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Cholesteryl ester transfer protein (CETP) increases postprandial triglyceridaemia and delays triacylglycerol plasma clearance in transgenic mice

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The CETP (cholesteryl ester transfer protein) is a plasma protein synthesized in several tissues, mainly in the liver; CETP reduces plasma HDL (high-density lipoprotein) cholesterol and increases the risk of atherosclerosis. The effect of CETP levels on postprandial intravascular metabolism of TAGs (triacylglycerols) is an often-overlooked aspect of the relationship between CETP and lipoprotein metabolism. Here, we tested the hypothesis that CETP delays the plasma clearance of TAG-rich lipoprotein by comparing human CETP expressing Tg (transgenic) and non-Tg mice. After an oral fat load, the postprandial triglyceridaemia curve was markedly increased in CETP-Tg compared with non-Tg mice (280 ± 30 versus 190 ± 20 mg/dl per 6 h respectively, $P < 0.02$). No differences in intestinal fat absorption and VLDL (very-low-density lipoprotein) secretion rates were observed. Kinetic studies of double-labelled chylomicron-like EMs (emulsions) showed that both [^{3}H]triolein and [^{14}C]cholesteryl oleate FCRs (fractional clearance rates) were significantly reduced (~20%) in CETP-Tg mice. Furthermore, TAG from lipid EM pre-incubated with CETP-Tg plasma had plasma clearance and liver uptake significantly lower than the non-Tg plasma-treated lipid EM. In addition, reductions in post-heparin plasma LPL (lipoprotein lipase) activity (50%) and adipose tissue mRNA abundance (39%) were verified in CETP-Tg mice. Therefore we conclude that CETP expression in Tg mice delays plasma clearance and liver uptake of TAG-rich lipoproteins by two mechanisms: (i) transferring TAG to HDLs and increasing CE content of the remnant particles and (ii) by diminishing LPL expression. These findings show that the level of CETP expression can influence the responsiveness to dietary fat and may lead to fat intolerance.

Key words: cholesteryl ester transfer protein (CETP), fat tolerance, lipoprotein lipase, postprandial lipoprotein, remnant, transgenic mice.

INTRODUCTION

Postprandial lipaemia is a physiological phenomenon occurring several times a day to cope with the almost complete absorption of dietary fat [1]. It is characterized by transient accumulation in plasma of potentially atherogenic particles of intestinal origin, named chylomicrons and their remnants. The potential atherogenic role of postprandial TAG (triacylglycerol)-rich lipoproteins was first hypothesized by Zilversmit [2]. These lipoproteins, particularly small chylomicrons and VLDL (very-low-density lipoprotein) remnants, may infiltrate and undergo retention in the vessel wall [3]. Several studies have shown that TAG-rich lipoprotein levels are coronary risk factors, independently of HDL (high-density lipoprotein) and LDL (low-density lipoprotein) levels [4].

CETP [CE (cholesteryl ester) transfer protein] takes part in the intravascular metabolism of both TAG-rich lipoproteins and HDL. Several studies have shown that postprandial lipaemia is accompanied by an increase in plasma CETP activity [5,6]. This mainly reflects the higher levels of substrates for the CETP-mediated reaction, which transfers CE from HDL to the ApoB (apolipoprotein B)-containing lipoproteins in exchange for TAG. Thus CETP transforms CE-enriched HDL into cholesterol-poor, TAG-enriched HDL, especially in the postprandial state. Because it reduces HDL cholesterol levels, CETP has been considered a major target for developing inhibitors aimed at reducing atherosclerosis risk [7,8]. However, recent drawbacks in clinical trials have seriously questioned this possibility [9,10].

Although the rise in plasma CETP activity in the fed state is well characterized, results from animal and human studies have not agreed on the variation of CETP mass in response to feeding or to a fat meal load. MacLean et al. [11] reported that plasma CETP concentration increased in fed compared with fasted CETP Tg (transgenic) mice. In contrast, Jiang et al. [12] showed that CETP expression levels in fasted hamsters fell after feeding. A fat load after an overnight fast did not change [13] or increased [14] plasma CETP concentration in normolipidaemic subjects.

