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% polyethyleneglycol (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.[1] Independently, HDL-2 fraction was isolated from plasma by ultracentrifugation as previously described.[1] While storage of human plasma samples may lead to alteration in apoE rich HDL,[2] earlier work clearly established that the increased apoE-rich HDL in subjects with CETP deficiency was mechanism-related.[3]

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% CO_2. 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*$^{-/-}$*Abcg1*$^{-/-}$ mice, were isolated and cultured in 10% FBS in DMEM media supplemented with macrophage-colony stimulating factor for 5-10 days before the experiment.[4] 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 [^{3}H]-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.[1,5]

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 pH6.8, 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

1. Yvan-Charvet L, Matsuura F, Wang N, Bamberger MJ, Nguyen T, Rinninger F, Jiang XC, Shear CL, Tall AR. Inhibition of cholesteryl ester transfer protein by torcetrapib modestly increases macrophage cholesterol efflux to HDL. *Arterioscler Thromb Vasc Biol.* 2007;27:1132-1138.

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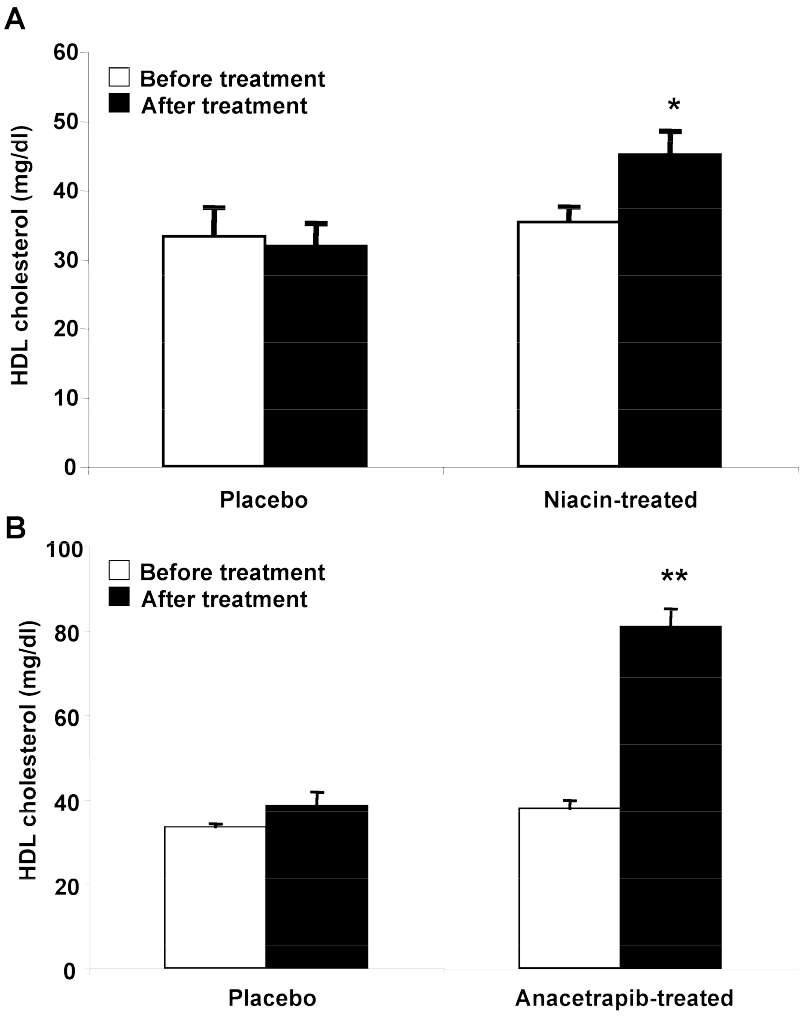
5. Matsuura F, Wang N, Chen W, Jiang XC, Tall AR. HDL from CETP-deficient subjects shows enhanced ability to promote cholesterol efflux from macrophages in an apoE- and ABCG1-dependent pathway. *J Clin Invest.* 2006;116:1435-1442.

