

# Apolipoprotein CI overexpression is not a relevant strategy to block cholesteryl ester transfer protein (CETP) activity in CETP transgenic mice

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ApoCI (apolipoprotein CI) is a potent inhibitor of plasma CETP [CE (cholesteryl ester) transfer protein]. The relevance of apoCI overexpression as a method for CETP blockade *in vivo* was addressed in the present study in CETPTg/apoCITg mice (mice expressing both human CETP and apoCI). Despite a significant reduction in specific CETP activity in CETPTg/apoCITg mice compared with CETPTg mice [transgenic mouse to human CETP;  $46.8 \pm 11.1$  versus  $101.8 \pm 25.7$  pmol · h<sup>-1</sup> · (μg of plasma CETP)<sup>-1</sup> respectively;  $P < 0.05$ ], apoCI overexpression increased both the CETP mass concentration (3-fold increase;  $P < 0.05$ ) and the hepatic CETP mRNA level (4-fold increase,  $P < 0.005$ ), leading to an increase in total plasma CE transfer activity (by 39%,  $P < 0.05$ ). The ratio of apoB-containing lipoprotein to HDL (high-density lipoprotein) CE was 10-fold higher in CETPTg/apoCITg mice than in apoCITg mice ( $P < 0.0005$ ). It is proposed that the increased CETP expression in CETPTg/apoCITg mice is a direct

consequence of liver X receptor activation in response to the accumulation of cholesterol-rich apoB-containing lipoproteins. In support of the latter view, hepatic mRNA levels of other liver X receptor-responsive genes [ABCG5 (ATP-binding cassette transporter GS) and SREBP-1c (sterol-regulatory-binding protein-1c)] were higher in CETPTg/apoCITg mice compared with CETPTg mice. In conclusion, overexpression of apoCI, while producing a significant inhibitory effect on specific CETP activity, does not represent a suitable method for decreasing total CE transfer activity in CETPTg/apoCITg mice, owing to an hyperlipidaemia-mediated effect on CETP gene expression.

**Key words:** apolipoprotein CI (apoCI), cholesteryl ester transfer protein (CETP), high-density lipoprotein (HDL), hyperlipidaemia, transgenic mice.

## INTRODUCTION

The CETP [CE (cholesteryl ester) transfer protein] mediates the exchange of neutral lipids, i.e. CEs and TGs (triacylglycerols), between plasma lipoproteins [1]. CETP will probably influence the atherogenicity of the lipoprotein profile through its action [2–4], and recent studies support a potential interest in inhibiting CETP activity *in vivo* by means of anti-CETP immunotherapy, antisense oligonucleotides or specific pharmacological inhibitors [5–10]. In addition to interventional studies with exogenous compounds, a number of studies indicated that plasma CETP activity can be modulated by many endogenous factors, among them being the lipid and apolipoprotein content of circulating lipoproteins [11–16]. High cholesterol intake was shown to increase CETP mass levels in humans [17] and in several animal species [18–20] as a result of increased CETP mRNA levels [18–22]. In agreement with the observations in hypercholesterolaemic monkeys [19,20], cholesterol feeding in transgenic mice expressing the human CETP gene under the control of its natural flanking region (CETPTg mice) also led to a significant increase in plasma CETP levels [22]. Similarly, hyperlipidaemia in genetically engineered mice, i.e. apoE0 and LDLR0, was also proven to produce increased CETP expression in CETPTg mice [23].

In addition to dietary manipulation and the knockout of apolipoprotein and lipoprotein receptor genes, overexpression of specific apolipoproteins, in particular apoCI (apolipoprotein CI),

was also shown to produce marked hyperlipidaemia in mice [24,25]. The latter point is of particular interest since recent studies demonstrated that apoCI constitutes a potent inhibitor of CETP. Indeed, apoCI can completely inhibit CETP activity with an IC<sub>50</sub> value that is compatible with its circulating concentrations [26]. Most importantly, *in vivo* studies in CETPTg/apoCI-KO mice (genetically engineered mice with complete apoCI deficiency) directly supported the ability of apoCI to inhibit CETP activity, and exacerbation of CETP-mediated neutral lipid transfers and associated lipoprotein changes were proven to be a direct consequence of apoCI deficiency [27].