While the consequences of postprandial lipaemia on CETP levels and cholesterol fluxes between lipoproteins have been substantially studied, the reciprocal effect of CETP levels on postprandial intravascular metabolism of TAG is an often-overlooked aspect of the relationship between CETP and lipoprotein metabolism. In the present study, we hypothesized that, by transferring TAG to HDL in exchange for CE, CETP delays plasma clearance of TAG-rich lipoproteins. This phenomenon would be better observed in the postprandial state, when plenty of TAG-rich lipoproteins are available. Since wild-type mice do not express CETP, Tg mice expressing the human CETP gene are useful models to study the consequences of CETP action on the TAG metabolism. Thus we compared human

Abbreviations used: CE, cholesteryl ester; CETP, CE transfer protein; EM, emulsion; NEFA, non-esterified fatty acid; FCR, fractional clearance rate; HDL, high-density lipoprotein; HL, hepatic lipase; LDL, low-density lipoprotein; LPL, lipoprotein lipase; LRP, LDL receptor related protein; Tg, transgenic; TAG, triacylglycerol; VLDL, very-low-density lipoprotein.

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CETP-expressing Tg mice with control wild-type littermates in terms of fat tolerance, fat absorption, VLDL secretion and TAG-rich lipoprotein plasma clearance.

MATERIALS AND METHODS

Animals

All animal protocols were approved by the university's CEEA (Committee for Ethics in Animal Experimentation) and this is in agreement with the guidelines of the COBEA (Brazilian College for Animal Experimentation) and conformed with the Guidelines on the Handling and Training of Laboratory Animals (published by the Universities Federation for Animal Welfare, U.K., in 1992). Hemizygous human CETP Tg mice (line 5203, C57BL6/J background) [15] expressing a human CETP minigene under the control of its natural flanking sequences used in the present study have been crossed with C57BL6 mice from the university's animal care centre (CEMIB/UNICAMP) for 10 years. Female CETP Tg and non-Tg littermates, between 12 and 16 weeks of age, were used in the present study. Mice were housed in a temperature-controlled room, on a 12 h light/12 h dark cycle, had free access to water and food and were placed on a standard chow diet (Nuvital CR1, Colombo, Brazil). CETP-expressing mice were genotyped by assaying the plasma CETP activity level, as previously described [16]. Plasma CETP activities expressed as percentage CE transferred from HDL to [VLDL+LDL] are: 36.6 ± 1.6 ($n = 24$) for CETP Tg mice and -0.5 ± 0.7 ($n = 30$) for non-Tg mice. In our assay, 36.6 % of HDL–CE transfer is equivalent to 14 μmol/ml per h. Terminal experiments were done in anaesthetized mice using 50 mg of ketamine/kg of body weight (Parke-Davis, SP, Brazil) and 10 mg of xylazine/kg of body weight (Bayer, SP, Brazil).

Plasma biochemical analyses

Blood samples were obtained from either the tail or the retro-orbital plexus of fasted anaesthetized mice. Plasma cholesterol and TAGs (Chod-Pap; Roche Diagnostic, Mannheim, Germany), NEFAs (non-esterified fatty acids; Wako Chemical, Neuss, Germany) and glucose (Glucose GOD; PAP-Laborlab, SP, Brazil) were determined using enzymatic colorimetric assays according to the manufacturer's instructions.

Mice fat tolerance test

Mice fasted for 12 h received a bolus of soya-bean oil (11 g/kg of body weight) through a gastric tube. Blood samples were obtained from the tail tip at 0, 60, 120, 180, 240, 300 and 360 min after the fat load (gavage) for TAG determination, as described previously [17].

Dietary fat absorption

Mice housed in individual cages had their food ingestion weighed and stools collected for 72 h. Fat content from dried and powdered food pellets and stools was measured by weighing the dried total lipid extracts [18]. Dietary fat absorption was estimated as the difference between the fat content in ingested food and that excreted in stools. Experiments were performed with two types of diets, a low-fat chow diet (4 % fat) and a high-fat diet (14 % fat). The high-fat diet was homemade and consisted of (%, w/w): 21 protein, 59 carbohydrates, 14 saturated fat (lard) and AIN93 mineral and vitamin mixtures. Mice were allowed to adapt to the high-fat diet during 1 week before the 3 day fat balance.

