZK1O/RAP ones (53%, $p < 0.01$) (Table 3), indicating that CETP is involved in a process which independently deliver HDL CE into the liver. We also noticed that there was significant increased of kidney-clearance rate in CETPTg/PDZK1O/RAP mice than that of controls (75%, $p < 0.001$). We measured [^3H] cholesterol in feces and found that there was a 35% increase in the radiolabeled cholesterol (Fig.6), indicating enhancement of hepatic uptake resulted in increased fecal sterol output,

To determine to what extent enhanced clearance of the HDL CE from plasma involves the CETP-mediated transfer of HDL CE to TRL, lipoproteins were isolated by ultracentrifugation of pooled plasma at $d < 1.063$ and the amount of [^3H]Cet present in the VLDL + LDL fraction was determined at 0.25, 0.5, 1, 2, 5, and 9 hours after tracer injection in the different groups of mice. Fifteen minutes after the injection of labeled HDL, 8.9% and 7.2% of the total

radioactivity present in plasma was detected in the $d < 1.063$ g/ml lipoproteins in CETPTg/PDZK1O/RAP and PDZK1O/RAP mice, respectively. This amount remained constant over the time interval studied (Fig.7) indicating that the changes observed in the plasma HDL-C and [3 H]CEt-HDL turnover rate are not reflected by an equivalent transfer of [3 H]CEt-HDL into the $d < 1.063$ g/ml fraction. This result confirmed a previous report (21).

Discussion

CETP in the circulation directly influences the efficiency of reverse cholesterol transport. Early kinetic study in rabbits has shown that CETP influences the overall movement of CE from plasma to the liver (25). It is known that NFR-CETPTg mice, which have human-like CETP concentrations in the circulation (22), have significantly lower plasma HDL CE levels than controls, and this phenotype is associated with increased catabolism of HDL CE due to enhanced clearance by the liver (20,21).

The aim of our studies here was to determine the mechanism of CETP-mediated lowering of HDL CE. It has been previously reported that *in vivo* remodeling of HDL by CETP enhanced uptake of HDL CE by cells expressing SR-BI (21), suggesting that SR-BI might be involved in this process. However, direct *in vivo* evidence was lacking.

SR-BI is an HDL receptor. In mice, SR-BI overexpression causes dramatic reduction of plasma HDL-C levels (26), while SR-BI deficiency results in a significant induction of plasma HDL-C levels (27). So far no human SR-BI deficiency was discovered. PDZK1 is a 70-kDa protein comprising four PDZ domains, and is primarily expressed in the kidney, liver, and lung (28, 29). It was recently reported that gene-targeted disruption of PDZK1 resulted in the near absence of SR-BI in mouse liver, proving that PDZK1 is essential for SR-BI expression primarily in the liver and to a smaller extent in the intestine (12,13). Therefore, PDZK1O mice approximate a liver-specific deficiency of SR-BI. Since blocking SR-BI by PDZK1 deficiency in the liver did not block the effect of CETP in lowering plasma HDL CE (Fig. 1, Table I), the involvement of SR-BI in this pathway could be excluded. Our data also suggest that humans with SR-BI deficiency but normal CETP levels will probably not exhibit high plasma HDL levels, as predicted from SR-BI-deficient mice (27), and this will probably explain the difficulty of identifying SR-BI-deficient humans.

We may also exclude the possibility that CETP expression increases HDL CE transfer into apoB-containing lipoproteins that are then taken up in mouse liver by receptor-mediated endocytosis. There are several reasons for this: 1) CETPTg mice did not show incremental transfer of HDL CE into apoB-containing lipoproteins (21, and Fig.7 in this study), suggesting that the enhanced clearance of HDL CE in mice expressing the human CETP transgene may not be mediated by enhanced transfer to $d < 1.063$ g/ml lipoproteins; 2) overexpression of RAP did not block the effect of CETP on decreasing plasma HDL CE levels (Fig.3), indicating that LRP was not essential for this pathway; 3) we could not completely rule out the effect of LDLR, since AdV-RAP might only partially block its function (16), but we have previously shown that NFR-CETP expression causes reduction, but not induction, of hepatic LDLR, owing to increased cholesterol return to the liver (30); 4) Gauthier et al. demonstrated that *in vitro* CETP enhances HDL CE uptake independent of known lipoprotein receptors, including SR-BI, LRP and LDLR (31); and 5) the plasma and liver clearance rate of [3 H]CEt-HDL in CETPTg/PDZK1O/RAP mice was significantly higher than that of PDZK1O/RAP mice (Fig. 5, Table 3), which was similar to previous report, i.e., CETPTg vs WT mice (20), indicating that CETP-mediated plasma HDL CE clearance is independent of background.

