Torcetrapib Differentially Modulates the Biological Activities of HDL2 and HDL3 Particles in the Reverse Cholesterol Transport Pathway

Giovanna Catalano, Zélie Julia, Eric Frisdal, Benoit Vedie, Natalie Fournier, Wilfried Le Goff, M. John Chapman, Maryse Guerin

Objective—Therapeutic strategies to raise low plasma HDL-cholesterol levels, with concomitant normalization of the intravascular metabolism, physicochemical properties, and antiatherogenic function of HDL particles, are a major focus in atherosclerosis prevention.

Methods and Results—Patients displaying Type IIB hyperlipidemia (n=14) and healthy controls (n=11) were recruited. After drug washout, dyslipidemic patients first received atorvastatin (10 mg/d) for 6 weeks and subsequently torcetrapib/atorvastatin (60/10 mg/d) for the same period. Partial CETP inhibition markedly reduced supranormal CE transfer rates to normal levels from HDL3 (−58%; P<0.0001) to apoB-lipoproteins; endogenous CE transfer rates from HDL2 to apoB-lipoproteins were markedly subnormal as compared to those in control subjects (0.7±0.9 versus 29.3±4.8 µgCE/h/mL plasma, respectively). Torcetrapib enhanced the subnormal capacity of HDL2 particles from dyslipidemic patients to mediate free cholesterol efflux via both SR-BI and ABCG1 pathways (38%; P<0.0003 and +35%; P<0.03, respectively) as compared to baseline. In vitro observations and in vivo studies in mice demonstrated that CETP inhibition was associated with an enhanced selective hepatic uptake of CE from HDL particles (1.7-fold; P<0.0003).

Conclusion—CETP inhibition partially corrected the abnormal physicochemical and functional properties of HDL2 and HDL3 particles in type IIB hyperlipidemia. Enhanced hepatic selective uptake of HDL-CE may compensate for attenuated indirect CE transfer to apoB-containing lipoproteins via CETP attributable to torcetrapib. (Arterioscler Thromb Vasc Biol. 2009;29:268-275.)

Key Words: CETP ■ torcetrapib ■ high density lipoprotein ■ cellular cholesterol efflux ■ HDL-CE uptake

Substantial evidence from prospective epidemiological studies attests to the cardioprotective role of elevated levels of HDL-cholesterol.1 The reverse cholesterol transport (RCT) pathway is frequently cited as the primary mechanism by which HDL protects against atherosclerosis and by which it may induce plaque regression.2 The atheroprotective nature of HDL particles reflects a composite of multiple mechanisms.3 In particular, HDL particles are intimately involved in the centripetal movement of free cholesterol from peripheral tissues, including the vessel wall, to the liver for its excretion, transformation or recycling.4

The antiatherogenic properties of HDL can however be compromised in metabolic diseases associated with elevated cardiovascular risk.5 Type IIB hyperlipidemia is a characteristic feature of metabolic diseases including type 2 diabetes and metabolic syndrome, and is frequently associated with premature atherosclerosis. Furthermore, Type IIB hyperlipidemia is characterized by an imbalance between excess circulating levels of proatherogenic apoB-lipoproteins in the form of VLDL, VLDL-remnants, and LDL, among which VLDL-1 and small dense LDL predominate, relative to subnormal levels of antiatherogenic apoAI-lipoproteins.5,6,7 In atherogenic dyslipidemias involving a low HDL phenotype associated with a moderate or marked degree of hypertriglyceridemia, HDL particles typically display pronounced structural, compositional, and functional alterations.1,5 Indeed, enrichment of HDL particles in triglyceride at the expense of cholesteryl ester is the most frequent abnormality in HDL lipid composition, and results directly from concomitant elevation in circulating levels of triglyceride-rich lipoproteins and in CETP activity.7,8 Accelerated plasma apoAI clearance and shedding of ApoAI from TG-rich HDL particles after lipolysis by hepatic lipase contribute to attenuated HDL particle stability, with reduction in plasma residence time,
and concomitantly in HDL-C and apoAI levels. In addition, modification of the core lipid content of HDL particles as a result of CETP-mediated CE–TG heteroexchange alters the conformation of ApoAI domains that are critical for HDL to act as lipid acceptors; equally, such modification impairs the capacity of TG-enriched HDL particles to deliver CE to the liver through the SR-BI pathway.

Therapeutic strategies based on raising plasma HDL levels with concomitant normalization of their intravascular metabolism, physicochemical properties, and function, have become a major focus of research for the treatment of atherosclerosis and CVD. At present, CETP inhibitors represent the pharmacological strategy of greatest efficacy for elevation of plasma HDL-C levels in both fasting and postprandial states. The indirect reverse cholesterol transport pathway, involving CETP-mediated transfer of CE from HDL to VLDL, IDL, and LDL, predominates quantitatively as the major mechanism for return of CE from peripheral tissues to the liver in human plasma. Pharmacological inhibition of CETP might therefore disrupt this pathway and might theoretically impair cholesterol flux from peripheral tissues to the liver. Inhibitors of CETP do not, however, alter fecal excretion of cholesterol in human subjects, thereby suggesting that any potential reduction in cholesterol flux through the indirect pathway might be counterbalanced by acceleration of cholesterol transport through the direct RCT pathway. Consistent with this hypothesis, torcetrapib therapy preferentially generates large CE-rich HDL particles which efficiently promote cholesterol efflux from macrophages through the ABCG1-dependent pathway. Large HDL particles have equally been shown to efficiently efflux cellular cholesterol via the SR-BI pathway, thereby suggesting that SR-BI-mediated cholesterol efflux might also be enhanced after CETP inhibition.

CETP inhibitors are of potential relevance to the therapeutic normalization of defects in both the quantitative and qualitative characteristics of HDL in common dyslipidemic phenotypes associated with premature atherosclerosis such as Type IIB hyperlipidemia. We therefore evaluated the impact of torcetrapib on the physicochemical and functional properties of HDL and its major subfractions, HDL2 and HDL3, in Type IIB hyperlipidemia. These studies have revealed that CETP inhibition significantly reduced the CE donor activity of HDL2 from type IIB patients to subnormal levels; by contrast, absolute CE transfer rates from HDL3 were normalized. Torcetrapib-mediated inhibition of CETP significantly enhanced the capacity of HDL2 particles from dyslipidemic patients to mediate cellular free cholesterol efflux via both SR-BI and ABCG1 pathways. Finally, torcetrapib-mediated CETP inhibition was associated with an enhanced capacity of HDL particles to mediate selective hepatic uptake of CE.

