CETP Inhibitor Torcetrapib Promotes Reverse Cholesterol Transport in Obese Insulin-Resistant CETP-ApoB100 Transgenic Mice

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Abstract

Insulin resistance and type 2 diabetes are associated with low HDL-cholesterol (HDL-c) levels, which would impair reverse cholesterol transport (RCT). A promising therapeutic strategy is to raise HDL with cholesteryl ester transfer protein (CETP) inhibitors, but their effects on RCT remains to be demonstrated *in vivo*. We therefore evaluated the effects of CETP inhibitor torcetrapib in CETP-apolipoprotein (apo)B100 mice made obese and insulin resistant with a 60% high-fat diet. High-fat diet over 3 months increased body weight and homeostasis model of insulin resistance index by 30% and 846%, respectively ($p < 0.01$ for both vs. chow-fed mice). Total cholesterol (TC) increased by 46% and HDL-c/TC ratio decreased by 28% (both $p < 0.05$). Compared to vehicle, high-fat-fed mice treated with torcetrapib (30 mg/kg/day, 3 weeks) showed increased HDL-c levels and HDL-c/TC ratio by 41% and 37% (both $p < 0.05$). Torcetrapib increased *in vitro* macrophage cholesterol efflux by 22% and *in vivo* RCT through a 118% increase in ^{3}H-bile acids fecal excretion after ^{3}H-cholesterol labeled macrophage injection ($p < 0.01$ for both). Fecal total bile acids mass was also increased by 158% ($p < 0.001$). In conclusion, CETP inhibition by torcetrapib improves RCT in CETP-apoB100 mice. These results emphasize the potential of CETP inhibition to prevent cardiovascular diseases. Clin Trans Sci 2011; Volume 4: 414–420

Keywords: reverse cholesterol transport, cholesteryl ester transfer protein, lipoprotein, dyslipidemia, atherosclerosis, mouse

Introduction

Dyslipidemia is a major characteristic of insulin-resistant and type 2 diabetic patients and contributes to the increased cardiovascular risk in these individuals. Although LDL-cholesterol therapies are known to reduce the risk of cardiovascular events,[1] additional therapies are required to further prevent cardiovascular diseases. As HDL-cholesterol (HDL-c) levels are inversely correlated with cardiovascular risk[2] and are known to be lower in diabetic dyslipidemia, a considerable interest has focused on the development of novel therapies that raises HDL-c.[3]

The cardioprotective effect of HDL-c would be related, at least in part, to its major role in macrophage-to-feces reverse cholesterol transport (RCT). This physiological process mediates the transport of excess cholesterol by HDL from peripheral tissues back to the liver for uptake of cholesteryl esters by the scavenger receptor class B type I (SR-BI). In the liver, cholesteryl esters are then metabolized as free cholesterol or bile acids for further excretion into the bile and ultimately in the feces.[4] In humans, another RCT pathway is represented by the cholesteryl ester transfer protein (CETP), which transfers cholesteryl esters from HDL to apolipoprotein (apo) B-containing lipoproteins VLDL/LDL for further hepatic uptake by the LDL-receptor. Increased CETP activity is thought to drive the lowering of HDL-c levels in diabetic dyslipidemia.[5] Hence, dyslipidemia would impair RCT and inhibition of CETP thus represents a relevant therapeutic strategy to raise HDL-c levels and potentially improve RCT. However, the CETP pathway is thought to be a major route for human RCT,[6] since it represents approximately 80% of the plasma cholesteryl esters output.[7] Hence, one of the major concerns with the development of CETP inhibitors is whether inhibiting the CETP pathway would alter the rate of RCT *in vivo*.

As no clinical data are available regarding the effects of CETP inhibition on human RCT, the use of animal models expressing CETP represents a relevant strategy to evaluate the effects of CETP inhibitors. Although the molecular mechanisms of RCT have been extensively investigated in mice, substantial differences exist between mouse and human RCT (e.g., mouse does not express CETP). However, introducing the expression of human CETP in mouse has been shown to induce a more human-like profile.[8,9] In this line, recent studies in transgenic mice that express both human apoB100 and human CETP (CETP-apoB100 mice) have demonstrated the relevance of this animal model to evaluate the effects of compounds raising HDL-c, such as niacin[10] and CETP inhibitor torcetrapib.[11] On another note, mouse fed a 60% high-fat diet has been described as a model of obesity and type 2 diabetes.[12]

Hence, to investigate the effects of CETP inhibition on RCT in the face of insulin resistance and dyslipidemia, we used CETP-apoB100 mice fed a 60% high-fat diet over 3 months and evaluated the effects of the CETP inhibitor torcetrapib in this animal model.