On the basis of previous observations, apoCI overexpression may predictably decrease the rate of transfer of plasma CEs between HDL (high-density lipoprotein) and apoB-containing lipoproteins through its specific inhibitory effect on CETP activity [26,27]. To determine the effect of apoCI overexpression on CETP activity levels and the plasma lipoprotein profile *in vivo*, it was necessary to evaluate the plasma total and specific CETP activities, CETP mass, hepatic CETP mRNA and the plasma lipoprotein profile in CETP/apoCI transgenic mice.

## EXPERIMENTAL

### Animals

Two distinct mouse lines in a homogeneous C57BL/6 genetic background were used in the present study: heterozygous mice

Abbreviations used: apoCI, apolipoprotein CI; apoCI-KO mouse, genetically engineered mouse with complete apoCI deficiency; apoCITg mouse, transgenic mouse to human apoCI; CE, cholesteryl ester; CETP, CE transfer protein; CETPTg mouse, transgenic mouse to human CETP; CETPTg/apoCITg mouse, transgenic mouse to both human CETP and apoCI; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LXR, liver X receptor; NBD, nitrobenzoxadiazole; SREBP-1c, sterol-regulatory-element-binding protein-1c; TBS, Tris-buffered saline; TG, triacylglycerol; VLDL, very-low-density lipoprotein.

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expressing human CETP under the control of natural flanking regions (CETPTg) [22] and heterozygous apoC1 transgenic mice (apoC1Tg) [28]. Wild-type controls, CETPTg mice, apoC1Tg mice and CETPTg/apoC1Tg mice were littermates, generated after cross-breeding of CETPTg and apoC1Tg heterozygotes. The mice had free access to water and food and were placed on a standard chow diet. Animal experiments were performed under the framework of the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

### Plasma samples

Blood samples from fasting mice were collected in heparin-containing test tubes. Plasmas were obtained by low-speed centrifugation and were stored at  $-80^{\circ}\text{C}$  until analysis.

### SDS/PAGE of HDL apolipoproteins

HDLs were isolated from control, apoC1Tg, CETPTg or CETPTg/apoC1Tg plasmas by sequential ultracentrifugation as described previously [27]. Isolated HDLs (protein, 0.25 g/l) were incubated for 15 min at  $80^{\circ}\text{C}$  in the presence of SDS (25 g/l) and dithiothreitol (33 g/l) in TBS (Tris-buffered saline; 100 mM; pH 7.4). Samples were then applied on to an SDS/polyacrylamide high-density gel (Phastsystem; Amersham Biosciences), and electrophoresis was performed according to the manufacturer's instructions. Protein bands were silver-stained as described previously [27]. Apparent molecular masses were determined by comparison with protein standards (Rainbow Markers; Amersham Biosciences) that were submitted to electrophoresis together with the samples.

### Measurement of CE transfer activity

Plasma CETP activity was measured in mouse plasmas by using a fluorescent assay that was performed in microplates by using donor liposomes enriched with NBD (nitrobenzoxadiazole)-labelled CEs (Roar Biomedical, New York, NY, U.S.A.) as described previously [27]. In brief, incubation media contained 5  $\mu\text{l}$  of donor liposomes and 10  $\mu\text{l}$  of total plasma. Final volumes were adjusted to 250  $\mu\text{l}$  with TBS (10 mM; pH 7.4) containing 2 mM EDTA, and incubations were performed in triplicate for 3 h at  $37^{\circ}\text{C}$  in a Victor<sup>2</sup> 1420 Multilabel Counter (Wallac-PerkinElmer Life Sciences, Boston, MA, U.S.A.). The CETP-mediated transfer of NBD-CEs from self-quenched donors to acceptor endogenous plasma lipoproteins was monitored by the increase in fluorescence intensity (excitation, 465 nm; emission, 535 nm). The amounts of NBD-CEs transferred (in pmol) were calculated by using a standard curve, which plotted fluorescence intensity and the concentration of NBD-CEs dispersed in propan-2-ol. Results were expressed as the amount of labelled CEs transferred after deduction of blank values.