Methods

Subjects and Blood Samples
Fourteen males aged between 36 and 59 years (mean: 46 ± 7 years) displaying a combined hyperlipidemia typical of the Type IIB lipid phenotype (fasting plasma levels of total cholesterol ≥ 230 mg/dL, triglycerides ≥ 150 mg/dL, and apolipoprotein B ≥ 140 mg/dL), and 11 healthy nondyslipidemic male volunteers aged between 27 and 62 years (mean: 42 ± 5 years) were selected for the study (supplemental Table I, available online at http://atvb.ahajournals.org). Patients were excluded if they displayed dysbetalipoproteinemia, diabetes mellitus, secondary causes of hyperlipidemia such as uncontrolled hypothyroidism, renal impairment or nephrotic syndrome, or known liver or muscle disease. Other exclusion criteria included uncontrolled hypertension, or a history of a major cardiovascular event. Type IIB patients and normolipidemic control subjects were nonobese and were matched for body mass index.

Dyslipidemic patients had ceased taking lipid-lowering drugs and stabilized their diet (AHA Step one diet or equivalent) over a 6-week period immediately before entering into the study. At week 0, a 6-week period of active treatment with atorvastatin only at 10 mg/d was initiated, followed by a 6-week period of active combination torcetrapib/atorvastatin therapy (60/10 mg/d).

Blood samples were obtained after an overnight fast at baseline (before treatment), after 6 weeks of atorvastatin alone (A) and after 6 weeks of torcetrapib/atorvastatin treatment (TA). Blood was collected by venipuncture from the antecubital vein into sterile EDTA-containing tubes (final concentration of EDTA, 1 mg/mL), and plasma separated immediately by low-speed centrifugation (2500 rpm) for 20 minutes at 4°C and stored at ~80°C until use.

The study was performed in accordance with the ethical principles set forth in the Declaration of Helsinki. The study protocol was reviewed and approved by an Ethics Committee. Written informed consent was obtained from all patients.

Details of biochemical analysis, lipoprotein fractionation and characterization, endogenous plasma CETP activity, free cholesterol efflux assays, in vitro and in vivo determination of selective uptake of HDL-CE, and statistical analysis are available in the supplemental Methods.

Results

The effects of CETP inhibition on plasma lipid and apolipoprotein levels and on HDL subfractions are presented in details in the supplemental Results.

Effects of CETP Inhibition on Cellular Free Cholesterol Efflux
To evaluate the impact of CETP inhibition on the capacity of whole plasma to mediate cellular free cholesterol efflux, we used 3 cellular models, each representative of a specific cholesterol efflux pathway (see supplemental Results).

The capacity of isolated HDL2 and HDL3 subfractions to mediate cellular free cholesterol efflux via SR-BI, ABCG1, and ABCA1 pathways is shown in Figure 1. HDL2 particles isolated from Type IIB dyslipidemic plasmas at baseline displayed an impaired capacity to mediate cholesterol efflux through both SR-BI (−32%; P < 0.0001) and ABCG1 (−20%; P < 0.02) pathways as compared to control subjects. However, torcetrapib-mediated inhibition of CETP significantly increased and restored the capacity of HDL2 particles from dyslipidemic patients to mediate free cholesterol efflux via both SR-BI (+38%; P < 0.003) and ABCG1 (+35%; P < 0.03) pathways as compared to baseline before drug therapy. By contrast, HDL3 particles isolated from Type IIB dyslipidemic plasmas displayed an impaired capacity to mediate cholesterol efflux through both SR-BI (−58%; P < 0.0001) and ABCA1 (−47%; P < 0.0001) pathways as compared to control subjects. Significantly, torcetrapib-mediated inhibition of CETP did not normalize the defective efflux capacities of HDL3 particles.
cholesterol ester transfer from HDL to apoB-lipoproteins was significantly reduced (−18%; \(P<0.001\)) in Type IIB patients. As expected, combination of torcetrapib with atorvastatin induced an additional significant reduction in total endogenous plasma CETP activity in Type IIB patients as compared to atorvastatin alone (−49%) and equally as compared to baseline (−60%). Interestingly, when individual donor HDL particle subfractions were considered, we observed that the HDL2 subfraction acted as the major CE donor in normolipidemic control subjects, accounting for 79% of total cholesterol ester transferred from HDL. By contrast, in Type IIB patients, the CE transfer rate from HDL2 was significantly reduced (−29%; \(P<0.001\)), whereas that from HDL3 was considerably increased (3.8-fold) as compared to normolipidemic controls. When the total CE mass transferred from HDL was expressed as a function of plasma lipoprotein donor concentration, the capacity of each HDL subfraction to donate CE can be estimated.21 Thus, in normolipidemic subjects, HDL2 particles displayed a 3-fold superior capacity to transfer CE in comparison with HDL3, whereas HDL2 and HDL3 subfractions in type IIB patients at baseline possessed an equivalent capacity to donate CE as a result of a 3.8-fold increase in the capacity of HDL3 from dyslipidemic patients to transfer CE as compared to those from control subjects.

In Type IIB patients receiving atorvastatin alone, the CE transfer rate from HDL2 to apoB-lipoproteins was significantly reduced (−26%; \(P<0.0001\)) as compared to baseline, whereas that from HDL3 remained unchanged (Table). Furthermore, the capacity of HDL2 subfractions from type IIB patients to transfer CE to apoB-lipoproteins was significantly lowered (−28%; \(P<0.001\)) by atorvastatin therapy as compared to baseline. Torcetrapib-mediated inhibition of CETP further reduced the CE transfer rate from HDL2 as compared to atorvastatin alone (−29%; \(P<0.02\)) such that it was 3-fold lower than that in control subjects; in addition, CE transfer from HDL3 was normalized (−58%; \(P<0.0001\)) only by combination torcetrapib/atorvastatin therapy. Moreover, CETP inhibition significantly lowered the capacity of HDL2 (−60%; \(P<0.0005\)) from type IIB patients to donate CE to atherogenic particles as compared to atorvastatin alone (2-fold less) and baseline (3-fold less). By contrast, combination therapy reduced the CE transfer capacity of HDL3 to levels typical of control subjects.

**Effects of CETP Inhibition on Selective CE-Uptake in the Liver**

The effect of torcetrapib-mediated inhibition of CETP on the capacity of HDL particles to deliver cholesterol esters to the liver was evaluated in vitro using hepatic cellular models of human origin (HepG2) and of murine origin (Fu5AH) (Figure 2), and in vivo in C57Bl/6 mice (Figure 3). In vitro, HDL particles isolated from the plasma of type IIB patients displayed a reduced capacity (\(P<0.05\)) to deliver CE to hepatic cells as compared to those isolated from the plasma of normolipidemic controls subjects (−25% and −21% in Fu5AH and HepG2 cells, respectively). By contrast, HDL particles isolated from type IIB patients treated with combination torcetrapib/atorvastatin therapy showed a significant elevation (1.7-fold; \(P<0.0003\)) in their capacity to deliver CE.
to hepatic cells as compared to those from patients at baseline.