Methods

Animals and diet

All animal protocols were approved by the local ethical committee (Comité regional d'éthique de Midi-Pyrénées). Seven-week-old male human CETP/human apoB100 (CETP-apoB100) transgenic mice (Taconic, Lille Skensved, Denmark) were housed in plastic cages (seven animals/cage) containing wood shavings and maintained in a room with a 12-hour light cycle with free access to food and water. Animals were adapted to these conditions and fed a rodent chow diet (Purina chow 5001, Research Diets, New Brunswick, NJ, USA) for 1 week. This rodent chow diet was defined as the control diet. Mice were then fed the control diet ($n = 7$) or a 60% high-fat diet ($n = 14$) pelleted by Research Diets (diet D12492) over 3 months.

After 3 months of diet, mice were food deprived for 3 hours at 08:00 am and blood (25 µL/EDTA) was collected from the tail tip to measure blood glucose and plasma total cholesterol. High-fat-fed mice were randomized to create two homogenous groups with the same mean values for total cholesterol (1.78 g/L) and blood glucose (188 mg/dL). Mice were then treated for 3 weeks with either vehicle (1% DMSO/99% of a saline solution of

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0.5% hydroxypropyl methylcellulose/1% Tween 80) or torcetrapib 30 mg/kg, once daily. To evaluate the effects of high-fat diet on biochemical parameters, chow-fed mice were also treated with vehicle over 3 weeks. After 3 weeks of treatment, mice were food deprived for 3 hours at 08:00 am and blood (50 µL/EDTA) was collected from the tail tip and a pool of plasma (1 pool/group) was kept aside for fast protein liquid chromatography (FPLC) profiles and macrophage-to-plasma cholesterol efflux. Mice were then fasted overnight and blood (50 µL/EDTA) was collected from the tail tip for biochemical analysis.

To evaluate the effects of CETP inhibition on RCT, mice fed the high-fat diet recovered over 2 days after blood collection and then underwent the *in vivo* macrophage-to-feces RCT experiment, as described below.

In vitro macrophage-to-plasma cholesterol efflux

J774 macrophages were seeded in triplicates in 24-well plates (1.5×10^6 cells/well) and incubated for 24 hours in complete RPMI medium. Oxidized LDL at a final concentration of 50 µg/mL were incubated over 0.5 hour at 37°C with 1 µCi/mL ^{3}H-cholesterol in RPMI. Macrophages were then ^{3}H-cholesterol labeled/cholesterol-loaded for 24 hours using this labeling medium. After the 24-hour incubation, cells were washed and equilibrated for 4 hours with RPMI medium supplemented with 0.2% BSA. Cellular cholesterol efflux was then stimulated by adding serum-free medium containing 2.5% of pooled plasma from each group of mice (vehicle or torcetrapib-treated mice). After 4 hours, medium was collected and cell lipids were subsequently extracted with 250 µL isopropyl alcohol. For each well, ^{3}H-cholesterol released in the medium and present in the total cell lipid extract was measured by liquid scintillation counting. Background cholesterol efflux (determined in the absence of plasma) was subtracted. The percentage of cholesterol efflux was calculated as the amount of ^{3}H-cholesterol recovered in the medium divided by the total label (cells + medium) in the wells multiplied by 100. Data presented are the mean of two experiments, which were performed in triplicate.

In vivo macrophage-to-feces RCT

Preparation of J774 cells and *in vivo* RCT study were performed as previously described.[13] J774 cells obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), were grown in suspension in RPMI/HEPES supplemented with 10% FBS and 0.5% gentamicin in suspension in Nalgene Teflon flasks. Cells were radiolabeled with 5 µCi/mL ^{3}H-cholesterol and cholesterol loaded with 50 µg/mL oxidized LDL over 48 hours. Radiolabeled cells were then washed with RPMI/HEPES and equilibrated for 4 hours in fresh RPMI/HEPES supplemented with 0.2% BSA and gentamicin. Cells were pelletted by low-speed centrifugation and resuspended in MEM/HEPES prior to injection into mice.