### Immunoassay of CETP mass levels

CETP mass levels in mouse plasmas were determined by a specific immunoassay with TP2 anti-CETP monoclonal antibodies. In brief, plasma samples were diluted (1:9, v/v) in TBS (100 mM; pH 6.8) containing SDS (25 g/l) and then incubated for 15 min at  $80^{\circ}\text{C}$ . Samples were subsequently applied on to 8–12% discontinuous polyacrylamide gels in a Mini Protean device (Bio-Rad Laboratories) and then transferred on to nitrocellulose membranes (Hybond ECL<sup>®</sup>; Amersham Biosciences). The resulting blots were blocked for 1 h in 5% low-fat dried milk in PBS (100 mM; pH 7.4) containing 0.1% Tween and then washed with PBS/Tween. Human CETP was revealed by successive incubations with TP2 anti-CETP antibodies (Heart Institute, Ottawa, Canada)

and horseradish peroxidase-coupled secondary antibodies as described previously [29]. Blots were finally developed by using an ECL<sup>®</sup> kit (Amersham Biosciences). The CETP mass level in each plasma sample was estimated by comparison with a calibration curve that was obtained with serial dilutions of a human plasma standard submitted to electrophoresis together with the samples.

### Quantification of CETP, ABCG5 (ATP-binding cassette transporter GS) and SREBP-1c (sterol-regulatory-element-binding protein-1c) mRNAs

Total RNA was extracted from the liver by the guanidium thiocyanate/phenol/chloroform method using the RNA Instapure reagent (Eurogentec, Liege, Belgium). The quality of the RNA samples was checked by electrophoresis on agarose gels and the quantities were normalized to the 28 S band. After reverse transcription, cDNAs were amplified by real-time PCR with the following primers: CETP, 5'-CAGATCAGCCACTTGTCCAT-3' and 5'-CAGCTGTGTGTTGATCTGGA-3'; ABCG5, 5'-TGGA-TCCAACACCTCTATGCTAAA-3' and 5'-GGCAGGTTTTCT-CGATGAACTG-3'; SREBP-1c, 5'-GGAGCCATGGATTGCAC-ATT-3' and 5'-GCTTCCAGAGAGGCCAG-3'; and 18 S, 5'-GGGAGCCTGAGAAACGGC-3' and 5'-GGGTCCGGAGT-GGGTAATTT-3'. The reactions were carried out in the presence of 200 mM each of the sense and antisense primers by using the SYBR Green amplification kit (Qiagen, Courtaboeuf, France) in an ABI PRISM 7700 Sequence Detection System instrument (Applied Biosystems, Courtaboeuf, France). Hepatic mRNA levels were normalized to the 18 S and were expressed as a fold induction compared with the CETPTg group.

### ELISA of human apoC1

Polyclonal antibodies to human apoC1 were raised in a rabbit injected four times with 250  $\mu\text{g}$  of human apoC1 that had been purified as described previously [27]. IgG was purified by affinity chromatography on HiTrap Protein A columns. Competitive ELISA for apoC1 was set up according to the general procedure used in our laboratory to quantify apoAIV [30], apoB [31] and CETP [32]. Purified apoC1 was used as a plate coating in the present study.

### Plasma lipid analysis

All assays were performed on a Victor<sup>2</sup> 1420 Multilabel Counter (Wallac-PerkinElmer Life Sciences). Total cholesterol was measured by the enzymic method using Cholesterol 100 reagent (ABX Diagnostics, Montpellier, France), and concentration of the unesterified cholesterol was determined by the CHOD-PAP method (Sigma). The concentration of esterified cholesterol was calculated from the difference between total and free cholesterol. The TG concentration was determined by the enzymic method using Infinity Triglyceride Reagent (Sigma). Phospholipid concentration was measured by the enzymic method using PAP 150 reagent (bioMérieux, Marcy L'étoile, France).

### Fractionation of plasma lipoproteins

Plasmas (200  $\mu\text{l}$ ) were injected on a Superose 6 HR 10/30 column (Amersham Biosciences), which was connected to an FPLC (fast protein liquid chromatography) system (Amersham Biosciences). Lipoproteins were eluted at a constant flow rate of 0.3 ml/min with TBS (50 mM; pH 7.4) containing 0.074% EDTA and 0.02% sodium azide. CE and TG concentrations were assayed in individual 0.3-ml fractions. VLDLs (very-low-density lipoproteins) are contained in fractions 5–15, LDLs (low-density lipoproteins) are contained in fractions 16–29 and HDLs are contained

in fractions 30–45. In the preliminary FPLC analysis, plasmas from mice overexpressing apoC1 were shown to produce column clogging systematically, owing to an increased content of buoyant TG-rich lipoproteins. To circumvent this problem, and before performing any FPLC analysis, individual plasmas were removed from TG-rich remnants by a brief, 1-h spin at 386 000 *g* at isodensity in a 100.2 rotor in a TL100 ultracentrifuge (Beckman, Palo Alto, CA, U.S.A.).