Metabolic studies of radiolabeled HDL were performed in C57Bl/6 mice to evaluate the impact of CETP inhibition on HDL-CE uptake by the liver in vivo. Time course curves of plasma radioactivity decay of human labeled HDL-CE injected into mice revealed that HDL particles isolated from torcetrapib/atorvastatin-treated type IIB patients displayed an enhanced activity for hepatic uptake of CE as compared to HDL isolated from atorvastatin-treated or untreated patients (Figure 3A). Indeed, analysis of plasma radioactivity disappearance revealed that 30% of the radioactivity was cleared between 2 hours and 6 hours after injection of HDL particles from type IIB patients at baseline or after atorvastatin alone, whereas the disappearance of radioactivity attained 44% when HDL from type IIB torcetrapib/atorvastatin-treated patients were injected (Figure 3B). Tissue uptake of radioactive CE derived from HDL was significantly increased (+52%; P<0.03) in the liver after injection of HDL particles isolated from type IIB patients receiving combination torcetrapib/atorvastatin as compared to those from the same patients before or after atorvastatin alone (Figure 3C). Thus, both our in vitro and in vivo observations are consistent in suggesting that HDL particles formed on torcetrapib-mediated CETP inhibition are endowed with an enhanced capacity to mediate selective hepatic uptake of CE.

**Discussion**

The present studies have focused on the identification of structural and functional anomalies in the major HDL subfractions, ie, HDL2 and HDL3, in patients displaying a mixed dyslipidemic phenotype (Fredrickson Type IIB), and have tested the hypothesis that efficacious inhibition of the indirect reverse cholesterol transport pathway targeted to CETP would favor normalization of such structural and functional defects. Indeed, we have demonstrated that large cholesteryl ester-rich HDL2 particles in such dyslipidemic patients displaying subnormal HDL-cholesterol levels are defective in their capacity to mediate cholesterol efflux through both the SR-BI and ABCG1 pathways when compared to the corresponding subfraction in normolipidemic subjects on a per particle basis. Equally, the capacity of HDL particles to deliver CE to the liver by selective uptake both in vivo and in vitro is impaired in this atherogenic hyperlipidemia. By contrast, the HDL3 subfraction from Type IIB patients displayed a supranormal capacity to transfer CE to apoB-lipoproteins through the action of CETP when evaluated by an assay dependent on the endogenous content of CE donor (HDL) and acceptor particles (VLDL, IDL, and LDL). Significantly, torcetrapib-mediated inhibition of CETP favored normalization of the major abnormal physicochemical properties of HDL particles in our dyslipidemic patients, including both the neutral lipid core ratio of CE/TG and their capacity to mediate cholesterol efflux through both the reverse cholesterol transport pathway targeted to CETP.
Treatment with a combination of torcetrapib/atorvastatin induced marked elevation in HDL2 levels; moreover, HDL2 particles on treatment were enriched in CE, phospholipids, and apoAI, but in contrast, depleted in triglycerides as compared to baseline and treatment with atorvastatin alone. Such modifications were consistent with torcetrapib-induced partial inhibition of plasma CETP activity.\textsuperscript{18,21} Inversely, CETP-mediated neutral lipid transfer from HDL to apoB lipoproteins is primarily associated with reduction in HDL particle size as well as with dissociation of apoAI from HDL.\textsuperscript{22} As large HDL can efficiently efflux cellular cholesterol via the SR-BI pathway,\textsuperscript{19} it was essential to evaluate whether such SR-BI-mediated cholesterol efflux might be enhanced after CETP inhibition. Indeed, large HDL2 particles generated under torcetrapib treatment displayed elevated capacity to mediate cellular cholesterol efflux via the SR-BI pathway in vitro. Moreover, and in agreement with earlier observations,\textsuperscript{18} torcetrapib-mediated CETP inhibition enhanced the capacity of HDL2 particles to mediate cellular free cholesterol efflux via the ABCG1 pathway. It is relevant, however, that Yvan-Charvet et al\textsuperscript{18} reported earlier that CETP inhibition by torcetrapib enhanced the ability of HDL to promote free cholesterol efflux via the ABCG1 pathway only at the highest dose (120 mg/d) of CETP inhibitor in moderate hypercholesterolemic subjects and that this dose was without effect on the SR-BI mediated efflux pathway. These apparently conflicting observations may, in all likelihood, result from differences in the subject populations between the two studies. Indeed, we presently report that HDL particles isolated from type IIB hyperlipidemic plasmas displayed an altered capacity to mediate cholesterol efflux via both SR-BI and ABCG1 pathways as compared to normolipidemic controls, and that such impaired capacity was essentially normalized on combination atorvastatin/torcetrapib therapy (see supplemental Discussion).

In human plasma, the major pathway for the return of HDL-CE to the liver occurs via apoB lipoproteins after CETP-mediated CE transfer.\textsuperscript{23} However, it is significant that torcetrapib-mediated partial inhibition of CETP resulted in elevation in the capacity of HDL particles to deliver CE to the liver, thereby facilitating hepatic uptake of CE via the direct reverse cholesterol transport pathway. Thus, the degree of CETP inhibition might incrementally influence cholesterol flux through this pathway in Type IIB hyperlipidemia. The relationship of the content of apoE in HDL under conditions of low CETP activity remains controversial. Several studies have reported increased levels of apoE-rich HDL particles in CETP-deficient subjects.\textsuperscript{24–26} Partial CETP inhibition by torcetrapib in monotherapy induces elevation in apoE content in large HDL particles at the highest dose (120 mg/d), whereas the lower dose (60 mg/d) was without effect on HDL-apoE in moderate hypercholesterolemic subjects.\textsuperscript{18} In the present study, plasma levels of apoE in type IIB patients were significantly decreased by 25% (\textit{P}<0.0001) after atorvastatin alone as compared to baseline. No significant variation in plasma apoE levels was, however, detected between atorvastatin-treated patients and those receiving combination atorvastatin/torcetrapib therapy. As large HDL particles containing apoE represent a minor lipoprotein subspecies within the circulating apoAI-containing HDL particle pool, as atorvastatin therapy leads to upregulation of hepatic LDL receptors, it is likely that large apoE-enriched HDL particles generated after CETP inhibition by torcetrapib therapy were rapidly cleared from the circulation.