The day of the experiment, mice were weighed and placed in individual cages. ^{3}H-cholesterol-labeled and oxidized LDL-loaded J774 cells (2.5×10^6 cells containing 10×10^6 dpm in 0.5 mL minimum essential medium) were then injected intraperitoneally. High-fat-fed mice had free access to food and water, and were treated daily with vehicle ($n = 7$) or torcetrapib 30 mg/kg twice daily ($n = 7$) over 48 hours. Blood (50 µL/EDTA) was collected from the tail tip at 24 and 48 hours to measure radioactivity released into the plasma (10 µL counted in a liquid scintillation counter). Radioactivity in HDL at 48 hours after injection was also measured after phosphotungstate/$MgCl_2$ precipitation. After 48 hours, mice were weighed, sacrificed by cervical dislocation, exsanguinated and liver was harvested from each animal and weighed. Samples of liver were then flash-frozen in liquid nitrogen and then stored at −80°C prior to western blot analysis. An approximately 50-mg piece of liver was also kept and homogenized using an ultrasounds probe in 500 µL water. The liver homogenate was then used for hepatic ^{3}H-cholesterol and ^{3}H-bile acids extraction as described for the feces analysis below.

Feces were collected over 48 hours and then stored at 4°C before extraction of cholesterol and bile acids. Fecal cholesterol and bile acids extraction was performed as previously described.[13] The total feces collected from 0 to 72 hours were weighed and soaked in Millipore water (1 mL water per 100 mg feces) overnight at 4°C. The following day, an equal volume of absolute ethanol was added, and the mixtures were homogenized. To extract the ^{3}H-cholesterol and ^{3}H-bile acid fractions, 1 mL of the homogenized samples was combined with 1 mL ethanol and 200 µL NaOH. The samples were saponified at 95°C for 1 hour and cooled to room temperature, and then ^{3}H-cholesterol was extracted two times with 3 mL hexane. The extracts were pooled, evaporated, resuspended in 500 µL toluene, and then 200 µL were counted in a liquid scintillation counter. To extract ^{3}H-bile acids, the remaining aqueous portion of the feces was acidified with concentrated HCl and then extracted two times with 3 mL ethyl acetate. The extracts were pooled together, evaporated, resuspended in 500 µL ethyl acetate, and 200 µL were counted in a liquid scintillation counter. For both cholesterol and bile acids extract, the remaining volume was evaporated and resuspended in ethanol and commercial kits were used to determine fecal total cholesterol (Biomerieux, Marcy l'Etoile, France) and total bile acids (Bioquant, San Diego, CA, USA) mass.

Results were expressed as a percentage of the radioactivity injected recovered in plasma, liver, and feces. The plasma volume was estimated as 3.5% of the body weight.

Biochemical analysis

Blood glucose was monitored using a glucometer (Roche Diagnostics, Meylan, France). Plasma insulin was determined with a commercial kit (Mercodia, Uppsala, Sweden). Total cholesterol and triglycerides were assayed using commercial kits (Biomerieux, Marcy l'Etoile, France). HDL-c was determined using the phosphotungstate/$MgCl_2$ precipitation method as previously described.[13] Non-HDL-c and HDL-c/total cholesterol ratio were calculated from HDL-c and total cholesterol values. Plasma CETP activity was measured by fluorescence using a commercial kit (Roarbiomedical, New York, NY, USA). FPLC lipoprotein profiles (total cholesterol) using pooled plasma from vehicle- or torcetrapib-treated mice were analyzed, as previously described (Briand et al., 2010). FPLC fractions corresponding to LDL and HDL were pooled into three (LDL) or four (HDL) aliquots. ApoB100, A-I, and E composition was then evaluated with coomassie staining after resolving LDL on a 3–8% Tris-Acetate gel and HDL on a Nu-PAGE 4–12% Bis-Tris gels (Invitrogen, Carlsbad, CA, USA) under reducing conditions.

