High-Density Lipoprotein (HDL) Particle Subpopulations in Heterozygous Cholesteryl Ester Transfer Protein (CETP) Deficiency: Maintenance of Antioxidative Activity

Sandrine Chantepie[1]*[¤], Andrea E. Bochem[2], M. John Chapman[1,3,4], G. Kees Hovingh[2], Anatol Kontush[1,3,4]

1 INSERM UMRS 939, Pitie-Salpetriere University Hospital, Paris, France, 2 Department of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands, 3 Department of Scientific Research, University Pierre and Marie Curie - Paris 6, Paris, France, 4 AP-HP, Groupe hospitalier Pitié-Salpétrière, Paris, France

Abstract

Cholesteryl ester transfer protein (CETP) deficiency causes elevated high-density lipoprotein-cholesterol (HDL-C) levels; its impact on HDL functionality however remains elusive. We compared functional and compositional properties of HDL derived from 9 Caucasian heterozygous CETP mutation carriers (splice-site mutation in intron 7 resulting in premature truncation) with those of 9 age- and sex-matched normolipidemic family controls. As expected, HDL-C levels were increased 1.5-fold, and CETP mass and activity were decreased by −31% and −38% respectively, in carriers versus non-carriers. HDL particles from carriers were enriched in CE (up to +19%, $p<0.05$) and depleted of triglycerides (TG; up to −54%, $p<0.01$), resulting in a reduced TG/CE ratio (up to 2.5-fold, $p<0.01$). In parallel, the apoA-I content was increased in HDL from carriers (up to +22%, $p<0.05$). Both the total HDL fraction and small, dense HDL3 particles from CETP-deficient subjects displayed normal antioxidative activity by attenuating low-density lipoprotein oxidation with similar efficacy on a particle mass basis as compared to control HDL3. Consistent with these data, circulating levels of systemic biomarkers of oxidative stress (8-isoprostanes) were similar between the two groups. These findings support the contention that HDL functionality is maintained in heterozygous CETP deficiency despite modifications in lipid and protein composition.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: sandrine.chantepie@u-pec.fr

¤ Current address: Université Paris Est Créteil, Créteil, France

Introduction

Low circulating levels of high-density lipoprotein-cholesterol (HDL-C) constitute a significant and independent predictor of cardiovascular disease (CVD). This association may reflect deficiency of multiple antiatherogenic activities of HDL particles, including the capacity to act as an acceptor for cellular cholesterol together with antioxidative and anti-inflammatory actions [1].

HDL-C levels are subject to strong genetic control, with heritability ranging between 40% and 60% [2,3,4]. Factors determining HDL-C levels can be either of monogenic, polygenic, environmental, or multifactorial nature [5]. The CETP gene is one of the major genes affecting HDL metabolism; the gene codes for cholesteryl ester transfer protein (CETP), a protein responsible for the transfer of cholesteryl ester (CE) from HDL to apolipoprotein (apo) B-containing particles, particularly very low-density lipoprotein (VLDL), in exchange for triglyceride (TG) [6]. As a corollary, CETP deficiency typically results in an antiatherogenic lipid profile characterized by elevated levels of HDL-C and decreased to normal concentrations of low-density lipoprotein-cholesterol (LDL-C) [7,8].

The CETP gene is highly polymorphic with several common polymorphisms as well as rare mutations [9]. Homozygous CETP deficiency associated with complete loss of CETP activity leads to the accumulation of large, CE-rich HDL and elevation of HDL-C

levels up to 5-fold. Such complete CETP deficiency is exceedingly rare in Caucasians, but frequently observed in subjects of Asian origin [10,11]. Heterozygous deficiency of CETP results in less pronounced increases in HDL-C levels of +10 to +30% [12].