Our present observations on the impact of torcetrapib-mediated CETP inhibition on key steps of the reverse cholesterol transport pathway in HDL and its apoE content are consistent with those of Yvan-Charvet et al\textsuperscript{18} in that CETP inhibition by torcetrapib induced an increase in apoE content in large HDL particles at the highest dose (120 mg/d) whereas the lower dose (60 mg/d) was without effect on HDL-apoE in moderate hypercholesterolemic subjects.\textsuperscript{18} In the present study, plasma levels of apoE in type IIB patients were significantly decreased by 25% (\textit{P}<0.0001) after atorvastatin alone as compared to baseline. No significant variation in plasma apoE levels was, however, detected between atorvastatin-treated patients and those receiving combination atorvastatin/torcetrapib therapy. As large HDL particles containing apoE represent a minor lipoprotein subspecies within the circulating apoAI-containing HDL particle pool, as atorvastatin therapy leads to upregulation of hepatic LDL receptors, it is likely that large apoE-enriched HDL particles generated after CETP inhibition by torcetrapib therapy were rapidly cleared from the circulation.
cholesterol pathway allow us to propose an integrated mechanism of action of CETP inhibitors on cholesterol metabolism in patients displaying the Type IIB phenotype (Figure 4). In the normolipidemic state, plasma free cholesterol efflux from peripheral tissue occurs via specific ABC membrane transporters, ABCA1 and ABCG1, or via receptors such as SR-BI/CLA-1 (Figure 4A). Free cholesterol is taken up by lipid poor-ApoAI complexes, preβ/HDL, or mature HDL particles according to the receptor/transporter involved. LCAT allows the transformation of lipid-poor preβ/HDL to HDL3 and subsequently to HDL2, thereby generating large CE-enriched HDL2. CETP redistributes CE from HDL to apoB-lipoproteins.3 In normolipidemic subjects, such CE transfer mainly involves HDL2 and LDL particles.27 The final step of the RCT pathway represents the return of CE to the liver, via selective HDL-CE uptake involving SR-BI/CLA-1 or via uptake of LDL by specific hepatic LDL-receptors. By contrast, key steps of the reverse cholesterol transport pathway are altered in Type IIB hyperlipidemia as compared to the normolipidemic state (Figure 4B). Firstly, the capacity of HDL particles to mediate free cholesterol efflux from peripheral tissues with subsequent CE delivery to the liver is impaired in this dyslipidemic state. Thus, rates of CE transfer from HDL to apoB-lipoproteins are accelerated as a result of an increase in both plasma CETP level and circulating number of apoB-containing CE acceptor particles.28 In contrast with normolipidemic subjects, type IIB patients are characterized by elevated rates of CE transfer from HDL3 to TRL particles which are the primary CE acceptors in this phenotype.29 Such elevated CETP-mediated heterotransfer of neutral lipid between HDL and TRL contributes to reduction in HDL-C levels and favors the formation of TG-enriched HDL particles. Type IIB hyperlipidemia also involves an atherogenic lipid triad characterized by elevated circulating levels of TRL, including VLDL, VLDL remnants, and IDL, and a predominance of atherogenic small dense LDL particles.30 This triad results in an imbalance attributable to an excess of cholesterol in atherogenic apoB lipoproteins relative to that in atheroprotective HDL, thereby favoring peripheral cholesterol deposition and enhanced atherogenesis.31 HMG-CoA reductase inhibitors, such as atorvastatin, primarily act by inhibiting cellular biosynthesis of cholesterol in the liver (Figure 4C). The reduction of intracellular cholesterol content induces hepatic LDL receptor expression and as a direct consequence, enhances uptake of all apoB lipoproteins by the liver.32 In this way, atorvastatin therapy decreases
plasma levels of TRL and equally those of LDL subfractions including small dense LDL. In addition, atorvastatin attenuates CETP-mediated CE transfer from HDL to TRL, mainly as a result of reduced CE transfer rates from HDL2, whereas that from HDL3 is not affected and remains elevated. Such statin-mediated reduction in endogenous plasma CETP activity results in retention of CE in HDL with induction of an elevation in numbers of large HDL2 particles, thereby enhancing the capacity of whole plasma to promote cellular cholesterol efflux via the SR-BI/CLA-1 pathway. By contrast, atorvastatin has no impact on the attenuated capacity of HDL particles from type IIB patients to mediate free cholesterol efflux from peripheral cells with subsequent CE delivery to the liver. Nonetheless, atorvastatin normalizes the quantitative and qualitative features of apoB lipoproteins in large part, but appears to lack impact on functionally defective HDL particles in Type IIb hyperlipidemia. Torcetrapib-mediated inhibition of CETP, on a background of atorvastatin therapy, normalizes several features of the functionality of defective HDL particles and of intravascular HDL remodeling in this phenotype (Figure 4D). Elevation in plasma levels of large HDL2 particles is associated with normalization of core neutral lipid content (CE/TG ratio) of both HDL2 and HDL3 subspecies, with potential correction of apoAI conformation and thence their biological activities. Partial CETP inhibition enhances the capacity of HDL2 particles to mediate free cholesterol efflux from peripheral cells via both SR-BI/CLA-1 and ABCG1 pathways, whereas altered efflux capacities of HDL3 from type IIB patients are not corrected. Equally, CETP inhibition enhances the capacity of HDL particles to deliver CE to the liver for selective uptake. Critically, partial CETP inhibition reduces supranormal CE transfer rates from HDL3 to apoB lipoproteins and thus attenuates their potential atherogenicity by reducing their core CE content and cholesterol load. Clearly then, pharmacological inhibition of CETP, in association with statin-mediated upregulation of hepatic LDL receptors, accelerates key steps of both the direct and indirect reverse cholesterol transport pathways, thereby maintaining efficient removal of cholesterol from peripheral tissues and the arterial wall with return to the liver.

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Disclosures
None.

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SUPPLEMENT MATERIAL

SUPPLEMENTAL METHODS

BIOCHEMICAL ANALYSIS

The lipid contents of plasma and isolated lipoprotein fractions, total protein and apoAI, apoAII and apoB were quantified with an Autoanalyzer (Konelab 20). Reagent kits from Roche Diagnostics and ThermoElectron were used for determination of total cholesterol and triglyceride levels, respectively. The levels of unesterified cholesterol and phospholipids were determined with commercial reagent kits (Wako Diagnostics). Cholesteryl ester (CE) mass was calculated as (TC-FC) x 1.67, and thus represents the sum of the esterified cholesterol and fatty acid moieties. The Bicinchoninic acid assay reagent (Pierce) was utilized for protein quantification. Fasting plasma LDL-C was calculated using the Friedewald formula. HDL-C levels were determined after dextran sulphate-magnesium precipitation of apoB-containing lipoproteins. Plasma ApoAI, ApoB and ApoAII concentrations were determined using immunoturbidimetric assays (ThermoElectron reagents and calibrators; Wako Diagnostics reagents and calibrators).

ISOLATION OF HDL SUBFRACTIONS

Lipoproteins were isolated from plasma by isopycnic density gradient ultracentrifugation in a Beckman Sw41 Ti rotor at 40000 rpm for 48 hours in a Beckman XL70 at 15°C and by a slight modification of the method of Chapman et al. as previously described. Lipoprotein mass concentration expressed in mg/dl was calculated as the sum of the concentrations in mg/dl of the individual lipid (free
cholesterol, esterified cholesterol, phospholipids and triglycerides) and protein (as total protein content) components for each lipoprotein fraction.

