Cholesterol Efflux Potential and Antiinflammatory Properties of High-Density Lipoprotein After Treatment With Niacin or Anacetrapib

Laurent Yvan-Charvet, Jelena Kling, Tamara Pagler, Hongna Li, Brian Hubbard, Tim Fisher, Carl P. Sparrow, Andrew K. Taggart, Alan R. Tall

Objective—To examine the effects of treatments with niacin or anacetrapib (an inhibitor of cholesteryl ester transfer protein) on the ability of high-density lipoprotein (HDL) to promote net cholesterol efflux and reduce toll-like receptor–mediated inflammation in macrophages.

Methods and Results—A total of 18 patients received niacin, 2 g/d, for 4 weeks; 20 patients received anacetrapib, 300 mg/d, for 8 weeks; and 2 groups (n = 4 and n = 5 patients) received placebo. HDL samples were isolated by polyethylene glycol precipitation or ultracentrifugation, tested for the ability to promote cholesterol efflux in cholesterol-loaded THP-1 or mouse peritoneal macrophages, or used to pretreat macrophages, followed by lipopolysaccharide exposure. HDL cholesterol levels were increased by 30% in response to niacin and by approximately 100% in response to anacetrapib. Niacin treatment increased HDL-mediated net cholesterol efflux from foam cells, primarily by increasing HDL concentration, whereas anacetrapib treatment increased cholesterol efflux by both increasing HDL concentration and causing increased efflux at matched HDL concentrations. The increased efflux potential of anacetrapib-HDL was more prominent at higher HDL cholesterol concentrations (>12 μg/mL), which was associated with an increased content of lecithin–cholesterol acyltransferase (LCAT) and apolipoprotein E and completely dependent on the expression of ATP binding cassette transporters (ABCA1 and ABCG1). Potent antiinflammatory effects of HDL were observed at low HDL concentrations (3 to 20 μg/mL) and were partly dependent on the expression of ABCA1 and ABCG1. All HDL preparations showed similar antiinflammatory effects, proportionate to the HDL cholesterol concentration.

Conclusion—Niacin treatment caused a moderate increase in the ability of HDL to promote net cholesterol efflux, whereas inhibition of cholesteryl ester transfer protein via anacetrapib led to a more dramatic increase in association with enhanced particle functionality at higher HDL concentrations. All HDLs exhibited potent ability to suppress macrophage toll-like receptor 4–mediated inflammatory responses, in a process partly dependent on cholesterol efflux via ABCA1 and ABCG1. (Arterioscler Thromb Vasc Biol. 2010;30:1430-1438.)

Key Words: ABC transporter ■ atherosclerosis ■ lipoproteins ■ macrophages

New therapies for increasing the high-density lipoprotein (HDL) level might be an effective way to reduce the significant burden of residual cardiovascular disease in patients treated with lipid-lowering therapies.1–3 However, current approaches for increasing HDL level are limited. Although niacin has been shown to reduce atherosclerotic cardiovascular disease in the Coronary Drug Project,4 and probably to reduce atherosclerotic plaque in the coronary and carotid arteries in the FATS and Arbiter studies,5,6 its ability to increase HDL cholesterol (HDL-C) levels is often associated with side effects such as flushing. New approaches to reduce niacin-induced flushing, such as the combination of niacin with prostaglandin D2 (PGD2) receptor (DP1) antagonists, are being evaluated.7 Inhibitors of cholesteryl ester transfer protein (CETP), such as torcetrapib or anacetrapib, can produce higher elevations of HDL levels than niacin (≥100%) and low-density lipoprotein lowering.8–10 However, the failure of the CETP inhibitor torcetrapib in a large clinical trial (ILLUMINATE) has called this approach into question.11,12 It is not known whether the excess of cardiovascular events and deaths occurring in subjects receiving torcetrapib was the result of mechanism-related defects, such as production of dysfunctional HDL or off-target toxicity. Although major off-target toxicity of torcetrapib, involving increased blood pressure and hyperaldosteronism, has been identified, it remains uncertain whether these or related
toxic effects\textsuperscript{13} were responsible for the adverse outcome.\textsuperscript{3,4,14} Consequently, there has been great interest in developing assays or biomarkers that may reflect the underlying antiatherogenic functions of HDL.\textsuperscript{15}