Supplemental Table. I

Baseline	**Placebo**	**Niacin-ER**	**Placebo**	**Anacetrapib**
Demographics				
Sex, female/male	0/4	10/8	1/4	12/8
Age (years)	49.3 ± 4.9	52.4 ± 9.0	54.6 ± 8.2	58.8 ± 7.0
BMI (kg/m2)	29.2 ± 3.0	31.7 ± 6.8	31.0 ± 5.0	32.1 ± 5.9
% on background statin or other LMT	25.0	50.0	0	0
Plasma lipids				
Total cholesterol (mg/dl)	173.3 ± 48.2	203.4 ± 52.3	200.3 ± 13.5	235.3 ± 35.2
LDL-C (mg/dl)	104.3 ± 36.8	120.2 ± 45.5	123.7 ± 13.5	148.5 ± 23.9
Triglyceride (mg/dl)	100.0 ± 48.7	170.8 ± 60.2	167.8 ± 42.7	197.7 ± 90.9

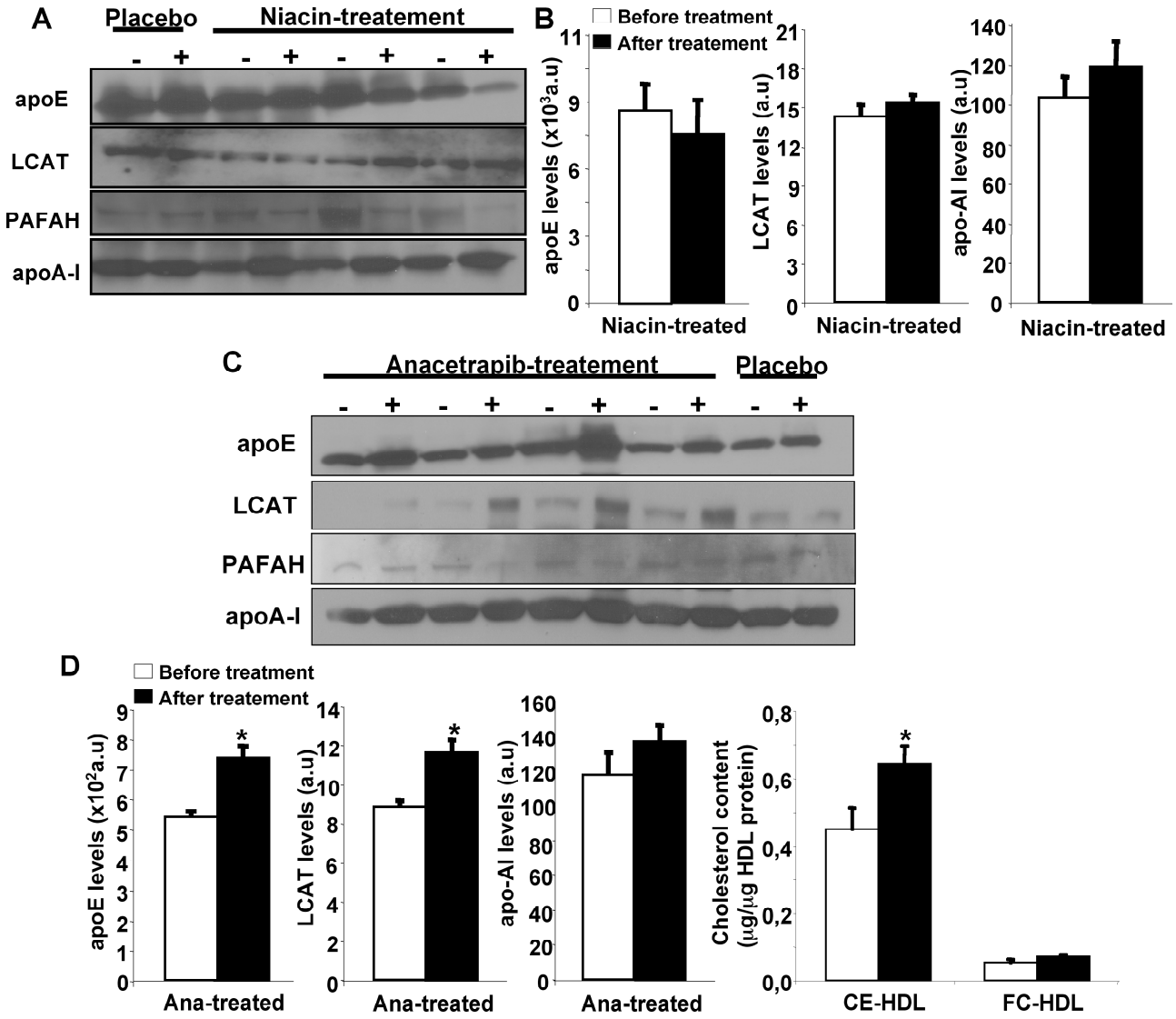
Data are presented as mean ± SD. BMI indicates body mass index and LMT, lipid modifying therapy. There were no significant differences between the groups.