**DETERMINATION CE TRANSFERRED FROM HDL**

Samples of radiolabeled $^3$H-HDL-CE were prepared from the plasma of each subject as previously described$^3$. Cholesteryl ester transfer was determined after incubation of whole plasma from individual subjects at 37°C or 0°C for 3 hours in the presence of radiolabeled HDL (less than 5% of the total HDL-CE mass present in 1 ml of subject’s plasma) and iodoacetamide (final concentration 1.5 mmol/l) for inhibition of Lecithin:Cholesterol Acyltransferase (LCAT)$^3$. Plasma lipoproteins were subsequently fractionated by isopycnic density gradient ultracentrifugation. The radioactive content of HDL subfractions was quantified by liquid scintillation spectrometry with a Trilux 1450 (Perkin Elmer). Cholesteryl ester transferred from HDL was calculated from the difference between the radioactivity transferred at 37°C and 0°C. The radioactive CE content of each isolated lipoprotein fraction was quantified and the rate of CE transfer was calculated from the known specific radioactivity of radiolabelled HDL-CE and expressed as $\mu$g CE transferred/h/ml plasma$^3$.

**FREE CHOLESTEROL EFFLUX ASSAYS**

Fu5AH cells were maintained in Eagle’s MEM containing 5% new-born calf serum, Raw264.7 cells were maintained in DMEM supplemented with 10% foetal bovine serum, and CHO-K1 cells (wild type and hABCG1 transfected cells), kindly provided by Dr W. Jessup, were maintained in Ham’s F-12 medium containing 10% foetal bovine serum. All media were supplemented with 1% L-glutamine and 0.75% penicillin-streptomycin.
Lipid efflux assays using Fu5AH, Raw264.7, CHO-K1 and CHO-hABCG1 were performed as described previously. **Fu5AH**: After plating, cells were labelled by incubation with [³H]-cholesterol (1µCi/ml) in serum-free medium supplemented with new-born calf serum (25%) for 48h. Subsequently, Fu5AH cells were incubated for 24h in serum-free medium supplemented with BSA (0.5%) to allow equilibration of the label. After equilibration, cholesterol acceptors (2.5% diluted plasma or 10µgPL/ml of isolated HDL subfractions) were added in serum-free medium and incubated with cells for 4 hours at 37°C. **Raw264.7 cells**: The day after cell plating, cells were loaded and labelled with acetylated LDL (50 µg/ml) and 0.5 µCi/ml [³H]-cholesterol for 24h in serum-free DMEM containing glucose (4.5 g/l) and BSA (0.2%) (DGGB). After incubation, Raw264.7 cells were incubated with DGGB in the absence or presence of cAMP (0.3 mM) for 16 hours to induce ABCA1 expression. Cholesterol acceptors (2.5% diluted plasma or 30µgApoAI/ml of isolated HDL subfractions) were added to Raw264.7 cells in serum-free DMEM for 4 hours at 37°C in the presence or absence of 0.3 mM 8-Br cAMP. Efflux is expressed as fractional efflux to cells in the presence or absence of 8-Br cAMP. **CHO-K1 cells (WT and hABCG1)**: Two days after plating, cellular cholesterol was labelled by incubation of cells with culture medium and 1 µCi/ml [³H]-cholesterol for 24h. Equilibration of the label was performed for 90 min in serum-free medium and BSA (0.1%). After equilibration of labelling, acceptors (2.5% diluted plasma or 5µgPL/ml of isolated HDL subfractions) were added to the cells in serum-free medium containing BSA (0.1%) for 4 hours at 37°C. Net efflux was expressed as the difference between efflux to hABCG1-transfected CHO-K1 cells and wild type CHO-K1 cells.

All efflux experiments were performed in triplicate for each sample. Fractional cholesterol efflux, (expressed as a percentage), was calculated as the amount of the
label recovered in the medium divided by the total label in each well (radioactivity in the medium + radioactivity in the cells) obtained after lipid extraction from cells in a mixture of 3:2 hexane-isopropanol (3:2 v/v). The background cholesterol efflux obtained in the absence of any acceptor was subtracted from the efflux values obtained with the test samples. Plasma derived from a single normolipidemic subject not included in the study was prepared in aliquots, frozen and used as internal standard to each experiment.

The capacity of HDL subfractions, HDL2 or HDL3, to mediate free cholesterol efflux is expressed as percentage of cholesterol efflux per mole of acceptor particle. Molecular weights of HDL subfractions were calculated by transforming concentration data (mg/dl) into absolute molar units using Mr of CE, FC, PL and TG of 650, 387, 750 and 850 respectively. The protein moiety was considered to consist of two apolipoproteins, apoAI and apoAII, and the molecular weight of the protein moiety in each HDL subfraction was calculated using the total protein content (mg/dl) converted to molarity on the basis of relative mass content of apoAI and apoAII as previously described. The total molecular weight of the protein moiety in each HDL subclass was assumed to be accounted for exclusively by apoAI and AII. The molecular weight of the protein moiety in each subclass was calculated as % from the chemically-measured composition of the total lipoprotein. We next assumed that HDL2 contain an average of 4 copies of apoAI, with an average of 3 copies in HDL3 (see Chapman and Kontush et al for the scientific findings supporting this assumption). The remainder of the molecular weight contribution of each protein moiety was then assumed to be represented by apoAII.

Optimal plasma dilutions or HDL concentrations to use were determined on the basis of dose response curves for the release of free cholesterol from each cellular model.
incubated 4 hours in the presence of human diluted plasma or isolated HDL particles (Supplemental Figure I). In the present study we used the release of labelled cell cholesterol to quantify efflux. Although cholesterol efflux can be mediated by a number of different mechanisms, the high-throughput measurement of isotopic release of cholesterol is accurate because only FC undergoes efflux\textsuperscript{9}. This methodological approach does not however allow evaluation of the question as to whether efflux of labelled cholesterol reflects net mass efflux. Therefore, in additional control experiments, we verified whether isotopic release of cellular cholesterol was associated with a net mass transfer of cholesterol from the cells to extra cellular acceptors (diluted plasma or HDL). Our approach was as follows: after 4 h incubation at 37°C in each cellular system, culture medium was collected, cells were washed with PBS and solubilised with 200µl of NaOH 0.2N for 15 minutes at room temperature with gentle mixing. The cell lysate was used for protein determination and for lipid extraction as previously described\textsuperscript{10}. The volume of cell lysate was made up to 1 ml with PBS containing BHT (20 µmol/L) and EDTA (2 mmol/L) and the total lipid content was extracted into methanol (2.5 ml) then hexane (5 ml). Cholesteryl heptadecanoate was added to the sample before extraction as internal standard. A sample of the hexane layer was evaporated and redissolved in the appropriate mobile phase. Separation of FC and cholesteryl esters was performed by reverse phase HPLC on a system Gold instrument (Beckman Instrument Inc, Palo Alto, CA) equipped with a LC-18 column (Supelco, Bellefonte, PA). Acetonitrile/isopropanol (30/70, vol/vol) was used as eluent at a flow rate of 1 ml/min, and detection was achieved by monitoring absorbance at 205 nm in a Beckman 168 detector. Data were analyzed with the system Gold software from Beckman and expressed as nmol cholesterol/mg cell protein\textsuperscript{10}.
The depletion of cellular cholesterol mass was evaluated after 4 hours incubation at 37°C in the presence of cholesterol acceptors from normolipidemic subjects (Supplemental Figure II). A reduction in cellular cholesterol content following incubation of cells in the presence of isolated HDL or diluted plasma was detected using both Fu5AH and RAW264.7 cells. Equally, the ABCG1 dependent cholesterol efflux was associated with a net mass transfer of cholesterol from cells to isolated HDL whereas no net mass transfer of cholesterol was detected when diluted plasma was used as cholesterol acceptor. These observations are entirely consistent with a previous report showing that for short periods of incubation (up to 4 hours) in the Fu5AH cellular efflux model, isotopic depletion of cellular cholesterol was tightly linked to reduction in cellular cholesterol mass as demonstrated by the maintenance of a constant cell cholesterol specific activity of cellular cholesterol\textsuperscript{11}. Furthermore, Gelissen et al.\textsuperscript{12} confirmed, by quantitation of cellular cholesterol content with HPLC technology, that the expression of hABCG1 in CHO cells stimulates net mass cholesterol export from cells to HDL2.