A principal antiatherogenic property of HDL is thought to be its ability to promote cholesterol efflux from macrophage foam cells.\textsuperscript{1,3,16,17} Researchers\textsuperscript{18–20} recently reported that large CE-rich HDL particles accumulating in CETP-deficient or torcetrapib-treated subjects showed increased ability to promote cholesterol efflux from macrophage foam cells. Central to these observations was the key role of LCAT and apolipoprotein E (apoE), present at high levels in CETP-deficient and torcetrapib-treated subjects, driving net cholesterol efflux in an ABCG1-dependent fashion.\textsuperscript{18,19} The increased cholesterol efflux potential of HDL from torcetrapib-treated subjects that was observed at higher doses and higher HDL levels\textsuperscript{19} appeared to correlate with the finding that subjects receiving torcetrapib who had the largest increase in HDL levels after therapy also had significant regression of coronary atherosclerosis, as assessed by intravascular ultrasound.\textsuperscript{21}

In addition to its role in promoting cholesterol efflux, HDL also exhibits antiinflammatory properties in endothelial cells and macrophages; these properties could contribute to its atheroprotective effects.\textsuperscript{3,17,22} Although multiple different mechanisms are likely to be involved, antiinflammatory effects may, in part, be related to ABCA1- and ABCG1-mediated cholesterol efflux because cholesterol accumulation in the plasma membrane of Abca1\textsuperscript{−/−}, Abcg1\textsuperscript{−/−}, and Abca1\textsuperscript{−/−}Abcg1\textsuperscript{−/−} macrophages has been shown to increase signaling of toll-like receptors (TLRs), enhancing the inflammatory response to lipopolysaccharide or other TLR ligands in a nuclear factor-\kappaB–dependent fashion.\textsuperscript{23–26} Murphy et al\textsuperscript{27} recently showed that the antiinflammatory effects of apoA-I in macrophages were likely mediated by cholesterol efflux via ABCA1; however, the potential involvement of macrophage ABC transporters in the antiinflammatory effects of HDL particles has not been assessed.

The present study was undertaken to compare the cholesterol efflux potential of HDL accumulating in subjects treated with niacin or a new class of CETP inhibitor, anacetrapib, currently being assessed in phase 3 clinical trials.\textsuperscript{10} A secondary goal of the study was to determine the consequences of these treatments on the antiinflammatory potential of HDL in macrophages and the role of ABCA1 and ABCG1 transporters in mediating these effects.

**Methods**

For details, see the online data supplement (available at http://atvb.ahajournals.org).

This study was conducted using archived plasma samples from 3 clinical trials (2 using niacin and 1 using anacetrapib). The niacin samples were from 2 different studies, the details of which have been published previously.\textsuperscript{28,29} Samples from a total of 22 dyslipidemic subjects were analyzed (18 received Niaspan, extended release of niacin for 8 to 16 weeks [2 g/d for the last 4 weeks] and 4 received placebo for 8 weeks). Patients in these studies were permitted to receive stable background lipid-modifying therapy in addition to placebo for 8 weeks). Blood was collected before and after treatment to allow comparison between each individual receiving therapy.

**Results**

**Patient Groups and HDL Responses**

Niacin treatment increased plasma concentrations of HDL by 28% compared with either baseline or placebo treatment (supplemental Figure IA), whereas 8-week anacetrapib treatment increased the HDL-C level by approximately 100% (supplemental Figure IB). In the niacin group, 2 subjects showed no change in HDL-C level; these subjects were included in all analyses. At baseline, the different groups did not show significant differences with respect to demographic and lipid characteristics (supplemental Table). Three independent pools of plasma from subjects receiving daily niacin before and after treatment; 4 pools of plasma from subjects receiving anacetrapib, 300 mg/d, for 8 weeks before and after treatment; and 1 pool of placebo control for each condition were made to isolate HDL particles by sequential-density ultracentrifugation to have sufficient material to analyze HDL-2 protein composition (supplemental Figure II). Immunoblot analysis of platelet-activating factor acetylhydrolase (PAFAH) in individual pooled HDL-2 samples from patients before and after niacin or anacetrapib treatment did not reveal any differences (supplemental Figure IIA and IIC). The level of apoA-I was also not significantly changed in the different groups as expected because samples were matched for total protein content and because apoA-I is the major protein of HDL (supplemental Figure IIA and IIC). In contrast, anacetrapib–HDL-2 exhibited a significant mean 1.4-fold increase in apoE and LCAT proteins compared with control HDL-2 (supplemental Figure IIC and IID). These findings are consistent with the increased cholesterol ester content of anacetrapib–HDL-2 (supplemental Figure IID) and with earlier findings using HDL samples from subjects with homozygous CETP deficiency or treatment with higher doses of torcetrapib, 120 mg.\textsuperscript{8,18,19} Interestingly, similar changes were not observed for niacin-HDL (supplemental Figure IIA and IIB).