It was reported that CETPTg mice have smaller HDL size than WT mice (20,32). We confirmed the previous observations (Fig.1 and Fig. 3). In this study, we also show that the organ FCR

for the kidney increased preferentially (by 75%) to account for 12.7% of ^3H -CEt clearance in CETP/PDZK10/RAP vs. PDZK10/RAP mice (Table 3). This finding is of relevance as it is known in man that under hypertriglyceridemic conditions, small HDL are formed due to CETP action and these particles are degraded by the kidney cubulin-megalin receptor pathway (33, 34). However, although the cublin/megalin pathway makes contribution to the HDL catabolism, such contribution is small, compared to the liver (Table 3). Moreover, we might exclude a pathway resembling a cubulin/megalin mechanism, since there is no cublin/megalin expression in the liver (33).

The molecular mechanism by which CETP directly promotes HDL CE delivery to the liver is unknown. Since CETP can transfer CE among lipoproteins, it is conceivable that CETP may also promote CE transfer from lipoprotein to the hepatocyte membrane, and this process may not need other associated proteins, since mice have no CETP expression (19,35). CETP could play a role in the circulation, or it could exist on the cell membrane and play a role locally. It has also been proposed that CETP mediates HDL CE uptake by hepatocytes through both CETP inhibitor-sensitive (exogenous CETP) and CETP inhibitor-insensitive (cell-associated CETP) mechanisms (31). The exact mechanisms deserve further investigation.

The limitation of this study is that mouse lipoproteins may not serve as optimal substrates for human CETP (20). The observation in this study showing that apoB-containing lipoproteins in the CETPTg mouse plasma did not incur a large increase in HDL-derived CE (Fig. 7) following the administration of labeled HDL is also in support of this idea. However, at least, the mouse model used in this study provided a unique opportunity for us to investigate the function of CETP, in terms of HDL catabolism.

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Glossary

Abbreviations used in this paper:

CETP	cholesteryl ester transfer protein
Tg	transgene
WT	wild type
NFR	natural flanking region
SR-BI	scavenger receptor BI
RAP	receptor associate protein
LRP	LDL receptor related protein
LDLR	

	LDL receptor
HDL	high density lipoprotein
FCR	fractional catabolism rate
FPLC	fast protein liquid chromatography
TG	triglyceride

REFERENCES

1. Miller NE, Miller GJ. Letter: High-density lipoprotein and atherosclerosis. *Lancet* 1976;1:1033. [PubMed: 48701]
2. Rhoads GG, Gulbrandsen CL, Kagan A. Serum lipoproteins and coronary heart disease in a population study of Hawaii Japanese men. *N Engl J Med* 1976;294:293–298. [PubMed: 173994]
3. Gordon DJ, Rifkind BM. High-density lipoprotein--the clinical implications of recent studies. *N Engl J Med* 1989;321:1311–1316. [PubMed: 2677733]
4. Glomset JA. The plasma lecithins: cholesterol acyltransferase reaction. *J Lipid Res* 1968;9:155–167. [PubMed: 4868699]
5. Hara H, Yokoyama S. Role of apolipoproteins in cholesterol efflux from macrophages to lipid microemulsion proposal of a putative model for the pre-beta high density lipoprotein pathway. *Biochemistry* 1992;31:2040–2046. [PubMed: 1536847]
6. Acton S, Rigotti A, Landschultz KT, Xu S, Hobbs HH, Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* 1996;271:518–520. [PubMed: 8560269]
7. Tall AR. Plasma cholesteryl ester transfer protein. *J Lipid Res* 1993;34:1255–1274. [PubMed: 8409761]
8. Barter PJ, Brewer HB, Chapman MJ Jr, Hennekens CH, Rader DJ, Tall AR. Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. *Arterioscler Thromb Vasc Biol* 2003;23:160–167. [PubMed: 12588754]
9. Mardones P, Quinones V, Amigo L, Moreno M, Miquel JF, Schwarz M, Miettinen HE, Trigatti B, Krieger M, Van Patten S, Cohe DE, Rigotti A. Hepatic cholesterol and bile acid metabolism and intestinal cholesterol absorption in scavenger receptor class B type I-deficient mice. *J Lipid Res* 2001;42:170–180. [PubMed: 11181745]
10. Braun A, Trigatti BL, Post MJ, Sato K, Simons M, Edelberg JM, Rosenberg DR, Schrenzel M, Krieger M. Loss of SR-BI expression leads to the early onset of occlusive atherosclerotic coronary artery disease, spontaneous myocardial infarctions, severe cardiac dysfunction, and premature death in apolipoprotein E-deficient mice. *Circ Res* 2000;90:270–276. [PubMed: 11861414]
11. Silver DL. A carboxyl-terminal PDZ-interacting domain of scavenger receptor B, type I is essential for cell surface expression in liver. *J Biol Chem* 2002;277:34042–7. [PubMed: 12119305]
12. Kocher O, Yesilaltay A, Cirovic C, Pal R, Rigotti A, Krieger M. Targeted disruption of the PDZK1 gene in mice causes tissue-specific depletion of the high density lipoprotein receptor scavenger receptor class B type I and altered lipoprotein metabolism. *J Biol Chem* 2003;278:52820–52825. [PubMed: 14551195]
13. Lan D, Silver DL. Fenofibrate induces a novel degradation pathway for scavenger receptor B-I independent of PDZK1. *J Biol Chem* 2005;280:23390–23396. [PubMed: 15837786]
14. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science* 1986;232:34–47. [PubMed: 3513311]