In vivo hepatic VLDL secretion rate

After a 12 h fasting period and 5 h after an oral fat load (gavage), anaesthetized mice were injected intravenously (femoral vein) with Triton WR1339 (500 mg/kg of body weight) [19]. At 0, 15, 30, 60, 90 and 120 min after the Triton injection, blood samples were drawn and analysed for cholesterol and TAG concentrations. Hepatic VLDL secretion rates were calculated from the slopes of the linear regression curves.

Plasma kinetics of labelled TAG-rich EM (emulsion)

The TAG-rich lipid EMs were prepared by sonication and purified by ultracentrifugation as previously described [20]. A lipid mixture containing 23 % phosphatidylcholine (Lipid Products, South Nutfield, Redhill, Surrey, U.K.), 2 % cholesterol, 6 % cholesteryl oleate and 69 % triolein (Nu-Chek Prep., Elysian, MN, U.S.A.), 13 μCi of [^{3}H]triolein and 11 μCi of [^{14}C]cholesteryl oleate (Amersham Biosciences UK) was evaporated under nitrogen flow, emulsified by sonication in 2.785 M NaCl solution at 55 °C and purified in two steps of ultracentrifugation in discontinuous gradients of NaCl and NaBr solutions in an SW 41 rotor at 22 °C (course lipid floating after 12 000 rev./min was discarded and EM particles were recovered after a second run at 36 000 rev./min for 25 min). The lipid EM was injected intravenously (femoral) into anaesthetized CETP Tg and non-Tg mice after a 12 h fasting period (1×10^6 d.p.m., 0.5 mg of TAG). In a separate set of experiments, lipid EMs were pre-incubated with plasma from CETP Tg or non-Tg mice during 20 min at 37 °C, re-isolated by 30 min ultracentrifugation and injected into non-Tg wild-type mice. Blood samples of 50 μl were taken from the tail tip at 1, 2, 3, 5 and 10 min.

Anaesthetized mice were killed by exsanguination through the retro-orbital plexus. Neutral lipid classes from plasma samples were separated by TLC in the solvent system of hexane/diethyl ether/acetic acid (70:30:1, by vol.). Bands corresponding to TAG and CE were then scraped into counting vials for radioactivity measurement in 3 ml of scintillation solution (ACS® aqueous counting scintillant; Amersham Biosciences UK) in a Beckman LS 6000 beta counter. Plasma FCRs (fractional clearance rates) of [^{3}H]triolein and [^{14}C]cholesteryl oleate were computed from monoexponential curve fitting.

Intravascular lipase activities

Total lipase activity was determined by the method of Ehnholm and Kuusi [21]. Overnight fasted mice plasmas, obtained 10 min after subcutaneous injection of heparin (100 units/kg of body weight), were incubated with a [^{3}H]triolein/Arabic-gum substrate ([9,10-^{3}H(N)]triolein; New England Nuclear, Boston, MA, U.S.A.) during 1 h. HL (hepatic lipase) activity was determined in tubes where the LPL (lipoprotein lipase) was inhibited by 2 M NaCl. The hydrolysed labelled NEFAs were extracted with methanol/chloroform/heptane (1.4:1.25:1, by vol.), dried under N_2, and their radioactivity was determined in an LS6000 Beckman beta counter. The LPL activity was calculated as the difference between the total lipase and the HL activities.

Statistical analysis

The results are presented as the means $\pm$ S.E.M. for the number of determinations (n) indicated. The statistical analyses were done with the GraphPad InStat software (version 3.00) using a Student's t test for two group comparisons. Statistical significance was defined as $P < 0.05$.