**Effects of Niacin and Anacetrapib on HDL-Mediated Cholesterol Efflux**

We evaluated the cholesterol efflux potential of HDL particles isolated from subjects receiving niacin and anacetrapib. Dose-response curves for cholesterol mass efflux, mediated by HDL in polyethylene glycol supernatants from niacin-treated subjects using 3 different concentrations of pooled HDL (12-, 36-, and 72-µg/mL cholesterol), showed similar levels of cholesterol efflux as control pretreatment HDL samples (Figure 1A). In contrast, the dose-response curve for cholesterol efflux, using 3 different concentrations of pooled control and anacetrapib-HDL (12-, 36-, and 72-µg/mL cholesterol), showed increased cholesterol efflux compared with pretreatment HDL, as reflected by increased cholesteryl ester (CE) and total cholesterol accumulation in media (Figure 1B). As previously observed,\textsuperscript{19} the increment over control for cholesterol efflux by anacetrapib-HDL tended to increase with increasing HDL concentration. At a higher cholesterol concentration (ie, 72 µg/mL), there was also a signifi-
cant increase in free cholesterol efflux with anacetrapib-HDL compared with control-HDL (Figure 1B). The comparison between control-HDL at 36-3926 g/mL cholesterol and anacetrapib-HDL at 72-3926 g/mL cholesterol also allowed us to estimate an approximately 2.4-fold increase in the efflux potential of anacetrapib-HDL, matched by volume compared with control-HDL (Figure 1B). To further test for any potential dysfunctional effects of niacin on HDL particles, we next performed a dose-response curve for cholesterol efflux matched by sample volume. This showed increased cholesterol efflux for the niacin-HDL samples compared with controls, which was significant at the 2 higher sample volumes, primarily reflecting increased CE accumulation in the media (Figure 2A and B). When control and anacetrapib-HDL were added at the same cholesterol concentration (50 μg/mL), anacetrapib-HDL exhibited a significant 4-fold increase in CE accumulation in media and a significant 1.6-fold increase in total cholesterol efflux (Figure 3). These findings suggested that both anacetrapib-HDL and niacin-HDL showed increased cholesterol efflux potential. However, the effect is more dramatic for anacetrapib-HDL and is likely to reflect increases in both particle functionality and concentration; niacin-HDL primarily reflects an increase in HDL concentration.

Role of ABCA1 and ABCG1 in Mediating Net Cholesterol Efflux to Control, Niacin, and Anacetrapib-HDL

To assess the role of ABCA1 and ABCG1 in mediating the increased cholesterol efflux potential of anacetrapib-HDL or niacin-HDL, we first obtained dose-response curves for both sets of samples, matched by either volume or HDL-C con-
Effects of Niacin-HDL and Anacetrapib-HDL on Inflammatory Responses Mediated by TLR4