15. Hobbs HH, Russell DW, Brown MS, Goldstein JL. The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. *Annu Rev Genet* 1990;24:133–170. [PubMed: 2088165]
16. Willnow TE, Sheng Z, Ishibashi S, Herz J. Inhibition of hepatic chylomicron remnant uptake by gene transfer of a receptor antagonist. *Science* 1994;264:1471–1474. [PubMed: 7515194]
17. Iadonato SP, Bu G, Maksymovitch EA, Schwartz AL. Interaction of a 39 kDa protein with the low-density-lipoprotein-receptor-related protein (LRP) on rat hepatoma cells. *Biochem J* 1993;296:867–875. [PubMed: 8280086]
18. Medh JD, Fry GL, Bowen SL, Pladet MW, Strickland DK, Chappell DA. The 39-kDa receptor-associated protein modulates lipoprotein catabolism by binding to LDL receptors. *J Biol Chem* 1995;270:536–540. [PubMed: 7822276]
19. Ha YC, Barter PJ. Differences in plasma cholesteryl ester transfer activity in sixteen vertebrate species. *Comp Biochem Physiol B* 1982;71:265–269. [PubMed: 7060347]
20. Hayek T, Chajek-Shaul T, Walsh A, Agellon LB, Moulin P, Tall AR, Breslow JL. An interaction between the human cholesteryl ester transfer protein (CETP) and apolipoprotein A-I genes in transgenic mice results in a profound CETP-mediated depression of high density lipoprotein cholesterol levels. *J Clin Invest* 1992;90:505–510. [PubMed: 1644921]
21. Collet X, Tall AR, Serajuddin H, Guendouzi K, Royer L, Oliveira H, Barbaras R, Jiang XC, Francone OL. Remodeling of HDL by CETP in vivo and by CETP and hepatic lipase in vitro results in enhanced uptake of HDL CE by cells expressing scavenger receptor B-I. *J Lipid Res* 1999;40:1185–1193. [PubMed: 10393203]
22. Jiang XC, Agellon LB, Walsh A, Breslow JL, Tall AR. Dietary cholesterol increases transcription of the human cholesteryl ester transfer protein gene in transgenic mice. Dependence on natural flanking sequences. *J Clin Invest* 1992;90:1290–1295. [PubMed: 1401066]
23. Matthews CME. The theory of tracer experiments with ¹³¹I-labeled plasma proteins. *Phys Med Biol* 1957;2:36–53. [PubMed: 13484460]
24. Rinninger F, Pittman RC. Regulation of the selective uptake of high density lipoprotein-associated cholesteryl esters. *J Lipid Res* 1987;28:1313–1325. [PubMed: 3430062]
25. Whitlock ME, Swenson TL, Ramakrishnan R, Leonard MT, Marcel YL, Milne RW, Tall AR. Monoclonal antibody inhibition of cholesteryl ester transfer protein activity in the rabbit. Effects on lipoprotein composition and high density lipoprotein cholesteryl ester metabolism. *J Clin Invest* 1989;84:129–137. [PubMed: 2738149]
26. Wang N, Arai T, Ji Y, Rinninger F, Tall AR. Liver-specific overexpression of scavenger receptor BI decreases levels of very low density lipoprotein ApoB, low density lipoprotein ApoB, and high density lipoprotein in transgenic mice. *J Biol Chem* 1998;273:32920–32926. [PubMed: 9830042]
27. Rigotti A, Trigatti BL, Penman M, Rayburn H, Herz J, Krieger M. A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc Natl Acad Sci USA* 1997;94:12610–12615. [PubMed: 9356497]
28. Ikemoto M, Arai H, Feng D, Tanaka K, Aoki J, Dohmae N, Takio K, Adachi H, Tsujimoto M, Inoue K. Identification of a PDZ-domain-containing protein that interacts with the scavenger receptor class B type I. *Proc Natl Acad Sci U S A* 2000;97:6538–6543. [PubMed: 10829064]
29. Wang S, Yue H, Derin RB, Guggino WB, Li M. Accessory protein facilitated CFTR-CFTR interaction, a molecular mechanism to potentiate the chloride channel activity. *Cell* 2000;103:169–179. [PubMed: 11051556]
30. Jiang XC, Masucci-Magoulas L, Mar J, Lin M, Walsh A, Breslow JL, Tall AR. Down-regulation of mRNA for the low density lipoprotein receptor in transgenic mice containing the gene for human cholesteryl ester transfer protein. Mechanism to explain accumulation of lipoprotein B particles. *J Biol Chem* 1993;268:27406–27412. [PubMed: 8262982]
31. Gauthier A, Lau P, Zha X, Milne R, McPherson R. Cholesteryl Ester Transfer Protein Directly Mediates Selective Uptake of High Density Lipoprotein Cholesteryl Esters by the Liver. *Arterioscler Thromb Vasc Biol* 2005;25:2177–2184. [PubMed: 16123327]