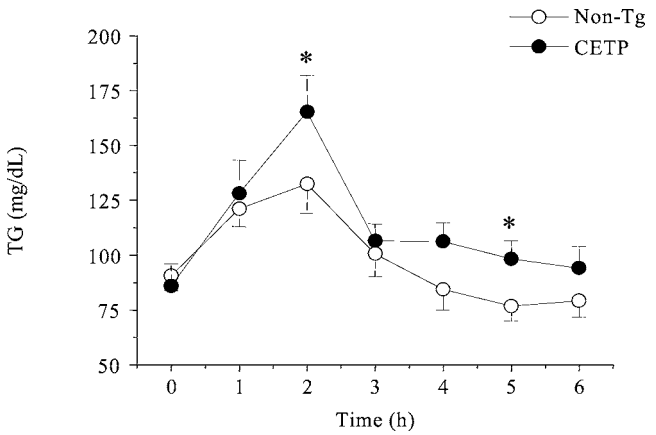

Figure 1 Oral fat tolerance test in CETP Tg and non-Tg mice

For details, see the Materials and methods section. Incremental areas under the triglyceridaemia curves are 280 ± 30 and 190 ± 20 mg/dl per 6 h for CETP and non-Tg respectively; $P = 0.02$. Results shown are means ± S.E.M. ($n = 9$–13). *$P < 0.05$.

RESULTS

Fasting plasma lipids and glucose

As expected, plasma total cholesterol levels were reduced by 20 % in CETP Tg mice compared with non-Tg mice (56 ± 2 and 71 ± 6 respectively). No differences in glucose, TAG and NEFA levels were found between the two groups (results not shown).

Fat tolerance test

The postprandial lipaemia after an oral fat load in CETP Tg and non-Tg mice is shown in Figure 1. The integrated 6 h incremental area under the triglyceridaemia curve was markedly increased in CETP Tg mice compared with their controls (280 ± 30 versus 190 ± 20 mg/dl per 6 h respectively, $P = 0.02$). A time point-by-point comparison of the curves shows statistical differences at 2 and 5 h after the fat load. The prolonged residence time of TAG in the plasma compartment of CETP Tg mice could be the result of (i) increased intestinal absorption, (ii) increased hepatic VLDL–TAG secretion and (iii) decreased clearance of TAG from circulation.

Fat absorption

A 3-day fat balance (ingestion–excretion) was performed in both groups of mice in order to estimate dietary fat absorption. The results presented in Table 1 show that there are no differences in intestinal absorption of dietary fat in both groups of mice under low-fat chow diet (4 % fat). When mice were submitted to a high-fat diet (14 % fat), we observed that non-Tg mice incorporated larger amounts of dietary fat than CETP Tg because they actually ate more food, not because they had greater intestinal fat absorption capacity, which was identical (96 %). The higher capacity of fat absorption from high-fat diet compared with low-fat diet is due to a lower content of non-digestible fibres in the high-fat diet (5 % versus 8 % respectively). The stool amount excreted by both groups when fed the low fat was much higher (~3 g) compared with high-fat diet (~0.8 g dried weight in 3 days). The important point is that there is no difference between both groups concerning the fat absorption efficiency under either diet. Thus higher postprandial plasma levels of TAG observed in CETP Tg mice (Figure 1) cannot be attributed to increases in their capacity of intestinal fat absorption.

Hepatic VLDL secretion rates

We evaluated the effect of CETP on hepatic TAG secretion rate in Triton-treated mice after an overnight fasting period (Figure 2A) and 5 h after the oral fat load (Figure 2B). No significant differences were observed in the TAG accumulation rates of CETP Tg and non-Tg mice either in fasted mice (3.2 ± 0.6 versus 3.1 ± 0.6 mg/dl per h) or 5 h after the oral fat load (11 ± 1.2 versus 12 ± 1.6 mg/dl per h). Likewise, cholesterol accumulation rates were not affected by the presence of CETP in mice in both conditions, fasting and post-fat load (results not shown).

In vivo plasma clearance of TAG-rich chylomicron-like EM particles

Previous works have shown that these protein-free lipid EMs are models to study native chylomicron metabolism [20,22,23]. These EMs promptly acquire plasma apolipoproteins and are readily hydrolysed by LPL. The plasma removal rates of EM double labelled with [^{3}H]triolein and [^{14}C]cholesteryl oleate injected intravenously into CETP Tg and non-Tg mice are shown in Figure 3. The plasma FCR of [^{3}H]triolein was significantly lower in CETP Tg than in non-Tg mice (0.157 ± 0.010 versus 0.187 ± 0.009 respectively, $P < 0.04$). The [^{14}C]cholesteryl oleate FCR was also lower in CETP Tg than in non-Tg mice (0.105 ± 0.003 versus 0.128 ± 0.006 respectively, $P < 0.004$). In a system without CETP, the CE moiety of the EM is a marker of remnant particle removal. However, in the presence of CETP, there may be considerable dilution of radioactive CE in the EM with cold CE coming from endogenous HDL, decreasing its specific activity as compared with the EM in the plasma compartment of non-Tg mice. This process may falsely indicate a decrease in [^{14}C]CE FCR in CETP-Tg. On the other hand, the plasma removal rate of [^{3}H]TAG is not subject to any artefact and results from the rate of TAG degradation by LPL, remnant tissue uptake and, in the case of CETP Tg mice, CETP-mediated transfer to HDL, which has a longer half-life in the plasma compartment.