Next, we compared the ability of the different HDL preparations to modulate the macrophage inflammatory response to TLR4 activation induced by lipid A treatment. To avoid the potential confounding effects of direct binding of lipid A to HDL, we used a protocol in which cholesterol-loaded macrophages were pretreated with HDL (matched by concentration or volume), extensively washed, and then challenged with a lipid A stimulus. The inflammatory response was monitored by measuring the mRNA levels of a battery of cytokines and chemokines. To assess the potential dependence of antiinflammatory effects of HDL on ABCA1 and ABCG1, we performed experiments in WT and Abca1<sup>−/−</sup>Abcg1<sup>−/−</sup> macrophages. Results for niacin-HDL are shown in Figure 5, and results for anacetrapib-HDL are shown in Figure 6. Treatment of cells with lipid A induced an inflammatory response, as shown by increased mRNA expression of tumor necrosis factor α, macrophage inflammatory protein (MIP)-1α, MIP-2, and interleukin 6 in WT cells. As previously reported,26 this response was exaggerated in Abca1<sup>−/−</sup>Abcg1<sup>−/−</sup> macrophages. All HDL samples showed a similar ability to suppress inflammatory responses. The only significant difference observed was increased suppression of inflammation in WT macrophages by anacetrapib-HDL for samples matched by volume (Figure 6B and supplemental Figure IV), likely reflecting the marked increase in HDL concentration in these samples. As expected from the cholesterol efflux experiments (Figure 4), this ability was lost in Abca1<sup>−/−</sup>Abcg1<sup>−/−</sup> macrophages. Interestingly, HDL samples were still able to suppress inflammatory responses in Abca1<sup>−/−</sup>Abcg1<sup>−/−</sup> macrophages; however, compared with WT macrophages, higher concentrations or volumes of HDL were required to bring the inflammatory responses in Abca1<sup>−/−</sup>Abcg1<sup>−/−</sup> macrophages back toward the baseline (prelipid A) values.
**Discussion**

This study was undertaken to assess the functionality of HDL accumulating in subjects treated with extended-release niacin or the CETP inhibitor, anacetrapib. Both niacin-HDL and anacetrapib-HDL were functional in supporting cholesterol efflux from macrophage foam cells and in inhibiting inflammatory responses induced by TLR4. However, only anacetrapib-HDL led to higher levels of cholesterol efflux when matched for particle cholesterol content. Increased functional capacity per particle was driven by an increase in media CE formation and probably reflects an increased content of apoE and LCAT in anacetrapib-HDL. Anacetrapib-HDL also exhibited an increased ability to reduce the inflammatory response induced by TLR4 ligand when samples were matched by volume. Although our macrophage assay only reflects 1 particular set of anti-inflammatory functions of HDL, these findings may help to allay concerns that CETP inhibitors induce the formation of a pro-inflammatory HDL particle. To our knowledge, our studies show for the first time, on a mechanistic level, that the anti-inflammatory effects of HDL in macrophages are likely mediated in part by cholesterol efflux via ABCA1 and ABCG1.

Although HDL from torcetrapib-treated subjects only modestly increased macrophage cholesterol efflux at the 60-mg dose used in the ILLUMINATE study, the efflux potential of HDL at the 300-mg anacetrapib dose appears to be larger. One likely reason is that the 300-mg dose probably causes complete inhibition of CETP activity, as judged by the fact that HDL increasing and low-density lipoprotein decreasing the anacetrapib level is equivalent to the level produced by homozygous genetic CETP deficiency. Niacin also increased the cholesterol efflux potential but only when samples were matched by volume, reflecting the approximately 30% increase in HDL-C levels, without any increase in apoE and LCAT protein levels in HDL-2. Although these subjects were also receiving lipid-lowering therapies, the increase in HDL-C levels was previously attributed to a direct effect of niacin. In contrast, increased apoE and LCAT protein levels were observed in anacetrapib–HDL-2, correlating with the finding of increased HDL particle cholesterol efflux.
potential. We cannot exclude that the storage of the samples may have modestly exaggerated these effects. However, in the present study, HDL was isolated from subjects with triglycerides in the normal range; the increase in apoE in CETP-deficient HDL was originally shown in fresh unfrozen samples. In earlier studies, removal of the apoE-rich fraction from CETP-deficient HDL greatly reduced its cholesterol efflux potential. Similar differential effects on macrophage cholesterol efflux were also seen when comparing 120-mg torcetrapib, which induced increased amounts of apoE-rich HDL in most subjects, with 60-mg torcetrapib, which did not induce increased amounts of apoE-rich HDL in most subjects. These results point to a role of apoE acting in conjunction with LCAT, possibly facilitating expansion of the CE core of HDL and permitting ongoing cholesterol efflux. The increased efflux potential of anacetrapib-HDL required the activity of ABCA1 and ABCG1 over a range of HDL-C concentrations. The increased cholesterol efflux potential was more apparent at higher cholesterol concentrations (≥12 μg/mL). This could reflect dissociation of apoE from HDL particles in diluted samples. Alternatively, it could reflect the relatively high apparent Michaelis constant values for ABCG1-mediated cholesterol efflux (approximately 30- to 50-μg/mL phospholipid) and the dependence of the increased efflux potential on activity of ABCG1.