32. Jiang XC, Beyer TP, Li Z, Liu J, Quan W, Schmidt RJ, Zhang Y, Bensch WR, Eacho PI, Cao G. Enlargement of high density lipoprotein in mice via liver X receptor activation requires apolipoprotein E and is abolished by cholesteryl ester transfer protein expression. *J Biol Chem* 2003;278:49072–49078. [PubMed: 12947111]
33. Hammad SM, Barth JL, Knaak C, Argraves WS. Related Articles, Megalin acts in concert with cubilin to mediate endocytosis of high density lipoproteins. *J Biol Chem* 2000;275:12003–12008. [PubMed: 10766831]
34. Barth JL, Argraves WS. Related Articles, Cubilin and megalin: partners in lipoprotein and vitamin metabolism. *Trends Cardiovasc Med* 2001;11:26–31. [PubMed: 11413049]
35. Hogarth CA, Roy A, Ebert DL. .Genomic evidence for the absence of a functional cholesteryl ester transfer protein gene in mice and rats. *Comp Biochem Physiol B Biochem Mol Biol* 2003;135:219–229. [PubMed: 12798933]

Fig 1.

Plasma cholesterol distribution analysis by FPLC in mice. A 200- μ l aliquot of pooled plasma (from five animals) was loaded into a Sepharose 6B column and eluted with 50 mM Tris, 0.15 M NaCl (pH 7.5). An aliquot of each fraction was used for the determination of cholesterol. *FPLC*, fast protein liquid chromatography.

Fig 2. SDS-PAGE analysis of apolipoproteins from ultracentrifugally isolated plasma lipoproteins. Plasma lipoproteins (VLDL, LDL and HDL, density = 1.006-1.21 g/ml) were separated by preparative ultracentrifugation as described. SDS-PAGE was performed on 3%-20% SDS-polyacrylamide gradient gel, and the apolipoproteins were stained by Coomassie brilliant blue and scanned as described. This experiment was the representative of three independent experiments. Panel A, a representative gel. Panel B, quantitative display of three independent experiments. Values are mean $\pm$ SD, *P<0.01.

Fig 3.

Plasma cholesterol distribution analysis by FPLC in PDZK1O/RAP and CETPTg/PDZK1O/RAP mice. A 200- μ l aliquot of pooled plasma (from five animals) was loaded into a Sepharose 6B column and eluted with 50 mM Tris, 0.15 M NaCl (pH 7.5). An aliquot of each fraction was used for the determination of cholesterol. *FPLC*, fast protein liquid chromatography.

Fig 4.