Western blot analysis

Western blot analysis was performed on liver from four individuals of each treatment group (vehicle or torcetrapib). Fifty microgram of total protein were resolved by 3–8% Tris-Acetate or 4–12% Bis-Tris gels (Invitrogen) and transferred to nitrocellulose membrane (Invitrogen). Commercial antibodies were used to detect the

	Chow + vehicle	High fat + vehicle	High fat + torcetrapib
Body weight (g)	33.2 ± 1.0	43.0 ± 2.0***	42.4 ± 1.3
Blood glucose (mg/dL)	142.8 ± 5.6	196.4 ± 10.7**	176.8 ± 4.3
Plasma insulin (µU/mL)	32.0 ± 4.4	208.5 ± 16.6***	101.2 ± 24.7††
HOMA-IR (mM*µU/mL/22.5)	11.3 ± 2.2	106.6 ± 10.4***	44.5 ± 11.3††
Total cholesterol (g/L)	1.01 ± 0.06	1.47 ± 0.14**	1.30 ± 0.06
HDL-c (g/L)	0.39 ± 0.04	0.43 ± 0.05	0.61 ± 0.04††
Non-HDL-c (g/L)	0.61 ± 0.08	1.04 ± 0.10**	0.69 ± 0.04††
HDL-c/total cholesterol ratio	0.40 ± 0.05	0.29 ± 0.01*	0.47 ± 0.02†††
Triglycerides (g/L)	0.79 ± 0.10	0.80 ± 0.07	0.70 ± 0.06
CETP activity (pmol/h/µL)	43.3 ± 4.0	60.2 ± 3.3**	36.6 ± 1.6†††

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ chow + vehicle versus high fat + vehicle; †$p < 0.05$, ††$p < 0.01$, †††$p < 0.001$ high fat + vehicle versus high fat + torcetrapib.

Table 1. Effects of high-fat diet and torcetrapib in CETP-apoB100 mice (mean ± SEM, ***n*** = 7 per group).

following proteins: SR-BI and ATP-binding cassette A1 (Novus, Littleton, CO, USA) and LDL-receptor (Biovision, Mountain View, CA, USA). Antibody for βactin (Abcam, Cambridge, MA, USA) was used as a loading control. Blots were visualized with the ECL chemiluminescent detection system (GE Healthcare, Piscataway, NJ, USA).

Statistics

Data are presented as mean ± SEM. An unpaired Student's *t*-test was used to compare chow-fed versus high-fat-fed mice or vehicle- versus torcetrapib-treated mice. A $p < 0.05$ was considered significant.

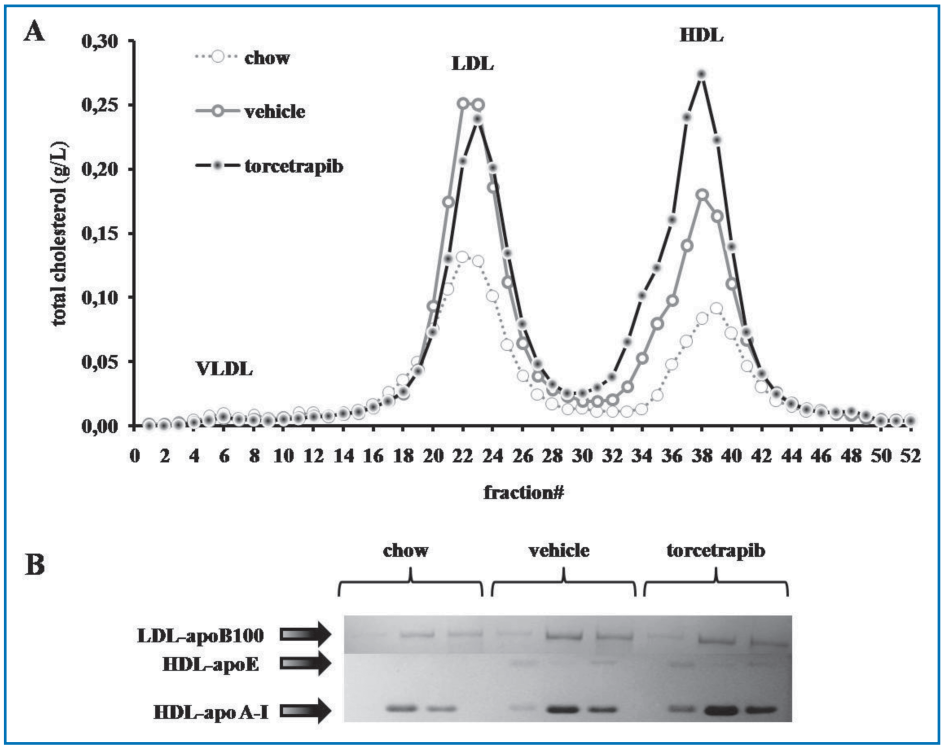

Figure 1. Fast protein liquid chromatography profiles for total cholesterol in chow-fed mice or high-fat-fed mice treated with vehicle or CETP inhibitor torcetrapib (A). Apolipoprotein (apo) B100, E, and A-I were detected by coomassie staining after pooling the fractions corresponding to LDL and HDL pics (B).