Accumulating evidence suggests that by removing excess free cholesterol from macrophages, HDL exerts anti-inflammatory effects. Our findings suggest that HDL from subjects treated with either niacin or anacetrapib has the same ability as control-HDL to decrease inflammation induced by TLR4 in macrophage foam cells when matched by HDL-C concentration. This antiinflammatory effect of HDL occurred at low HDL-C concentrations, consistent with a recent report from Murphy et al. It is not clear why the antiinflammatory properties of HDL occur at such low HDL concentrations. One possibility is that proinflammatory receptors, such as MD2-TLR4 complexes, are sensitive to small changes in membrane cholesterol content, perhaps reflecting disruption of lipid rafts. Interestingly, maximum antiinflammatory effects of anacetrapib and other HDLs required activity of ABCA1 and ABCG1. Overall, the antiinflammatory effects of HDL in this study correlate well with
cholesterol efflux data. Thus, over the concentration range at which HDL exerted increasing antiinflammatory effects (3 to 20 μg/mL), there were parallel increases in cholesterol efflux and both antiinflammatory effects and cholesterol efflux were reduced in Abca1−/− Abcg1−/− macrophages. All types of HDL particles exerted antiinflammatory effects in proportion to their cholesterol efflux properties in the same concentration range. Anacetrapib-HDL were more efficient antiinflammatory agents, matched by volume but not by concentration, mirroring the effects on cholesterol efflux in this low HDL concentration range. Together, this suggests that a major antiinflammatory mechanism of HDL relates to cholesterol efflux via ABC transporters. HDL is still an antiinflammatory agent at higher HDL concentrations in Abca1−/− Abcg1−/− cells, probably reflecting less efficient passive cholesterol efflux mechanisms or possibly other antiinflammatory properties of HDL. Our findings showing that the antiinflammatory effects of HDL are partly mediated by cholesterol efflux via ABCA1 and ABCG1 link 2 previous sets of independent observations; these data show that HDL exhibits antiinflammatory properties in macrophages and that the activities of ABCA1 and ABCG1 are antiinflammatory.

The ability of HDL, ABCA1, and ABCG1 to suppress inflammation mediated by toll receptor signaling is likely relevant to atherogenesis. Seminal studies have indicated the roles of TLR4 and myeloid differentiation primary response (Myd)-88 in atherogenesis in hypercholesterolemic animal models. There are likely other antiinflammatory effects of niacin and anacetrapib that were not assessed in this study. For instance, HDL may exhibit antiinflammatory effects in endothelial cells by reducing the expression of vascular cell adhesion molecule 1 and intercellular adhesion molecule-1 and leading to suppression of monocyte adhesion and transmigration across the endothelium. HDL may become proinflammatory in such assays, possibly reflecting accumulation of oxidized lipids or altered protein cargo. McGrath et al recently proposed a novel antiinflammatory effect of HDL mediated by an upregulation of 3β-hydroxysteroid-δ 24 reductase that inhibits the nuclear factor-κB binding site. Other effects include the modulation of the protein content of paraoxonase, glutathione peroxidase, or other complement proteins directly in HDL particles. PAFAH protein content in HDL-2, known to exert antiinflammatory property such as inhibition of the biological