Western blot of plasma RAP after AdV-RAP administration. Some 6 days after virus administration, samples (0.5 μ l) were separated by SDS PAGE, and the proteins were transferred to nitrocellulose filters. Western blot analysis for RAP was performed using polyclonal antimouse RAP antibody. Horseradish peroxidase-conjugated rabbit polyclonal antibody to mouse IgG was used as a secondary antibody for RAP. The SuperSignal West detection kit was used for detection.

Fig 5.

Plasma fractional catabolic rates for ^3H Cet-labeled HDL in mice NFR-CETPTg/PDZK1 and PDZK1 mice ($n = 4$) were injected with AdV-RAP (2×10^{11} viral particles) intravenously (through femoral vein). Some 6 days after the adenovirus injection, both groups of mice were injected intravenously with ^3H Cet-HDL (2×10^6 cpm). Blood (70 μl) was collected from the tail vein at 10 min, 0.5, 1, 2, 4, 8, and 24 hours for determination of radioactivity. Values are mean $\pm$ SD.

Fig 6.

Mouse feces [^3H]Cholesterol measurement. NFR-CETPTg/PDZK1 and PDZK1 mice ($n = 4$) were injected with AdV-RAP (2×10^{11} viral particles) intravenously (through femoral vein). Some 6 days after the adenovirus injection, both groups of mice were injected intravenously with [^3H]CET-HDL (2×10^6 cpm). Feces were collected after 24 hours. Radioactivity was counted. Values are mean $\pm$ SD. * $P < 0.02$.

Fig 7.

Transfer of [^3H]CET-labeled HDL to apoB-containing lipoproteins. Pooled plasma from CETPTg/PDZK1O/RAP and PDZK1O/RAP mice (n=5) was collected after the injection of labeled HDL and apoB-containing lipoproteins were isolated by ultracentrifugation. The amount of [^3H]CET present in apoB-containing lipoproteins was determined at 0.25, 0.5, 1, 2, 5, and 9 hours, after tracer injection in the different groups of mice.

Table 1

Plasma lipid analysis in mice

	Chol	PL	TG mg/dl	HDL-C	HDL-PL	CETP mass µg/ml
WT	101±9 ^a	153±15 ^a	57±9 ^a	80±5 ^a	122±11 ^a	--
PDZK10	135±29 ^b	201±35 ^b	6±13 ^a	110±15 ^b	160±20 ^b	--
CETPT ^g	89±3 ^c	129±19 ^c	62±15 ^a	69±5 ^c	99±11 ^c	2.8±0.2
CETPT ^g /PDZK10	87±5 ^c	133±11 ^c	70±20 ^a	70±6 ^c	97±21 ^c	2.9±0.3

Values are mean ± SD, n=5, P<0.001 by ANOVA. Columns labeled with different lower-case letters are statistically different, p<0.05. Chol, cholesterol; PL, phospholipids; TG, triglyceride; HDL-C, high density lipoprotein cholesterol. Differences among multiple groups were tested by ANOVA followed by the Post-Hoc test. Columns labeled with different lower-case letters are statistically different, P<0.05.

Table 2

Plasma lipid analysis in mice

	Chol	PL	TG mg/dl	HDL-C	Non-HDL-C	CETP mass µg/ml
PDZK10/RAP	136±36	210±23	123±27	78±12	57±9	--
CETPTg/PDZK10/RAP	90±16*	160±19*	132±39	39±5*	51±10	2.9±0.1

Values are mean ± SD.

* $p < 0.01$. n = 5. Chol, cholesterol; PL, phospholipids; TG, triglyceride; HDL-C, high density lipoprotein cholesterol.

Table 3
Plasma decay of ^3H -CEt-HDL in mice and tracer uptake rates by tissues

	PDZK10/RAP	CETPTg/PDZK10/RAP
Plasma-FCRs(pools/h)	0.105±0.031	0.158±0.037 [*]
Organ-FCRs($\times \text{h}^{-1} \times 10^3$)		
Liver	38.3±5.5	58.5±11.1 [*]
Heart	3.7±0.6	3.9±1.1
Kidney	5.2±0.2	9.1±0.5 [*]

Autologous of [^3H]-CEt-HDL was injected in WT or aP2-CETPTg mice. During the subsequent 24-hour interval, blood was harvested periodically to determine the plasma fractional catabolic rate (FCR). Some 24 hours after tracer injection, tissues were analyzed for radioactivity. Plasma-FCR's and organ-FCR's for [^3H]-CEt were calculated as described in Methods. Values are mean $\pm$ SD of n = 4 mice in each group.

^{*}
 $p < 0.01$.