Results

High-fat diet induces obesity, insulin resistance, and dyslipidemia in CETP-apoB100 mice

The effects of the 60% high-fat diet on body weight and biochemical parameters are shown in *Table 1*. As expected, mice fed the high-fat diet showed a 29% higher body weight ($p < 0.001$ vs. chow-fed mice). Blood glucose and plasma insulin levels were significantly increased by 37% and 551%, respectively. This induced an 846% increase of the HOMA-IR index ($p < 0.001$). Total cholesterol levels and non-HDL-c levels were significantly increased by 45% and 70% in mice fed the high-fat diet ($p < 0.01$ vs. chow-fed mice). This resulted in a 28% decrease of the HDL-c/total ratio ($p < 0.05$ vs. chow). Moreover, CETP activity was increased by 39% in mice fed the high-fat diet ($p < 0.01$ vs. chow).

The overall data indicate that CETP-apoB100 mice fed the high-fat diet are obese, insulin resistant, and dyslipidemic.

Torcetrapib improves dyslipidemia in CETP-apoB100 mice under high-fat diet

The effects of CETP inhibition by torcetrapib were measured in mice fed a high-fat diet (see *Table 1*). After 3 weeks of treatment, torcetrapib decreased blood glucose by 10%, although not significantly. Unexpectedly, plasma insulin levels were reduced by 51% ($p < 0.01$ vs. vehicle), which led to a 58% reduction of the HOMA-IR index ($p < 0.01$ vs. vehicle). CETP inhibition with torcetrapib significantly increased HDL-c levels by 42% ($p < 0.01$ vs. vehicle) and raised HDL-c/total cholesterol ratio by 61% ($p < 0.001$). As expected, CETP activity was significantly reduced by 39% with torcetrapib ($p < 0.001$ vs. vehicle).

To further evaluate the effects of torcetrapib, total cholesterol lipoprotein profiles were measured by FPLC using pooled plasma from nonfasted mice (see *Figure 1*). Torcetrapib tended to reduce the increased LDL pic by the high-fat diet (*Figure 1A*). As expected, CETP inhibition by torcetrapib increased the levels of HDL and led to enlarged particles. Apo's composition was evaluated by coomassie staining of FPLC fractions corresponding to LDL and HDL (see *Figure 1B*). Expectedly, high-fat diet tended to increase LDL-apoB100 bands, but no major effect was seen with torcetrapib. High-fat