**IN VITRO SELECTIVE HEPATIC UPTAKE OF HDL-CE**

In vitro selective HDL-CE uptake was performed as previously described\textsuperscript{13}. HepG2 cells were maintained in DMEM supplemented with 10% foetal bovine serum, 1% L-glutamine and 0.75% penicillin-streptomycin. HepG2 or Fu5AH cells were plated in 24-well tissue culture plates (10\textsuperscript{6} cells/well). Two days after plating, cells were washed 3 times with PBS and once with serum-free medium. Cells were subsequently incubated in the presence of \textsuperscript{3}H-CE labelled HDL (60µg protein) diluted in serum-free medium at 37°C for 5 hours. At the end of incubation, the medium was removed and cells were washed 4 times with PBS and incubated in the presence of
an excess of unlabelled HDL (100µg protein) for 30 minutes. Cells were then washed 4 times with PBS and solubilised with 200µl of NaOH 0.2N for 15 minutes at room temperature with gentle mixing. Protein content (20µl) from each well was measured using the Bicinchoninic acid protein reagent (Pierce). The radioactive content of 100µl of each cell lysate was measured by liquid scintillation counting. Selective uptake was calculated from the known specific radioactivity of radiolabelled HDL-CE and is expressed in µgHDL-CE/µg cell protein.

**IN VIVO METABOLIC STUDIES**

*In vivo* studies of the selective hepatic uptake of HDL-CE were performed as previously described by Harada et al. In vivo studies of the selective hepatic uptake of HDL-CE were performed as previously described by Harada et al. In vivo studies of the selective hepatic uptake of HDL-CE were performed as previously described by Harada et al.14. Wild type C57Bl/6 mice, aged 9 weeks, were housed in a temperature-controlled room under a 12 hour light-dark cycle with free access to standard chow diet and water. 3H-CE labelled HDL was administered intraperitoneally (5.10^5 dpm/animal). The injected HDL mass was less than 5% of the total mouse HDL pool. Blood samples (100µl) were drawn by retro-orbital puncture into heparinized capillary tubes at 2h, 6h and 24h and plasma was used for radioactivity determination. After 24h, mice were anesthetized, exsanguinated and infused with saline buffer through the left ventricle. The liver, spleen, adipose tissue and adrenals were collected and stored at -80°C. Frozen tissues (10- 80 mg) were minced, transferred into counting vials and liquid scintillation solution was added for radioactivity measurement.

**STATISTICAL ANALYSIS**

The effects of atorvastatin or combination torcetrapib/atorvastatin therapy on plasma levels of lipids, apolipoproteins and on HDL2 and HDL3 subfractions, on CE mass
transferred from HDL, on cellular free cholesterol efflux and on HDL-CE selective uptake were determined by comparing these parameters at baseline with those after 6 weeks of drug therapy by ANOVA using Student’s paired t-test. The differences between Type IIb patients at baseline, after atorvastatin, or after torcetrapib/atorvastatin therapy versus control subjects were analyzed by ANOVA using the Student’s unpaired t-test. The results were considered to be statistically significant at p<0.05.
SUPPLEMENTAL RESULTS

EFFECTS OF CETP INHIBITION ON PLASMA LIPID AND APOLIPOPROTEIN LEVELS

At baseline, dyslipidemic type IIB patients displayed elevated plasma total cholesterol, LDL-C, triglyceride and apoB levels, but subnormal plasma HDL-cholesterol and apoAI concentrations as compared to normolipidemic control subjects (Supplemental Table I). Combination torcetrapib/atorvastatin therapy induced marked elevations in plasma HDL-cholesterol and apoAI levels not only as compared to baseline (+65% and +23%; respectively) but also as compared to atorvastatin therapy alone (+47%; p<0.0001). As compared to normolipidemic subjects, dyslipidemic patients who received combination torcetrapib/atorvastatin therapy displayed substantial reductions in their elevated baseline levels of total cholesterol, LDL-C, apoB and triglycerides. In addition, plasma levels of apoE in type IIB patients were significantly decreased by 25% (p<0.0001) after atorvastatin alone as compared to baseline (4.1±0.2 mg/dl and 3.1±0.2 mg/dl in type IIB patients before and after atorvastatin alone, respectively). Furthermore, no significant variation in plasma apoE levels was detected between atorvastatin-treated patients and those receiving the combination atorvastatin/torcetrapib therapy (2.9±0.1 mg/dl after atorvastatin/Torcetrapib therapy).

EFFECTS OF CETP INHIBITION ON PLASMA HDL SUBFRACTIONS

At baseline, the total HDL mass concentration was significantly lower (-12%) in type IIB patients compared with normolipidemic control subjects (306±13 mg/dl and 347±13 mg/dl, respectively; p=0.05). This difference was mainly due to lower baseline plasma HDL2 levels (-19%) in type IIB patients as compared to control
subjects (138±9 mg/dl and 171±11 mg/dl, respectively; p<0.05). The total HDL mass concentration in type IIB patients significantly increased (+28%; p<0.0001) as a consequence of Torcetrapib-mediated CETP inhibition (306±13 mg/dl, 309±16 mg/dl and 394±23 mg/dl in type IIB patients before and after atorvastatin alone or after combination torcetrapib/atorvastatin therapy, respectively). CETP inhibition induced major elevations in large HDL2 particles (+48%; 144±13 mg/dl and 213±22 mg/dl in type IIB patients receiving atorvastatin alone and after combination Torcetrapib/atorvastatin therapy, respectively). In addition, it is important to note that plasma HDL2 levels in type IIB patients were markedly increased following torcetrapib/atorvastatin therapy relative to the normal range in normolipidemic control subjects (+24%; p<0.04). In contrast, only a minor increment in plasma levels of HDL3 particles was detected in subjects receiving combination Torcetrapib/atorvastatin therapy (+10%; 165±7 mg/dl and 181±6 mg/dl in type IIB patients after atorvastatin alone and after combination Torcetrapib/atorvastatin therapy, respectively) thereby resembling those in the control group (176±5 mg/dl).