### Native PAGE

Total lipoproteins were separated by ultracentrifugation as the  $d < 1.21$  g/ml plasma fraction with a 5.5 h, 386 000 *g* spin in a TLA100 rotor in a TLX ultracentrifuge (Beckman). Lipoproteins were then applied on to a 15–250 g/l polyacrylamide gradient gel (Spiragel 1.5–25.0; Spiral, Coutron, France), and electrophoresis was conducted according to the manufacturer's instructions. Gels were subsequently submitted to staining with Coomassie Brilliant Blue G (Sigma), and the distribution profiles of HDL were obtained by analysis with a Bio-Rad GS-670 imaging densitometer. The mean apparent diameters of HDL were determined by comparison with globular protein standards (HMW kit; Amersham Biosciences), which were submitted to electrophoresis together with the samples [33].

### Statistical analyses

The significance of the difference between mean values was determined by using either the ANOVA or Mann–Whitney *U* test, as appropriate. Coefficients of correlation  $\rho$  were calculated by using the Spearman correlation rank analysis.

## RESULTS

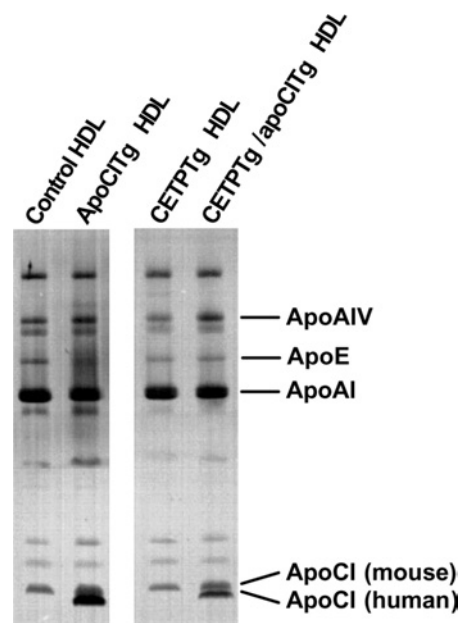
### Effect of human apoC1 overexpression on the apoprotein and lipid composition of plasma lipoproteins

As shown in Figure 1 and in contrast with the observations made after overexpression of apoA1 [34], accumulation of apoC1 in the HDL of apoC1Tg mice occurred with no major changes in the relative amounts of other HDL apolipoproteins (Figure 1). Quantitative analysis by ELISA indicated that human apoC1 mass levels in plasmas from apoC1Tg or CETPTg/apoC1Tg mice were significantly higher than the mean values in normolipidaemic human plasma, with no significant difference between the two mouse lines ( $97.5 \pm 20.1$  mg/l in apoC1Tg and  $109.8 \pm 16.3$  mg/l in CETPTg/apoC1Tg versus  $68.6 \pm 18.8$  mg/l in humans;  $P < 0.0001$  in both cases).

In agreement with earlier studies [28], apoC1Tg mice displayed a marked hyperlipidaemia compared with control mice. Detailed analysis by FPLC gel permeation chromatography showed that the lipid composition of individual lipoproteins was considerably modified by apoC1 expression (Figure 2 and Table 1). Cumulative analysis of TG-rich remnant-, VLDL-, LDL- or HDL-containing fractions from apoC1Tg mice revealed that CEs are significantly increased in all the lipoproteins compared with control mice (Table 1). A significant enrichment with TGs also occurred in large-sized TG-rich remnants, VLDL and LDL, but not in HDL (Table 1).

### Effect of CETP on lipoprotein parameters in CETPTg and CETPTg/apoC1Tg mice

CETP expression tended to decrease the cholesterol content of HDL in CETPTg mice compared with controls, and the difference was statistically significant in some of the HDL-containing fractions isolated by FPLC (Table 1; Figure 2). Concomitantly, cumu-