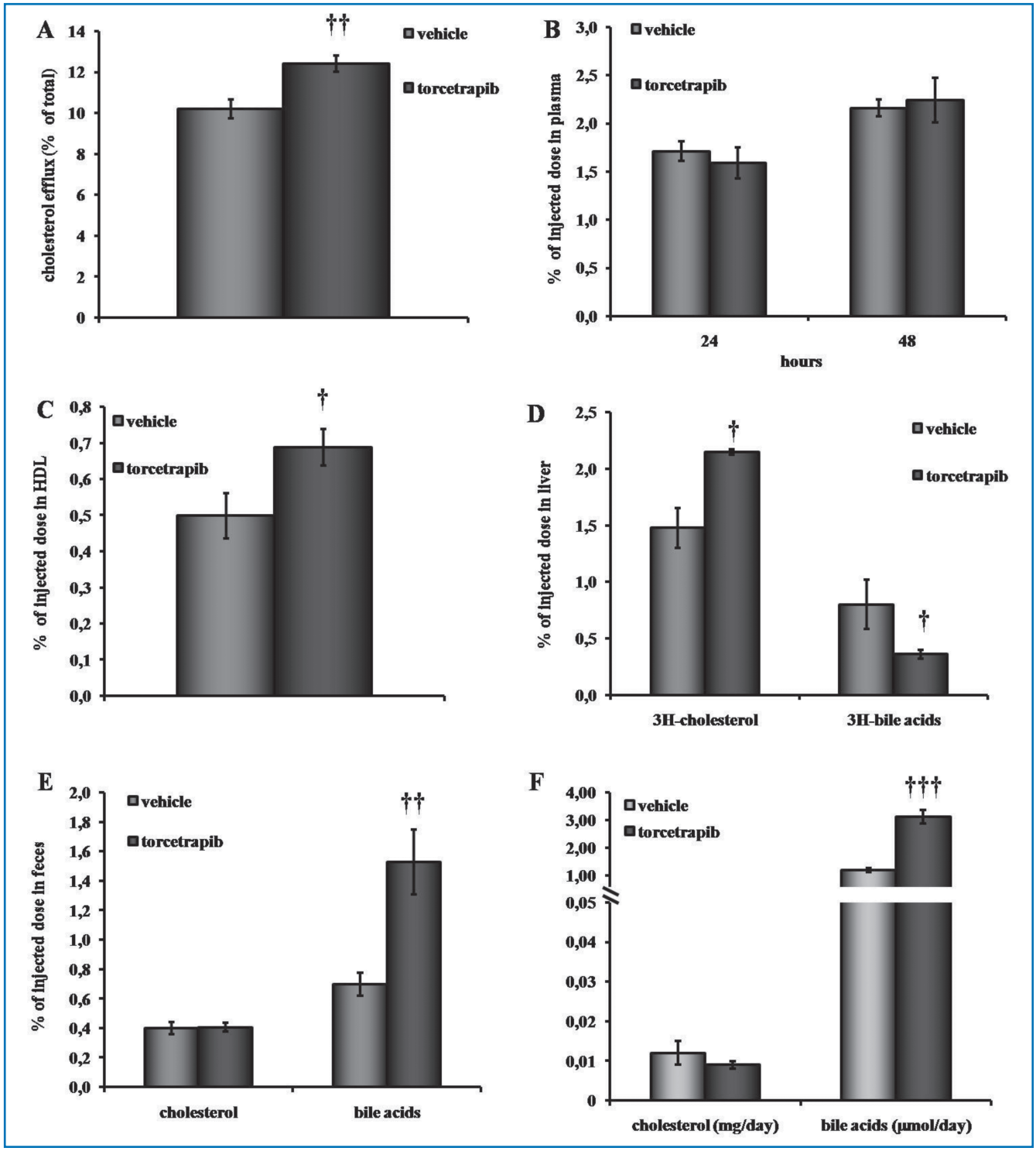

Figure 2. *In vitro* cholesterol efflux (A), *in vivo* ^{3}H-tracer appearance in plasma (B), HDL (C), liver (D), feces (E), after injection of ^{3}H-cholesterol-labeled macrophages, and fecal cholesterol/bile acids mass (F) in high-fat-fed mice treated with vehicle or torcetrapib. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ versus vehicle.

diet tended to increase the levels of apo A-I and somewhat apo E. As CETP inhibition further enlarged the HDL particles, the increase in apo A-I and also apo E was more evident in mice treated with torcetrapib.

Taken together, these data suggest that torcetrapib treatment improves dyslipidemia in obese insulin-resistant CETP-apoB100 mice.

Torcetrapib promotes macrophage-to-feces RCT in obese insulin-resistant CETP-apoB100 mice

To test whether CETP inhibition by torcetrapib promotes macrophage-to-feces RCT, ^{3}H-cholesterol-labeled/oxLDL-cholesterol-loaded macrophages were incubated *in vitro* with plasma collected from vehicle- or torcetrapib-treated mice. As shown in *Figure 2A*, macrophage-to-plasma cholesterol efflux

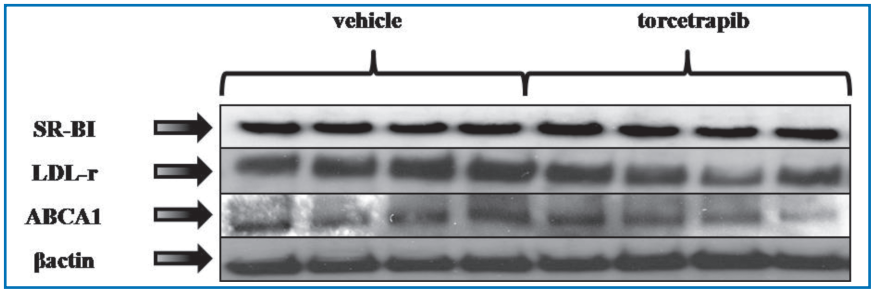

Figure 3. Expression of hepatic SR-BI, LDL-r, and ABCA1 (western blot analysis) in high-fat-fed mice treated with vehicle or torcetrapib.