The major HDL subfractions, ie HDL2 and HDL3, from type IIB hyperlipidemic patients were distinct in their chemical composition as compared with their counterparts in normolipidemic control subjects (Supplemental Table II). Indeed, both HDL2 and HDL3 subfractions from type IIB patients at baseline were enriched in triglyceride and to a minor degree depleted in CE, thereby resulting in a significant reduction (p<0.001) in the CE/TG ratio. Significantly however, Torcetrapib-mediated inhibition of CETP induced normalisation in core neutral lipid content of both HDL2 and HDL3 particles as a result of enhanced CE content and depletion in TG. In addition, CETP inhibition significantly increased HDL2-phospholipid content from 24% at baseline to 27% (p<0.008) following T/A therapy. Equally, the apoAI/protein
ratio in HDL2 and HDL3 from Type IIB patients was significantly increased following Torcetrapib therapy and indeed this ratio was normalised in HDL3. By contrast, HDL2 particles were deficient in apoAII at baseline, but this was not corrected by Torcetrapib/atorvastin treatment.

**Effects of CETP inhibition on cellular free cholesterol efflux**

In order to evaluate the impact of CETP inhibition on the capacity of whole plasma to mediate cellular free cholesterol efflux, we used three cellular models, each representative of a specific cholesterol efflux pathway. Overall plasma from Type IIB patients displayed diminished capacity (-21%, p<0.01) to mediate cellular free cholesterol efflux via the SR-BI pathway as compared to plasma from normolipidemic control subjects (Supplemental Table III). Indeed, using 40-fold diluted plasma, a significant elevation (+13%; p<0.05) in the capacity of plasma from type IIB patients to mediate free cholesterol efflux from Fu5AH cells was observed after atorvastatin treatment. Following combination torcetrapib/atorvastatin therapy, plasma from type IIB patients displayed an additional significant increase (+26%; p<0.001) in its capacity to mediate cholesterol efflux via SR-BI as compared to plasmas from patients at baseline. In addition, the capacity of total plasmas from Torcetrapib-treated patients to mediate efflux via SR-BI was similar to that of plasmas from normolipidemic control subjects; these findings indicate that CETP inhibition normalized the capacity of plasma from dyslipidemic patients to mediate cellular cholesterol efflux via the SR-BI pathway.

The capacity of whole plasma from type IIB patients at baseline to mediate free cholesterol efflux via both ABCA1 and ABCG1 transporters was not altered as compared to plasma from control subjects, and in addition, was not influenced by
atorvastatin alone or by combination Torcetrapib/atorvastatin therapy (Supplemental Table III).
SUPPLEMENTAL DISCUSSION

It is important to note that our dyslipidemic type IIIB patients displayed a low HDL-cholesterol phenotype, with a high CE/TG ratio consistent with their defective functions\textsuperscript{15}, whereas the study of Yvan-Charvet\textsuperscript{16} involved moderate hypercholesterolemic subjects who displayed an HDL-C level similar to that in our normolipidemic control group. Potentially therefore, the capacity of HDL particles to mediate cellular cholesterol flux via both the SR-BI and the ABCG1 pathways may not have been abnormal in moderate hypercholesterolemic patients. We therefore conclude that CETP inhibition represents an efficient therapeutic approach to restore functional alterations of HDL particles in atherogenic dyslipidemia involving a low HDL-C phenotype, and that this effect may be dependent upon the degree of CETP inhibition observed.
CONFLICT OF INTEREST STATEMENT:

This study was performed with official financial support from Pfizer in the form of a collaborative agreement between INSERM and Pfizer. There is no conflict of interest between any of the authors at INSERM Unit 551 and Pfizer. None of the authors have any form of financial interest at or in Pfizer. The salaries of the principle investigators were paid by INSERM. All biochemical analyses were independently performed at INSERM Unit 551.
SUPPLEMENTAL REFERENCES


torcetrapib modestly increases macrophage cholesterol efflux to HDL.

SUPPLEMENTAL LEGEND

Supplemental Figure I: Dose dependent curves for efflux of labelled cholesterol from cells incubated 4 hours in the presence of indicated plasma dilutions, HDL-Phospholipids (HDL-PL) or HDL-apolipoprotein Al (HDL-apoAl) concentrations. Panel A: SR-BI dependent efflux determined in Fu5AH cells (closed triangles), ABCG1 dependent efflux determined as the difference between efflux to hABCG1 transfected CHO-K1 cells and wild type CHO-K1 cells (open circles) and ABCA1 dependent efflux determined as the difference between efflux to RAW264.7 cells incubated in the presence and in the absence of 0.3 mM 8Br-cAMP (closed circles). Panel B: Cholesterol efflux from Fu5AH cells incubated as a function of HDL-PL concentrations. Panel C: Cholesterol efflux from RAW264.7 cells incubated in the presence (solid line) or in the absence (dotted line) of 0.3 mM 8Br-cAMP as a function of HDL-apoAI concentrations. Panel D: Cholesterol efflux from CHO-hABCG1 (solid line) and CHO-wild type (dotted line) cells as a function of HDL-PL concentrations. Arrows indicate the selected plasma dilution and HDL-PL or HDL-apoAI concentrations used in cholesterol efflux experiments in various cell types. Experimental values (mean±SE) are expressed as the fractional efflux of $^3$H-cholesterol of at least 3 independent experiments. When not apparent, errors bars are included.