**Figure 1** Relative composition of HDL apolipoproteins from control, apoC1Tg, CETPTg and CETPTg/apoC1Tg mice

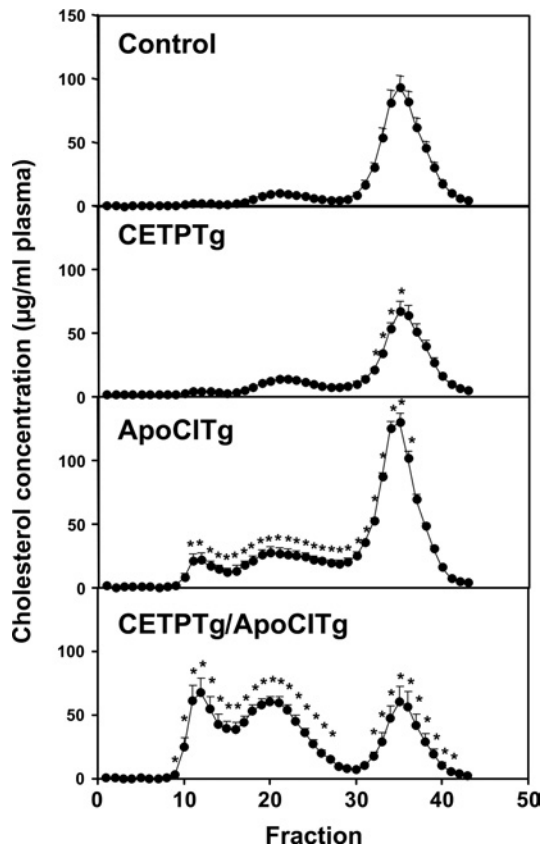
HDLs from control, apoC1Tg, CETPTg and CETPTg/apoC1Tg plasmas were isolated by ultracentrifugation, and their apolipoproteins were separated by SDS electrophoresis in a homogeneous polyacrylamide gel (High-Density Gel; Amersham Biosciences). Apolipoprotein bands were visualized after silver staining, and molecular masses were calculated by comparison with the relative mobility of protein standards (see the Experimental section).

lative analysis of LDL- and VLDL-containing fractions showed non-significant increases in the CE content of these lipoproteins for CETPTg mice compared with control mice. The effect of CETP on the lipid composition of individual lipoproteins was considerably magnified in CETPTg/apoC1Tg mice. In the apoC1-mediated hyperlipidaemic context, CETP led to a profound redistribution of cholesterol between lipoprotein fractions compared with apoC1Tg mice. Cholesterol tended to localize mainly in the VLDL and LDL fractions of CETPTg/apoC1Tg mice (Figure 2), and this occurred at the expense of HDL, which displayed a 51% decrease in CE content (Table 1). In contrast, HDL from CETPTg/apoC1Tg mice was clearly enriched with TGs (Table 1). Overall, the ratio of apoB-containing lipoprotein to HDL CE was 10.6-fold higher in CETPTg/apoC1Tg mice compared with apoC1Tg mice (Table 1).

Finally, alterations in the lipid composition of individual plasma lipoproteins were associated with significant modifications of the particle size in CETPTg/apoC1Tg mice. As shown by native PAGE (Figure 3), the combination of apoC1 and CETP produced a significant reduction in the mean apparent diameter of HDL ( $8.6 \pm 0.1$  nm) compared with control ( $8.8 \pm 0.1$  nm;  $P < 0.005$ ), apoC1Tg ( $8.9 \pm 0.1$  nm;  $P < 0.005$ ) or CETPTg ( $8.8 \pm 0.1$  nm;  $P < 0.05$ ) mice. The ratio of HDL CE to TG was significantly lower in CETPTg/apoC1Tg mice compared with control ( $-69\%$ ;  $P < 0.005$ ), apoC1Tg ( $-66\%$ ;  $P < 0.005$ ) or CETPTg ( $-62\%$ ;  $P < 0.005$ ) mice.

### Effect of apoC1 overexpression on total CE transfer and specific activity of plasma CETP in CETPTg/apoC1Tg mice

To determine whether apoC1 overexpression can modulate CE exchange between plasma lipoproteins, total CE transfer activity was measured in whole plasma from CETPTg and CETPTg/apoC1Tg mice. ApoC1 overexpression produced a significant



**Figure 2** Cholesterol distribution in plasmas from control, CETPTg, apoCITg and CETPTg/apoCITg mice

Plasmas were depleted from large-sized TG-rich remnants by brief ultracentrifugation as described in the Experimental section. Plasmas were subsequently passed through a Superose 6-HR column on an FPLC system, and the cholesterol levels in individual fractions were determined as described in the Experimental section. Fractions 5–15, 16–29 and 30–43 contain VLDL, IDL (intermediate-density lipoprotein)/LDL and HDL respectively. Each point is the mean  $\pm$  S.D. for seven (control and CETPTg mice) or five (apoCITg and CETPTg/apoCITg mice) distinct mice. \* $P < 0.05$ , significantly different from control mice; ANOVA.