Figure 6. Dose-response curve for suppression of toll-like receptor 4–mediated inflammation induced by a similar concentration or volume of ultracentrifugated control and anacetrapib-HDL in WT and Abca1−/− Abcg1−/− macrophages. Bone marrow–derived macrophages from WT and Abca1−/− Abcg1−/− mice were treated for 16 hours with 50-μg/mL ac low-density lipoprotein. A and B, Then, increased concentrations of pooled HDL-2 (3-, 8-, and 20-μg/mL cholesterol) from 300 mg of anacetrapib treatment (A) or increased volumes of pooled control HDL-2 (38±2 mg/dL of cholesterol) and anacetrapib–HDL-2 (81±4 mg/dL of cholesterol) (B) were added to 500 μL of 0.2% BSA media for 3 hours before lipid A treatment for 3 or more hours, as described in the “Methods” section. At the end of the incubation, inflammatory transcript levels (tumor necrosis factor α, interleukin 6, MIP1α, and MIP2) were quantified and normalized to m36B4. Data are given as the mean±SEM and expressed as arbitrary units from an experiment performed in triplicate. *P<0.05 vs control HDL-2.
activity of minimally oxidized low-density lipoproteins and associated reduction in dendritic cell migration, was unchanged by either niacin or anacetrapib treatment in this study.

In the ILLUMINATE study that used the CETP inhibitor torcetrapib to increase HDL levels, there was an unexpected excess of deaths from acute sepsis. Although this result could reflect off-target toxicity of torcetrapib, it is also possible that the HDL-mediated dampening of the immediate-phase response during sepsis, as reflected in its antiinflammatory properties measured in this study, could lead to an adverse outcome.

To summarize, our data indicate that there was no evidence for an adverse effect of niacin or anacetrapib on HDL-mediated cholesterol efflux or antiinflammatory responses using our assays. Whether such findings can be translated into clinical benefit can only be answered by ongoing randomized clinical trials involving niacin, anacetrapib, and dalcetrapib.

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Disclosures
Dr Tall is a consultant for Merck, Roche, Bristol Myers Squibb, Arisaph, and Kowa Pharmaceuticals.

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Supplemental Material

**Plasma HDL preparation.** Blood samples were collected from all subjects after 12-hour fasting. ApoB-containing particles was precipitated from plasma by adding 100µl of plasma to 40µl of 20% polyethylene glycol (PEG, Sigma P-2139 in 200mM glycine, pH10) solution. This mixture was incubated at room temperature for 15 min. After this incubation, the solution was centrifuged at 4,000rpm for 20 min. The supernatant, containing HDL fractions, was removed and used for experiments as previously described. Independently, HDL-2 fraction was isolated from plasma by ultracentrifugation as previously described. While storage of human plasma samples may lead to alteration in apoE rich HDL, earlier work clearly established that the increased apoE-rich HDL in subjects with CETP deficiency was mechanism-related.

**Human THP-1 macrophages.** THP-1 monocytes were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) at 37 °C in 5% CO₂. Cells were treated with 100nM PMA (Phorbol myristate acetate) for 24 h to facilitate differentiation into macrophages. Then, adherent macrophages were incubated with 50µg/mL acetyl-LDL and 3µM LXR agonist (TO901317) for 24 hours before cholesterol efflux studies.

**Mouse macrophage harvest and treatments.** Bone-marrow-derived macrophages from wild-type and Abca1<sup>−/−</sup>Abcg1<sup>−/−</sup> mice, were isolated and cultured in 10% FBS in DMEM media supplemented with macrophage-colony stimulating factor for 5-10 days before the experiment. Cells were treated overnight with 50µg/mL acetyl-LDL. The following day, foam cells were preincubated with different HDL concentrations or volumes for 3h in 0.2%BSA DMEM, washed extensively and equilibrated for 5min in 0.2% BSA DMEM (to remove any HDL left in the wells), before incubation with 100ng/mL lipidA (Toll-like receptor ligand) in 0.2% BSA DMEM for 3 more hours.

**Isotopic cholesterol efflux assay.** Bone-marrow-derived macrophages were cultured for 24h in 10% FBS in DMEM containing 50µg/mL acetyl-LDL and 2µCi/mL of [3H]-cholesterol. Cholesterol efflux was performed for 3h in 0.2% BSA DMEM containing different concentrations or volumes of HDL as acceptors. The cholesterol efflux was expressed as the percentage of the radioactivity in cells plus medium.