In order to verify whether reduction in plasma TAG FCR was directly related to the action of circulating CETP on lipid EM particles, we performed another set of experiments where [^{3}H]TAG-labelled chylomicron EMs were pre-incubated with plasma from CETP Tg (EM_{CETP}) or from non-Tg mice (EM_{NTg})

Table 1 Intestinal absorption* of dietary fat in female mice expressing CETP and control non-Tg mice on a low- or high-fat diet

*Estimated as the difference between food fat ingestion and fat excretion in stools during 72 h. Low-fat chow diet: 4 % fat, and high-fat diet: 14 % fat. The same letters indicate the pair comparisons that are significantly different: a, b: $P < 0.05$ (Student's t test). Values are means ± S.E.M. ($n = 6$). 'Fat' means total lipids extracted with a chloroform/methanol Folch solution.

	Low-fat diet			High-fat diet		
	Fat ingestion (mg)	Fat absorption (mg)	%	Fat ingestion (mg)	Fat absorption (mg)	%
Non-Tg	510 ± 29.1	327 ± 35.1	64 ± 4	1546 ± 31.8[a]	1485 ± 36.6[b]	96 ± 1
CETP	512 ± 12.6	302 ± 16.0	59 ± 3	1356 ± 56.4[a]	1304 ± 61.9[b]	96 ± 1

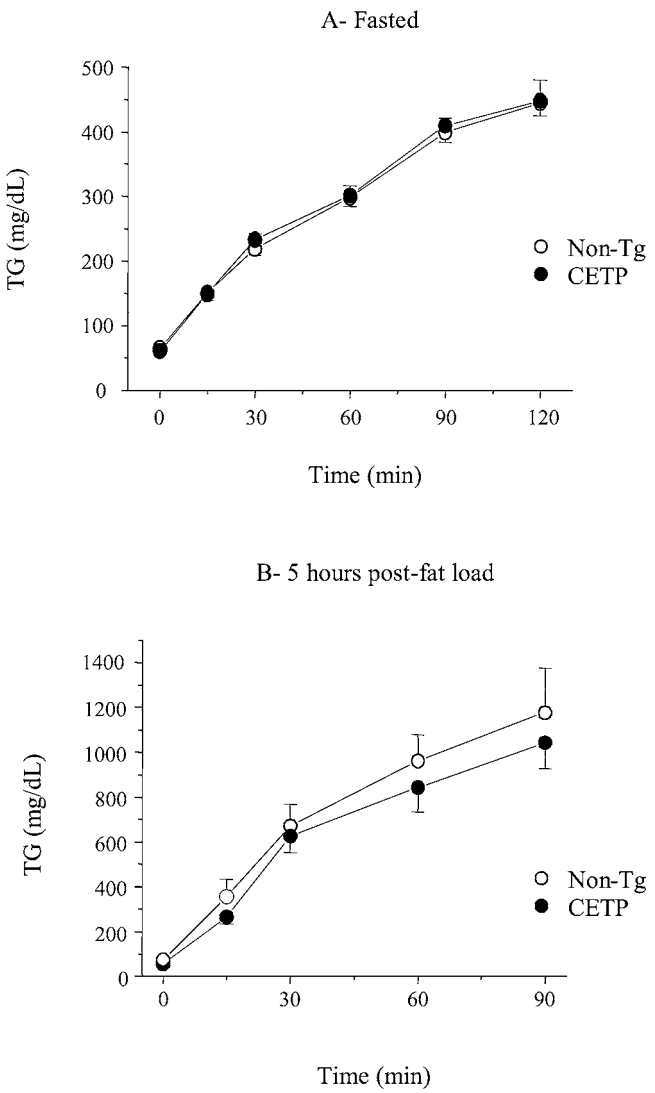

Figure 2 VLDL–TAG secretion in CETP Tg and non-Tg mice after an overnight fasting (A) or 5 h after a fat load (B)

Mice were injected with Triton WR1339 as described in the Materials and method section. Results shown are means ± S.E.M. ($n = 7$–9). Slopes of the triglyceridaemia curves are: (**A**) 3.2 ± 0.6 and 3.1 ± 0.6 mg/dl per h and (**B**) 11 ± 4 and 12 ± 3 mg/dl per h for CETP and non-Tg mice respectively.