was increased by 22% with plasma from torcetrapib-treated mice ($p < 0.01$ vs. vehicle). To evaluate the effect of CETP inhibition *in vivo*, mice were then injected with ^{3}H-cholesterol-labeled/oxLDL-cholesterol-loaded macrophages. As shown in *Figure 2B*, ^{3}H-tracer appearance in plasma was not different at time 24 and 48 hours after injection. However, when plasma HDL was isolated at time 48 hours (*Figure 2C*), ^{3}H-tracer appearance was increased by 38% in mice treated with torcetrapib ($p < 0.05$ vs. vehicle). As shown in *Figure 2D*, hepatic ^{3}H-cholesterol recovery after 48 hours was also increased by 45% ($p < 0.05$ vs. vehicle), while hepatic ^{3}H-bile acids were reduced by 55% ($p < 0.05$ vs. vehicle). No difference was seen in ^{3}H-cholesterol fecal excretion (*Figure 2E*). However, torcetrapib treatment significantly increased fecal ^{3}H-bile acids excretion by 118% ($p < 0.01$ vs. vehicle). To confirm the effects of torcetrapib on fecal excretion, the mass of fecal total cholesterol and bile acids was then measured. As shown in *Figure 2F*, fecal cholesterol mass remained unchanged. However, fecal total bile acids mass increased by 158% with torcetrapib ($p < 0.001$ vs. vehicle).

Overall, these data indicate that CETP inhibition with torcetrapib promotes macrophage-to-feces RCT through an increased fecal bile acids excretion in CETP-apoB100 mice.

Torcetrapib treatment does not affect liver ABCA1, SR-BI, and LDL-r expression

To test whether the increased rate of RCT could be related to hepatic transporter and receptors involved in lipoprotein/cholesterol metabolism, the expression of ATP-binding cassette A1, SR-BI, and LDL-receptor was evaluated by western blot. As shown in *Figure 3*, no change was observed with torcetrapib treatment, suggesting that improvement of RCT by CETP inhibition might be more related to its effect on plasma lipoprotein metabolism.

Discussion

Our data demonstrate that CETP inhibition with torcetrapib promotes macrophage-to-feces RCT in obese insulin-resistant CETP-apoB100 mice. One of the major concern that raised with the development of CETP inhibitors is whether the enlargement of HDL (due to cholesteryl esters enrichment) are functional particles to promote RCT.[14,15] In the CETP-apoB100 mouse model, CETP inhibition with torcetrapib also resulted in the enlargement of HDL particles (see *Figure 1*), which contained higher levels of both apo A-I and apo E. As *in vitro* macrophage cholesterol efflux was significantly increased with plasma from torcetrapib-treated mice, these changes in apo composition might have beneficial effects on the HDL quality and capacity to promote cholesterol efflux. Since apo A-I-containing particles are efficient in removing cholesterol from macrophages,[16] increased levels of apo A-I may contribute to the upregulation of *in vitro* macrophage cholesterol efflux. Meanwhile, apo E may have a substantial role in the upregulation of macrophage-to-plasma cholesterol efflux. In another study, HDL isolated from patients treated with the CETP inhibitor anacetrapib showed an increased efflux potential, which was concomitant with higher content of apo E, but not apo A-I.[17] As well, HDL from subjects with homozygous CETP deficiency[18] or moderately hyperlipidemic patients treated with torcetrapib[19] have increased apo E content, which enhances the ability to promote macrophage cholesterol efflux.

The assessment of *in vivo* RCT also indicated an increased ^{3}H-tracer appearance in the HDL fraction. This effect could be both related to increased macrophage-cholesterol efflux and changes in HDL particles turnover. For technical reasons, we were not able to evaluate the effects of CETP inhibition on HDL catabolism (large volume of plasma from vehicle- and torcetrapib-treated mice would be required to radiolabel HDL particles prior injection into other mice). In a larger animal model, other investigators demonstrated a reduced turnover of HDL particles from rabbits treated with a CETP inhibitor, while the removal of HDL-cholesteryl esters was not compromised by CETP inhibition.[20] Although this was not investigated in this study, it is possible that CETP inhibition by torcetrapib could have reduced HDL-cholesteryl ester turnover, which would contribute to increase HDL-c levels and result in higher ^{3}H-tracer appearance in the HDL fraction.