Supplemental Fig. I



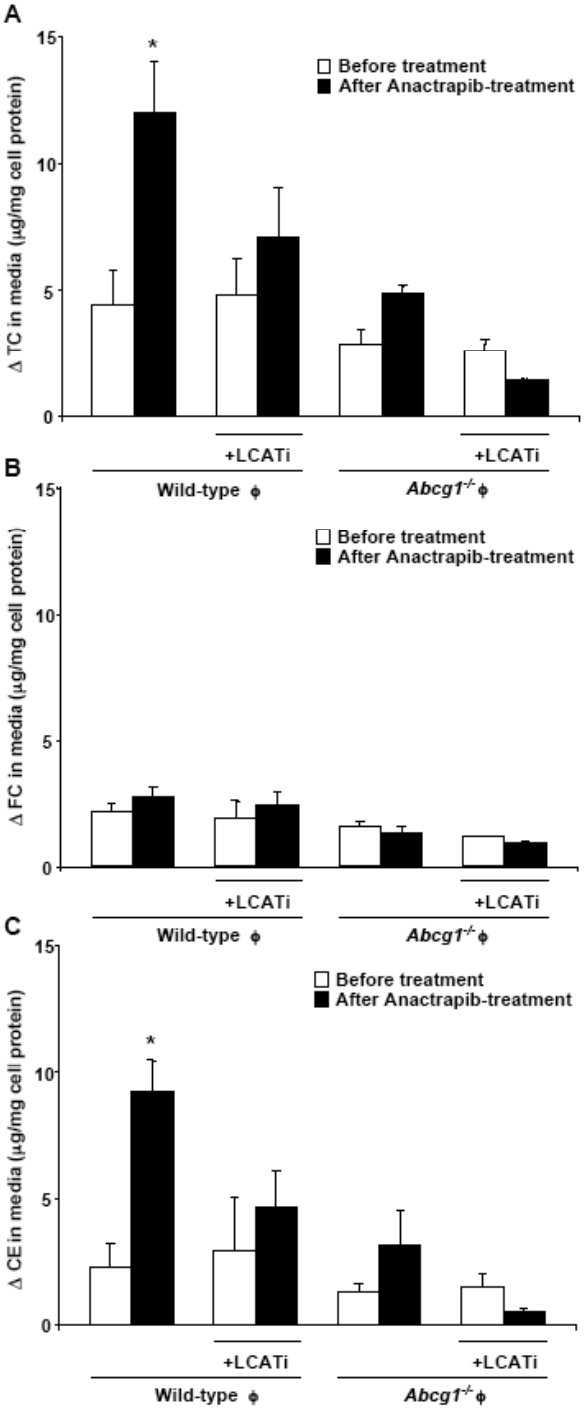
Supplemental Figure I: Effect of niacin and anacetrapib on plasma concentrations of HDL cholesterol (HDL-C). Relative to baseline, niacin increased plasma HDL-C levels by a mean of 28% from 35±2 to 45±3 mg/dL (A) and anacetrapib doubled plasma HDL-C levels (B). 18 subjects received niacin treatment compared to 4 placebo and 20 subjects received anacetrapib compared to 5 respective placebo. *P<0,01; **P<0.001 for comparison with baseline.