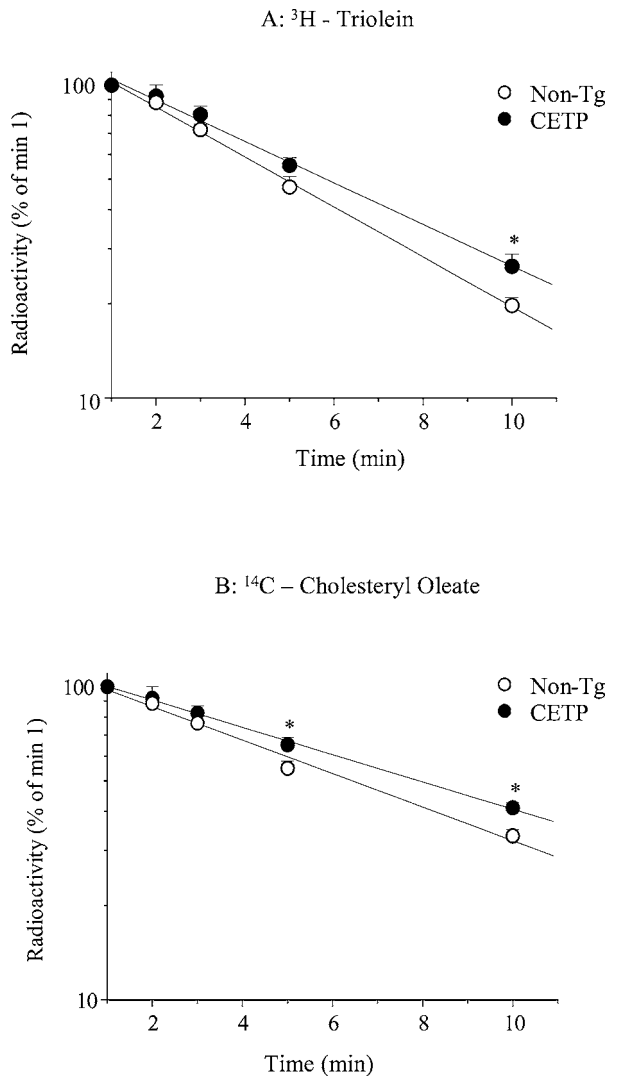

Figure 3 Plasma clearance of chylomicron-like EMs double labelled with [3H]triolein (A) and [14C]CE (B) injected intravenously in CETP Tg and non-Tg mice

Plasma FCRs are (**A**) −0.157 ± 0.010 and −0.187 ± 0.009 ($n = 8$, $P < 0.04$) and (**B**) −0.105 ± 0.003 and −0.128 ± 0.006 ($n = 8$, $P < 0.004$) for CETP and non-Tg mice respectively.

during 20 min, re-isolated and then injected into the bloodstream of wild-type (non-Tg) recipient mice. The CE content in EM_{CETP} increased by 50 % and the TAG/CE ratio decreased by 22 %. The concentrations of TAG and CE, in mg/dl, were 459 and 28 for EM_{NTG} and 562 and 42 for EM_{CETP}. The results shown in Figure 4 confirmed that CETP short time action on these particles can contribute to delaying their plasma clearance. In addition, 10 min after EM injection, the content of [3H]TAG retained in liver from CETP-modified particles (EM_{CETP}) was reduced compared with particles pre-incubated with non-Tg plasma (EM_{NTg}): 62 662 ± 2474 versus 107 224 ± 12 683 c.p.m./g respectively ($P < 0.01$, $n = 5$), although the amount of [3H]TAG found in adipose tissue was similar for both EM particles: 10 840 ± 1077 versus 10 935 ± 1434 c.p.m./g.