At the hepatic level, an increased ^{3}H-cholesterol recovery was observed after radiolabeled macrophage injection. However, no major change was seen regarding the expression of hepatic receptors SR-BI and LDL-receptor. Hence, upregulation of RCT upon CETP inhibition by torcetrapib might be more related to change in HDL composition rather than expression of hepatic receptors. Actually, since apoE is an effective ligand for LDL-receptor,[21] enlarged/apo E-rich HDL itself may promote the uptake of HDL-derived cholesterol. Although this remains to be determined in the CETP-apoB100 mouse model, previous studies performed in the perfused rat liver showed that hepatic uptake of apo E-rich HDL was 10-fold higher compared to apo A-I-rich HDL.[22] Moreover, *in vitro* and *in vivo* studies in mice demonstrated that hepatic uptake of HDL isolated from hyperlipidemic patients treated with torcetrapib was significantly enhanced.[23]

In regard with the last step of RCT, CETP inhibition by torcetrapib-stimulated macrophage-to-feces RCT through an increased bile acids excretion (*Figure 2D* and *E*). Although torcetrapib treatment was not associated with increased fecal sterol excretion in humans with low HDL-c levels,[24] the increased bile acids excretion by torcetrapib has been already described by others in a hamster model.[25] However, the mechanisms leading to higher bile acids excretion remain unknown. In this study, we observed a significant reduction of ^{3}H-bile acids in the liver. Hence, it is possible that enlarged/apo E-rich particles would drive HDL-derived cholesterol toward bile acids

synthesis for further biliary and fecal excretion. This hypothesis is supported by previous studies in cultured rat hepatocyte, in which stimulation of bile acid synthesis was found to be dependent on both cholesteryl esters and apo E content of the HDL particle.[26]

Taken together, these data suggest that the change in HDL composition upon CETP inhibition might be beneficial at multiple steps of macrophage-to-feces RCT in CETP-apoB100 mice.

Unexpectedly, another benefit of CETP inhibition with torcetrapib in the obese insulin-resistant CETP-apoB100 mice was the reduction of hyperinsulinemia and, to a lesser extent, blood glucose, which led to a significant reduction of the HOMA-IR index. In mice treated with CETP inhibitor torcetrapib, higher apo A-I content in HDL could have contributed to the improvement of insulin resistance. The effects of apo A-I on glucose homeostasis have been demonstrated *in vitro*[27-29] as well as *in vivo* in mouse.[27] Apo A-I stimulates phosphorylation of both AMPK and ACC and thus elevates glucose uptake *in vitro*,[27,28] while apo A-I-deficient mice have both increased plasma glucose and insulin, and exhibit impaired glucose tolerance.[27] On the contrary, apo A-I overexpression in mice appears to slightly improve insulin sensitivity.[30] As the reduction of insulin resistance was unexpected in this study, further *in vivo* experiments would be required to definitely demonstrate a beneficial effect of CETP inhibition on glucose homeostasis, such as *in vivo* hyperinsulinemic euglycemic clamp and tissue glucose uptake. On another note, it should be reminded that the clinical development of the CETP inhibitor torcetrapib was halted due to off-target effects (e.g., increased blood pressure), independent of CETP inhibition.[31,32] In this study, we cannot exclude that modulation of glucose metabolism could be due to torcetrapib itself. Hence, the effects of other CETP inhibitors should be also evaluated in this animal model to rule out this possibility.

Conclusion

Overall, our data suggest that CETP inhibition promotes RCT in a mouse model of obesity and insulin resistance with a human-like lipoprotein profile. Despite the failure of torcetrapib in the clinical setting, the development of other CETP inhibitors is still ongoing.[33,34] While larger clinical trials will help to demonstrate the relevance of CETP inhibition in reducing cardiovascular risks, preclinical experiments are also needed to test whether a given CETP inhibitor has the potential to promote RCT. While CETP inhibitors, including torcetrapib, have shown some positive effects in normolipidemic animal models,[25] it remains important to evaluate whether CETP inhibition is still beneficial in the face of diabetic dyslipidemia for better translation toward the clinical setting. These data suggest that the obese insulin-resistant CETP-apoB100 mouse could be a useful model in this line of investigations.

Conflict of Interest

François Briand, Quentin Thieblemont, and Thierry Sulpice are employees of Physiogenex. Agnès André and Khadija Ouguerram have no conflict of interest.

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