increase in the total plasma CE transfer rates in CETPTg/apoCITg mice compared with CETPTg mice ( $122.3 \pm 26.9$  versus  $87.7 \pm 3.1$  pmol/h respectively;  $P < 0.05$ ). Conversely, when expressed per  $\mu\text{g}$  of CETP, initial transfer rates were actually 54% lower in CETPTg mice co-expressing apoCI compared with CETPTg mice [ $46.8 \pm 11.1$  pmol  $\text{h}^{-1} \cdot (\mu\text{g of CETP})^{-1}$  in CETPTg/apoCITg plasma versus  $101.8 \pm 25.7$  pmol  $\text{h}^{-1} \cdot (\mu\text{g of CETP})^{-1}$  in CETPTg plasma;  $P < 0.05$ ].

#### Effect of apoCI overexpression on CETP mass and CETP mRNA levels in CETPTg/apoCITg mice

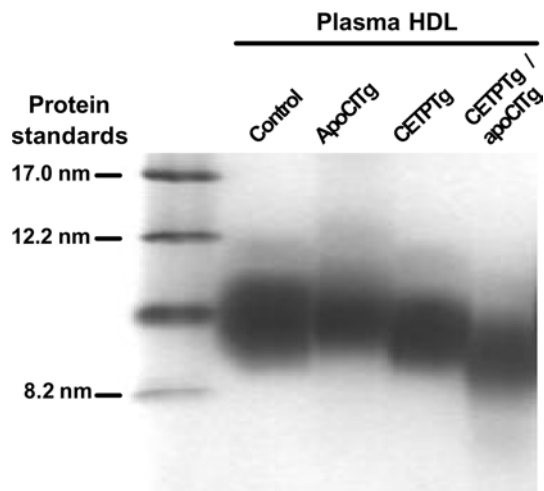
Plasma CETP mass levels in CETPTg mice were similar to those observed in normolipidaemic humans ( $3.57 \pm 0.73$  mg/l and  $2.79 \pm 0.58$  mg/l respectively; non-significant). ApoCI overexpression was associated with a 3-fold increase in plasma CETP mass levels in CETPTg/apoCITg mice compared with CETPTg mice ( $10.71 \pm 1.39$  versus  $3.57 \pm 0.73$  mg/l respectively;  $P < 0.05$ ). As shown in Figure 4, and in further support of an apoCI-mediated overexpression of CETP, CETPTg/apoCITg mice displayed a 4-fold increase in hepatic CETP mRNA levels compared with CETPTg mice ( $P < 0.005$ ). Similar tendencies towards an increase in hepatic mRNA levels were also observed for other LXR (liver X receptor)-responsive genes, e.g. ABCG5

**Table 1** CE and TG levels in large-sized TG-rich remnants, VLDL, LDL and HDL from control, CETPTg, apoCITg and CETPTg/apoCITg mice

Lipid contents were determined as described in the Experimental section. Values are expressed in mmol/litre and they are the means  $\pm$  S.E.M. for seven (control and CETPTg mice) or five (ApoCITg and CETPTg/apoCITg) animals. The significance between mean values was determined by using ANOVA; ND, not detectable.

	Mice			
	Control	CETPTg	ApoCITg	CETPTg/apoCITg
TG-rich remnants				
CE	ND	ND	$0.16 \pm 0.06$	$1.09 \pm 0.42$ ¶¶
TG	ND	ND	$0.66 \pm 0.27$	$1.16 \pm 0.40$
VLDL				
CE	$0.01 \pm 0.01$	$0.03 \pm 0.01$	$0.12 \pm 0.03^*$	$0.47 \pm 0.08$ †§¶¶
TG	$0.15 \pm 0.04$	$0.21 \pm 0.06$	$0.65 \pm 0.19$ †‡	$0.86 \pm 0.14$ †§
LDL				
CE	$0.13 \pm 0.01$	$0.20 \pm 0.02$	$0.45 \pm 0.06$ †§	$0.86 \pm 0.06$ †§¶¶
TG	$0.20 \pm 0.02$	$0.19 \pm 0.01$	$0.61 \pm 0.11$ †§	$0.85 \pm 0.14$ †§
HDL				
CE	$1.08 \pm 0.11$	$0.86 \pm 0.08$	$1.44 \pm 0.07$ †§	$0.70 \pm 0.16$ *¶¶
TG	$0.06 \pm 0.01$	$0.09 \pm 0.01$	$0.10 \pm 0.03$	$0.18 \pm 0.03$ †§
Ratio of non-HDL to HDL CE	$0.14 \pm 0.03$	$0.28 \pm 0.02$	$0.56 \pm 0.09$	$5.92 \pm 2.30$ †§¶¶
Ratio of HDL CE to TG	$14.5 \pm 1.4$	$11.9 \pm 2.7$	$13.2 \pm 2.6$	$4.5 \pm 1.4$