Supplemental Fig. II



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 paticles 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



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 Fig. IV

Anacetrapib treatment

Fig. 6A	***P, C-HDL effect in WT***			***P, A-HDL effect in WT***			***P, A-HDL vs.C-HDL***		
	3μgmL	**8μgmL**	**20μgmL**	**3μgmL**	**8μgmL**	**20μgmL**	**3μgmL**	**8μgmL**	**20μgmL**
TNFa	*NS*	*NS*	*<0.05*	*NS*	*NS*	*<0.05*	*NS*	*NS*	*NS*
IL-6	*NS*	*<0.05*	*<0.001*	*NS*	*<0.05*	*<0.001*	*NS*	*NS*	*NS*
MIP-1a	*NS*	*NS*	*<0.01*	*NS*	*NS*	*<0.01*	*NS*	*NS*	*NS*
MIP-2	*<0.05*	*<0.001*	*<0.001*	*<0.05*	*<0.001*	*<0.001*	*NS*	*NS*	*NS*
Fig. 6B	***P, C-HDL effect in WT***			***P, A-HDL effect in WT***			***P, A-HDL vs.C-HDL***		
	5μL	**10μL**	**25μL**	**5μL**	**10μL**	**25μL**	**5μL**	**10μL**	**25μL**
TNFa	*NS*	*NS*	*<0.05*	*NS*	***<0.05***	*<0.05*	*NS*	***<0.05***	*NS*
IL-6	*NS*	*NS*	*<0.001*	***<0.05***	***<0.01***	*<0.001*	***<0.05***	*NS*	*NS*
MIP-1a	*NS*	*NS*	*<0.05*	*NS*	***<0.05***	*<0.05*	***<0.05***	*NS*	*NS*
MIP-2	*NS*	*NS*	*NS*	***<0.05***	***<0.05***	***<0.01***	*NS*	*NS*	***<0.01***

Fig. 6A	***P, C-HDL effect in DKO***			***P, A-HDL effect in DKO***			***P, A-HDL vs.C-HDL***		
	3μgmL	**8μgmL**	**20μgmL**	**3μgmL**	**8μgmL**	**20μgmL**	**3μgmL**	**8μgmL**	**20μgmL**
TNFa	*NS*	*<0.05*	*<0.001*	*NS*	*<0.05*	*<0.001*	*NS*	*NS*	*NS*
IL-6	*NS*	*NS*	*<0.001*	*NS*	*NS*	*<0.001*	*NS*	*NS*	*NS*
MIP-1a	*NS*	*<0.05*	*<0.01*	*NS*	*<0.05*	*<0.01*	*NS*	*NS*	*NS*
MIP-2	*<0.05*	*<0.001*	*<0.001*	*<0.05*	*<0.001*	*<0.001*	*NS*	*NS*	*NS*
Fig. 6B	***P, C-HDL effect in DKO***			***P, A-HDL effect in DKO***			***P, A-HDL vs.C-HDL***		
	5μL	**10μL**	**25μL**	**5μL**	**10μL**	**25μL**	**5μL**	**10μL**	**25μL**
TNFa	*NS*	*NS*	*<0.001*	*NS*	*<0.05*	*<0.001*	*NS*	*NS*	*NS*
IL-6	*NS*	*NS*	*<0.001*	*NS*	*NS*	*<0.001*	*NS*	*NS*	*NS*
MIP-1a	*NS*	*<0.05*	*<0.001*	*NS*	*<0.05*	*<0.001*	*NS*	*NS*	*NS*
MIP-2	*NS*	*<0.01*	*<0.001*	*NS*	*<0.01*	*<0.001*	*NS*	*NS*	*NS*

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*$^{-/-}$*Abcg1*$^{-/-}$ (DKO) macrophages