Plasma LPL activity

In order to investigate a possible involvement of intravascular lipolysis in the delay of [3H]TAG FCR, we measured the maximal plasma activity of LPL in post-heparin plasma from both groups of mice. We found a 50 % decrease in heparin-stimulated LPL activity of CETP Tg as compared with non-Tg plasma (228 ± 19 versus 466 ± 46 nmol/ml per h respectively, $P < 0.001$) and no significant differences in HL activity (887 ± 83 versus 1041 ± 101 nmol/ml per h). Since reduction in plasma LPL activity in CETP-expressing animals was not previously suspected, we have additionally confirmed this effect at the level of LPL mRNA in the adipose tissue: there is a 39 % decrease in LPL/β-actin mRNA ratio in CETP Tg mice as measured by RT–PCR (reverse transcription–PCR; $P = 0.0057$, $n = 6$).

LRP (LDL receptor related protein)

Since LRP is the main liver receptor for TAG-rich lipoprotein remnants, we also evaluated the mass of LRP in the liver of CETP and non-Tg mice by Western-blot analysis (Figure 5). No significant differences were observed in the relative abundance of LRP mass in both groups of mice.

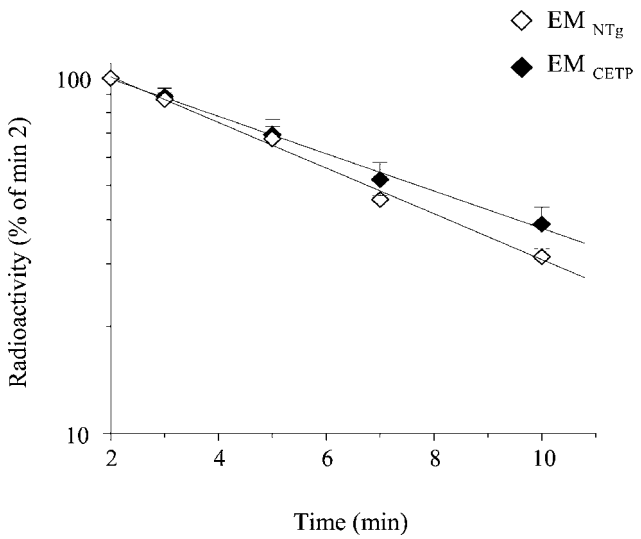

Figure 4 Plasma clearance of chylomicron-like EMs labelled with [^{3}H]triolein, pre-incubated with plasma from EM_{CETP} or from EM_{NTg} and injected into the bloodstream of non-Tg mice

Plasma FCRs are -0.122 ± 0.01 and -0.148 ± 0.006 ($n = 5$, $P < 0.05$) for EM_{CETP} and EM_{NTg} respectively.

DISCUSSION

Although genes coding for some apolipoproteins and LPL are well characterized as determinants or potential markers for postprandial lipaemic responses, studies implicating CETP in this process are lacking. In the present study, we performed functional studies designed to show direct evidence that plasma CETP activity modifies the postprandial response of TAG-rich lipoproteins. This was done by comparing TAG response in a biological context where the only genetic variation was the introduction of the human CETP gene in the mouse genome. Thus CETP levels varied as all-or-nothing in CETP Tg mice and CETP-non-expressing littermates (non-Tg) respectively. We demonstrated here that elevated levels of plasma CETP activity are associated with dietary fat intolerance. *In vitro* action of plasma CETP on TAG-rich lipoprotein models confirmed these findings.

Interestingly, human subjects with TaqI polymorphism of the gene encoding CETP that is associated with low HDL cholesterol and high CETP activities (B1 allele) also have higher plasma TAG concentrations [24]. In addition, simvastatin treatment of normotriglyceridaemic patients with coronary heart disease decreased CETP activity dose-dependently and improved the TAG response to an oral fat loading test [25]. Although improvement in the postprandial TAG plasma removal is certainly dependent on the up-regulation of LDL receptors promoted by simvastatin, our results indicate that lower CETP plasma levels may also have contributed to this effect.