\* $P < 0.05$ , † $P < 0.005$ , significantly different from control mice.  
‡ $P < 0.05$ , § $P < 0.005$ , significantly different from CETPTg mice.  
|| $P < 0.05$ , ¶ $P < 0.005$ , significantly different from apoCITg mice.



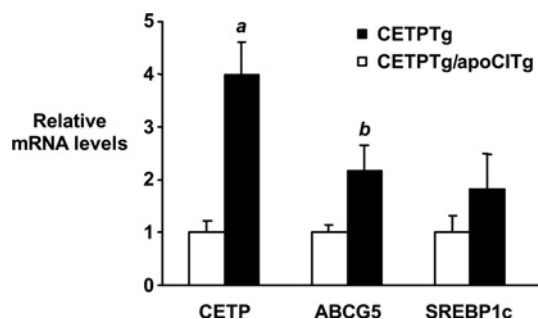
**Figure 3** HDL size distribution in the plasma from control, apoCITg, CETPTg and CETPTg/apoCITg mice

Total plasma lipoproteins were submitted to electrophoresis on 15–250 g/l polyacrylamide gradient gels that were stained for proteins as described in the Experimental section. The gel shown is representative of six distinct experiments, which were performed with plasma samples from six distinct mice for each genotype.

(2.2-fold increase,  $P < 0.05$ ) and SREBP-1c (1.8-fold increase,  $P = 0.08$ ) (Figure 4).

#### Relative effect of apoCI overexpression on the mass and specific activity of CETP in plasma from CETPTg/apoCITg mice

The balance between positive and negative effects of apoCI expression on CETP activity was studied in plasma from CETPTg/apoCITg mice with different levels of human apoCI expression over the range 80.3–140.2 mg/l. As shown in Figure 5, increase



**Figure 4** Abundance of hepatic CETP, ABCG5 and SREBP-1c mRNAs in CETPTg and CETPTg/apoCITg mice

Abundance of CETP, ABCG5 and SREBP-1c mRNAs was determined in livers from CETPTg and CETPTg/apoCITg mice. After extraction of total RNA from the liver, mRNA levels were estimated by real-time PCR. Results are expressed as means  $\pm$  S.D. for ten distinct mice. Significantly different from CETPTg mice, <sup>a</sup> $P < 0.005$ , <sup>b</sup> $P < 0.05$ ; Mann-Whitney test.

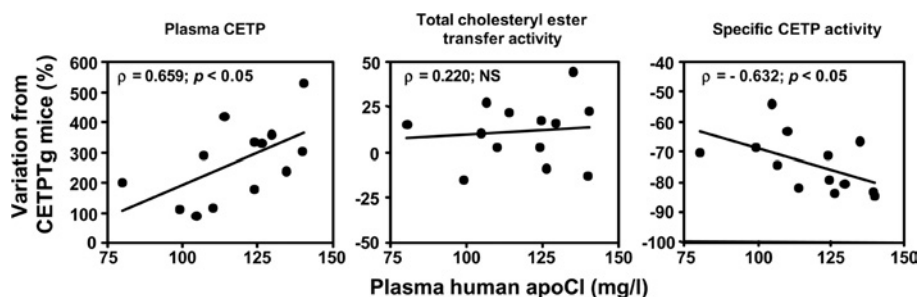
in plasma apoCI concentration in these mice correlated positively with the variation in plasma CETP mass concentration, and it correlated negatively with the variation in specific CETP activity, whereas no significant relationship with total CE transfer activity was observed when corrected by total neutral lipid levels (Figure 5). With the highest levels of apoCI expression, an approx. 5-fold increase in CETP mass concentration was accompanied by an approx. 5-fold decrease in specific CETP activity (Figure 5), indicating that a decrease in specific CETP activity compensates gradually for the apoCI-mediated increase in CETP mass concentration.