Supplemental Figure II: Cellular cholesterol content following 4 hours incubation in the absence (open bar) or in the presence of isolated HDL particles (solid bar) or diluted plasma (hatched bar). The SR-BI dependent depletion in cellular cholesterol content determined in Fu5AH cells was observed following incubation of cells in the presence of 10µg/ml HDL-PL and 40-fold diluted plasma. The ABCA1 dependent
depletion in cellular cholesterol content determined in RAW264.7 cells was observed following incubation of cells in the presence of 0.3 mM 8Br-cAMP and of 30µg/ml HDL-apoAI or 20-fold diluted plasma. The ABCG1 dependent depletion in cellular cholesterol content using hABCG1 transfected CHO-K1 cells was observed following incubation of cells in the presence of 5µg/ml HDL-PL or 20-fold diluted plasma.
Supplemental Table I:

Plasma Lipid and Apolipoprotein Levels in Control subjects and in Type IIB patients Before and After Drug Phases

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls Subjects (n=11)</th>
<th>Before Treatment</th>
<th>Atorvastatin Alone (10mg/d)</th>
<th>Torcetrapib/Atorvastatin (60/10 mg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>203±4*‡</td>
<td>252±7</td>
<td>189±6*</td>
<td>181±8*</td>
</tr>
<tr>
<td>LDL</td>
<td>131±4*†‡</td>
<td>174±7</td>
<td>117±5*</td>
<td>88±6†‡</td>
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<tr>
<td>HDL</td>
<td>56±3*†</td>
<td>40±2</td>
<td>45±2*</td>
<td>66±5†</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>79±4</td>
<td>190±14</td>
<td>137±10*</td>
<td>136±9*</td>
</tr>
<tr>
<td>Apolipoprotein (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-I</td>
<td>144±4*†</td>
<td>127±3</td>
<td>127±4</td>
<td>156±10*†</td>
</tr>
<tr>
<td>A-II</td>
<td>42±1*†</td>
<td>35±1</td>
<td>36±1</td>
<td>42±1*†</td>
</tr>
<tr>
<td>B</td>
<td>107±5*†</td>
<td>145±5</td>
<td>102±4*</td>
<td>82±4†</td>
</tr>
</tbody>
</table>

Values are mean±SE. LDL: Low Density Lipoprotein. HDL: High Density Lipoprotein.

Plasma lipid and apolipoprotein levels were determined in control subjects and in type IIB patients before, after 6 weeks of Atorvastatin alone and after 6 weeks of combination therapy with Torcetrapib/Atorvastatin.

*p<0.05 versus Type IIB patients at Baseline

†p<0.05 versus Type IIB patients following therapy with atorvastatin alone.

‡p<0.05 versus Type IIB patients following combination torcetrapib/atorvastatin therapy.
Supplemental Table II:

Percentage Weight Chemical composition of HDL particles in Control subjects and in Type IIB patients Before and After Drug Phases

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls Subjects (n=11)</th>
<th>Before Treatment</th>
<th>Atorvastatin Alone (10mg/d)</th>
<th>Torcetrapib/Atorvastatin (60/10 mg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HDL2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC, %</td>
<td><strong>4.0±0.3</strong></td>
<td>4.6±0.2</td>
<td>4.8±0.2</td>
<td>5.0±0.2</td>
</tr>
<tr>
<td>CE, %</td>
<td><strong>23.7±0.8</strong></td>
<td>23.4±0.8</td>
<td>23.0±0.5</td>
<td>25.3±0.6</td>
</tr>
<tr>
<td>TG, %</td>
<td><strong>3.2±0.2</strong></td>
<td>5.5±0.4</td>
<td>4.9±0.5</td>
<td>3.6±0.4</td>
</tr>
<tr>
<td>PL, %</td>
<td><strong>26.4±0.5</strong></td>
<td>24.5±1.2</td>
<td>25.4±1.3</td>
<td>27.4±1.2</td>
</tr>
<tr>
<td>Protein, %</td>
<td><strong>42.7±1.1</strong></td>
<td>42.0±0.8</td>
<td>41.9±0.9</td>
<td>38.7±1.3</td>
</tr>
<tr>
<td>CE/TG</td>
<td><strong>7.41</strong></td>
<td>4.25</td>
<td>4.69</td>
<td>7.03</td>
</tr>
<tr>
<td>AI/Protein</td>
<td><strong>0.77</strong></td>
<td>0.49</td>
<td>0.56</td>
<td>0.67</td>
</tr>
<tr>
<td>All/Protein</td>
<td><strong>0.26</strong></td>
<td>0.17</td>
<td>0.20</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>HDL3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC, %</td>
<td><strong>2.0±0.2</strong></td>
<td>2.3±0.2</td>
<td>2.5±0.2</td>
<td>2.6±0.2</td>
</tr>
<tr>
<td>CE, %</td>
<td><strong>17.9±0.9</strong></td>
<td>16.3±0.5</td>
<td>16.8±0.5</td>
<td>19.2±0.4</td>
</tr>
<tr>
<td>TG, %</td>
<td><strong>2.4±0.2</strong></td>
<td>4.6±0.3</td>
<td>3.9±0.4</td>
<td>3.1±0.3</td>
</tr>
<tr>
<td>PL, %</td>
<td><strong>19.2±0.5</strong></td>
<td>21.2±1.1</td>
<td>21.0±1.0</td>
<td>21.8±0.8</td>
</tr>
<tr>
<td>Protein, %</td>
<td><strong>58.5±1.2</strong></td>
<td>55.6±1.0</td>
<td>55.8±0.7</td>
<td>53.3±1.0</td>
</tr>
<tr>
<td>CE/TG</td>
<td><strong>7.46</strong></td>
<td>3.54</td>
<td><strong>4.31</strong></td>
<td>6.19</td>
</tr>
<tr>
<td>AI/Protein</td>
<td><strong>0.63</strong></td>
<td>0.55</td>
<td>0.58</td>
<td>0.63</td>
</tr>
<tr>
<td>All/Protein</td>
<td>0.21</td>
<td>0.20</td>
<td>0.19</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Values are mean±SE. HDL: High Density Lipoprotein.

*†p<0.05 versus Type IIB patients following therapy with atorvastatin alone.

*‡p<0.05 versus Type IIB patients following combination torcetrapib/atorvastatin therapy
### Supplemental Table III:

**Plasma Cellular Free Cholesterol Efflux capacities in Control subjects and in Type IIB patients Before and After Drug Phases**

<table>
<thead>
<tr>
<th>Efflux pathway</th>
<th>Cellular model</th>
<th>Control subjects (n=11)</th>
<th>Before Treatment</th>
<th>Atorvastatin (10 mg/d)</th>
<th>Torcetrapib/Atorvastatin (60/10 mg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR-BI</td>
<td>Fu5AH cells</td>
<td>23.6±3.3* †</td>
<td>18.7±2.2</td>
<td>21.2±3.0*</td>
<td>23.6±4.0* †</td>
</tr>
<tr>
<td>ABCA1</td>
<td>RAW264.7(+/-cAMP)</td>
<td>18.2±4.7</td>
<td>16.9±5.5</td>
<td>16.3±5.7</td>
<td>16.6±7.6</td>
</tr>
<tr>
<td>ABCG1</td>
<td>CHO (+/- hABCG1)</td>
<td>26.9±3.2</td>
<td>28.4±3.8</td>
<td>29.4±3.3</td>
<td>29.4±4.4</td>
</tr>
</tbody>
</table>

Values are mean ± SD and are expressed in fractional efflux (%). Free cholesterol release from cells were determined after 4 hours incubation in the presence of 40-fold diluted plasma.

* p<0.05 from Type IIB patients at Baseline (Before Treatment)
† p<0.05 from Type IIB patients following atorvastatin alone
‡ p<0.05 from Type IIB patients following combination torcetrapib/atorvastatin therapy