**Cholesterol mass analysis.** Cholesterol efflux was performed in DMEM containing 0.2% BSA in presence of HDL as described in figure legends. After incubation with HDL, the lipid fractions were extracted from the collected media with hexane in presence of β-sitosterol (5µg per sample) added as the internal standard. The mass of total cholesterol dissolved in hexane was subject to gas-liquid chromatography. The HDL mediated cholesterol efflux was calculated by subtraction of cholesterol
mass of the medium cultured with or without cells. This allows the determination of
the net cholesterol efflux driven by HDL particles reflecting the ability of HDL to
remove cellular cholesterol.  

**Western Blot analysis.** Aliquots of 20µg of HDL-2 were boiled at 95°C for 10
minutes in SDS buffer (6.25·10⁻³ M Tris-HCl pH 6.8, 2% SDS, 5% 2-mercaptoethanol,
10% sucrose and 0.002% Coomassie blue). Then, HDL proteins were resolved by
10% SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose
membranes. Primary antibodies for LCAT (400-107A2), PAFAH (160603), ApoE
(ab1906) or ApoA-I (ab17278) were purchased from Novus Biologicals (Littleton,
CO), Cayman Chemical (Ann Arbor, MI) and Abcam (Santa Cruz, CA). Anti-mouse
and rabbit secondary antibodies were obtained from GE Healthcare. Specific protein
signals were revealed using the ECL detection system (Amersham Biosciences).
References


**Supplemental Table. I**

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Data are presented as mean ± SD. BMI indicates body mass index and LMT, lipid modifying therapy. There were no significant differences between the groups.
Supplemental Figure I: Effect of niacin and anacetrapib on plasma concentrations of HDL cholesterol (HDL-C). Relative to baseline, niacin increased plasma HDL-C levels by a mean of 28% from 35±2 to 45±3 mg/dL (A) and anacetrapib doubled plasma HDL-C levels (B). 18 subjects received niacin treatment compared to 4 placebo and 20 subjects received anacetrapib compared to 5 respective placebo. *P<0.01; **P<0.001 for comparison with baseline.
Supplemental Figure II: HDL analysis. 20μg HDL-2 protein was subjected to SDS-PAGE and analyzed for LCAT, PAFAH, apoE and apoA-I mass in HDL-2. A representative autoradiogram is shown for HDL-2 from placebo, niacin group (A) and anacetrapib group (C) before and after 8-16weeks or 8weeks treatment, respectively. No significant change was observed for niacin-HDL-2 from three pools of plasma from subjects receiving daily niacin (3 pools each from 5 different subjects + 1 placebo pool) (B). Quantification of the change in HDL-2 composition for 300mg anacetrapib group is shown in D as well as cholesterol composition of these HDL-2 particles determined by gas chromatography. Values are means±SEM of four pools each from 5 different subjects + 1 placebo pool from 5 different subjects. *P<0.05, significant difference vs control HDL-2. CE : cholesteryl ester, FC : free cholesterol.
Supplemental Fig. III: Effect of LCAT inhibition and ABCG1 deficiency on anacetrapib-PEG-HDL-mediated cholesterol efflux. Peritoneal macrophages were treated with 50µg/mL acLDL and 3µmol/L TO901317 for 24h. LCAT inhibitor (2mM of LCAT-I; E600) was preincubated with PEG-HDL for 30min at room temperature and then, directly added to the efflux medium (final concentration of E600 in the media 0.2mM). The TC, FC, and CE mass in media were determined 6h after incubation of similar concentration of PEG-HDL (25µg/mL cholesterol) from control or anacetrapib group. Values are means±SEM of an experiment performed in triplicate. *P<0.05, significant difference vs control PEG-HDL.
Supplemental Figure IV: One-way ANOVA analysis with Bonferroni correction for the anti-inflammatory response of control and anacetrapib-HDL performed in Figure 6. C-HDL, control-HDL (HDL isolated from subjects before anacetrapib treatment); A-HDL, anacetrapib-HDL (HDL isolated from subjects after anacetrapib treatment). The upper panel represents the analysis of the effect of C-HDL and A-HDL in WT macrophages and the lower panel represents the effect of C-HDL and A-HDL in Abca1<sup>−/−</sup>Abcg1<sup>−/−</sup> (DKO) macrophages