Contrary to our results, Ritsch et al. [26] reported one case of a CETP-deficient patient with very high postprandial plasma levels of TAG and impaired TAG tolerance after an oral fat meal. However, in this case the CETP mutant exhibited an ApoE3/E2 phenotype (ApoE is apolipoprotein E), which is probably the main cause of the TAG intolerance. Further support for the present results is found in a recent work by Inazu et al. [27]. They showed that, among ApoE3/3 carriers, patients with CETP deficiency presented an approx. 2-fold reduction in postprandial TAG response when compared with controls.

Possible mechanisms involved in these CETP effects were further investigated in CETP Tg and non-Tg mice. Differences in intestinal fat absorption and in hepatic TAG secretion rate were ruled out. Nonetheless, kinetic studies with labelled chylomicron-like EMs showed that the lack of CETP expression accelerates TAG-rich lipoprotein plasma removal rates by approx. 20 %. The reasons for CETP-mediated delay in TAG plasma clearance can be explained by at least two mechanisms. One is related to a direct action of CETP on the particle lipid core, i.e. TAG transfer to HDL and CE enrichment of the remnant particles. This can decrease receptor affinity for remnant particles. The second mechanism may be related to a reduction in LPL expression in CETP mice. In agreement with our results, Zhou et al. [28] also found that adipocytes from adipose-tissue-specific-CETP-expressing mice presented reduced LPL expression. In addition, we showed a reduced amount of LPL in the plasma of CETP mice. At present, it is not possible to distinguish whether reduced LPL expression is a direct or indirect effect of CETP. Decreased LPL could delay TAG clearance through both enzymatic and non-enzymatic actions, since it can act as an adaptor for lipoprotein uptake. A putative reduction in the number of the main remnant receptor, LRP, was excluded since Western-blot analysis showed no significant differences between CETP and non-Tg mice LRP mass. Therefore both CETP and LPL may markedly affect the receptor affinity for

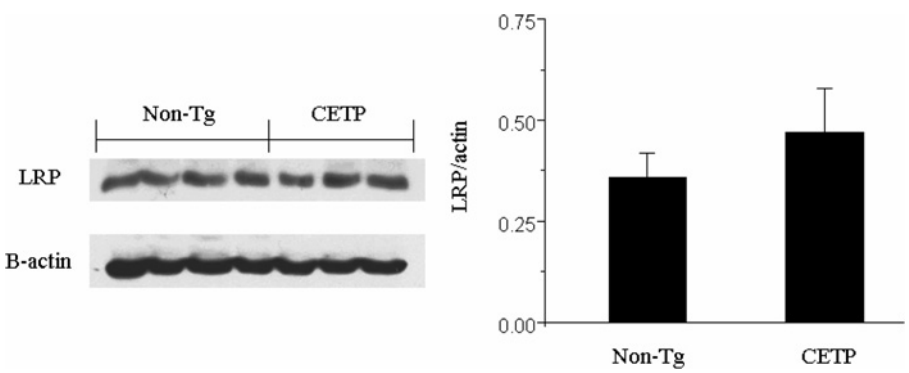

Figure 5 LRP liver mass in CETP Tg and non-Tg mice evaluated by Western blotting

Left panel: the membrane-specific bands for LRP and β-actin; right panel: densitometry of band intensities. Primary mouse monoclonal LRP (5A6; Abcam) antibody was used according to the manufacturer's instructions and subsequently incubated with secondary goat anti-mouse horseradish peroxidase-conjugated antibody. Protein bands were developed with chemiluminescence reagents.

TAG-rich lipoprotein remnants and compromise the efficiency of their tissue uptake.

The mechanisms underlying the differential lipaemic responses verified in CETP-expressing and -non-expressing isogenic littermate mice could also be valid for humans with high and low CETP activities. Accordingly, two human studies presented similar positive associations between CETP and TAG levels [24,27]. However, because of the ample genetic background variability in humans, direct confirmation with additional studies is needed.

The implications of these results for health risk may be anticipated, since postprandial hypertriglyceridaemia induces endothelial dysfunction by generating an oxidative stress [29]. These effects might be of importance in the process of atherosclerosis initiation and development, as long ago postulated by Zilversmith [2].

Our findings disclose a new aspect related to the effects of CETP expression and contribute to an improved understanding of the influence of specific genes on lipoprotein responsiveness to dietary fat.

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