## DISCUSSION

Previous studies indicated that apolipoprotein CI constitutes a potent physiological inhibitor of CETP and that the inhibitory potency of circulating HDL from human, wild-type mouse or apoCI-KO mouse plasmas is directly linked to their apoCI content [26,27]. The aim of the present study was to determine whether apoCI overexpression might constitute a relevant strategy for CETP inhibition *in vivo*. To this end, CETP mass and activity levels were compared in CETPTg mice overexpressing human apoCI or not.

In agreement with previous studies [24,25,28], overexpression of apoCI in apoCITg mice was associated with a severe combined

hyperlipidaemia. The accumulation of apoB-containing lipoproteins and TG was linked to the ability of apoCI to inhibit both lipoprotein lipase activity [35] and the binding of apoE-containing lipoproteins to specific cellular receptors, i.e. the VLDL receptor [36], the LDL-receptor-related protein [28,37,38] and the LDL receptor [39]. Hyperlipidaemia tends to increase CETP expression when the CETP gene is placed under the control of its natural promoter [18–22]. Thus high cholesterol intake has been shown to increase CETP mRNA levels in several animal models, including transgenic mice expressing human CETP. In the present study, both CETP gene expression and plasma CETP mass concentration were increased in response to the apoCI-mediated hyperlipidaemia in CETPTg/apoCITg mice fed with normal chow. The resulting increase in the total plasma CE transfer activity led to typical increases in the CE content of apoB-containing lipoproteins and decreases in the size and the CE to TG ratio of HDL. Moreover, the accumulation of apoB-containing lipoprotein acceptors in CETPTg/apoCITg mouse plasma is also likely to have contributed to the increase in total plasma CE transfer activity. In support of this view, increased levels of apoB-containing lipoproteins in patients with either primary hypertriglyceridaemia [40] or Type II diabetes [41] have been associated with increased CETP activity without the need to alter the CETP mass level. Moreover, the apoCIII-mediated accumulation of TG-rich lipoproteins was shown to potentiate the CE transfer activity of CETP, which was expressed at a constant level under the control of the metallothionein promoter in CETPTg/apoCIIITg mice [42]. In the present study, and in spite of the hyperlipidaemic state, specific activity of CETP as reported for plasma CETP concentration was significantly lower in the plasma of CETPTg/apoCITg mice compared with normolipidaemic CETPTg mice. Complementary analysis revealed that the significant increase in CETP mass concentration in CETPTg/apoCITg mice was compensated proportionally by a gradual reduction in specific CETP activity (Figure 5). The molecular mechanism of the apoCI-mediated increase in CETP expression appears to be due to the hyperlipidaemic state itself and not due to a specific regulatory property of apoCI at the CETP gene promoter [43]. In support of this view, CETP expression was proven to be increased to a similar extent in CETPTg/apoE0 (apoE knocked-out) and CETPTg/LDLR0 (LDL receptor knocked-out) mouse models with a similar degree of hyperlipidaemia [23]. The hyperlipidaemia-mediated increase in CETP expression might well reflect, at least in part, the LXR-mediated activation of the CETP gene [44], an hypothesis that was sustained by increased expression of the



**Figure 5** Correlation of CETP mass concentration, total CE transfer activity and specific CETP activity with apoCI mass concentration in CETPTg/apoCITg mice

Plasma CETP and apoCI mass concentrations were determined by specific immunoassays in CETPTg/apoCITg mice ( $n = 13$ ) (see the Experimental section). CE transfer activity was determined as the initial transfer rate of fluorescent NBD-CEs from labelled liposome donors to plasma lipoprotein acceptors, and values were normalized to plasma neutral lipid levels (see the Experimental section). Specific CETP activity in individual plasmas was obtained after correction of the total CE transfer rates by CETP mass. Variation in CETP mass and activity (y-axis) was calculated as compared with mean reference values, which were determined in parallel in CETPTg mice ( $n = 6$ ). Coefficients of correlation  $\rho$  were calculated by using the Spearman correlation rank analysis.

ABCG5 and SREBP-1c genes, i.e. two other LXR responsive genes [45,46], in CETPTg/apoCITg mice.

In conclusion, the present study of CETPTg/apoCITg mice indicates that the blockade of specific CETP activity via apoCI overexpression is counterbalanced by an opposite, hyperlipidaemia-mediated effect on CETP gene expression. Thus overexpression of full-length apoCI is unlikely to represent a suitable method for decreasing plasma CE transfer activity *in vivo*.

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