Despite elevated HDL-C levels, data on coronary risk in CETP-deficient subjects are conflicting [13,14,15,16,17,18,19,20], potentially reflecting defective atheroprotective functionality of HDL in this condition. CETP deficiency does not appear to compromise the capacity of HDL to efflux and transport cholesterol [21,22,23]; little is however known of other atheroprotective activities of HDL. To evaluate the effect of CETP deficiency on HDL function, we characterised compositional properties of HDL particles and their antioxidative activity in a hyperalphalipoproteinemic Dutch family with heterozygous CETP deficiency resulting from a splice-site mutation and premature protein truncation. Our findings further support the contention that HDL functionality is conserved in heterozygous CETP deficiency.

Subjects and Methods

Subjects

Nine Dutch subjects with heterozygous CETP deficiency due to a splice-site mutation in intron 7 resulting in premature truncation [24] were recruited at the Academic Medical Center, Amsterdam (The Netherlands). Age- and gender-matched family controls with

HDL-C levels between the 10th and the 90th percentiles for the general population were recruited at the same center. Subjects displaying severe hypertriglyceridemia (defined as plasma levels of TG >400 mg/dl) were excluded from the study.

Blood Samples

Venous blood was collected after an overnight fast. Routine biologic analyses were performed within 3 hours of blood sampling following centrifugal isolation of plasma. EDTA plasma and serum were immediately isolated, mixed with sucrose (final concentration 0.6%) as a cryoprotectant for lipoproteins [25], and frozen at $-80°C$ under nitrogen until using for lipoprotein analysis (see below). Total cholesterol (TC) and TG concentrations were determined by automated enzymatic methods (Konelab, Thermo-clinical Labsystems, Cergy Pontoise, France, and Biomerieux, Marcy L'Etoile, France, respectively). HDL-C was determined by a direct method using Konelab (Thermo Scientific, Waltham, MA, USA) [26]. LDL-C was calculated using Friedewald's equation [27]. Exogenous plasma CETP activity was determined using the Roar Biomedical kit (NY, New York) in which intra- and inter-assay coefficients of variation were less than 3% [28]. Plasma CETP concentration was determined by ELISA [29].

Isolation of Lipoproteins

Lipoproteins were fractionated by single-spin equilibrium density gradient ultracentrifugation [30] for 48 h at $40,000×g$ in a SW41-Ti rotor (Beckman Coulter, Pasadena, CA, USA) at 4°C. Five HDL subfractions were isolated: large, light HDL2b (density 1.063–1.087 g/ml) and 2a particles (density 1.088–1.110 g/ml), and small dense HDL3a (density 1.110–1.129 g/ml), 3b (density 1.129–1.154 g/ml) and 3c particles (density 1.154–1.170 g/ml) [30]. Reference LDL was isolated using the same procedure from one healthy, normolipidemic donor for all oxidation experiments. After dialysis against Dulbecco's PBS (pH 7.4) for 24 h at 4°C to remove EDTA and KBr, lipoproteins were maintained at 4°C and used within 1 week.

Characterisation of Lipoproteins

Total cholesterol (TC), free cholesterol (FC), phospholipid (PL) and TG concentrations were measured using commercially available kits (CHOP-PAP, Biomerieux, France). CE content was calculated by multiplying the difference between TC and FC by 1.67 [30]. Total protein was measured using the BCA assay. Total lipoprotein mass was calculated as the sum of the mass of total protein, CE, FC, PL and TG. Apolipoproteins apoA-I and apoA-II were quantified by immunonephelometry [31].

Enzymatic Activities of HDL Subpopulations

PON1 activity was determined photometrically at 270 nm as arylesterase activity using phenyl acetate as a substrate [31]. Inter- and intra-assay coefficients of variation were 2.2 and 9.8%, respectively.

Antioxidative Activity of HDL3b and HDL3c

Reference LDL (TC, 0.26 mmol/l, equivalent to 10 mg/dl) was incubated at 37°C in PBS in the presence of a water-soluble azo-initiator of oxidation, 2,2′-azo-bis-(2-amidinopropane) hydrochloride (AAPH; 1 mmol/l). Individual HDL subpopulations (10 mg/dl) or total HDL (40 mg total mass/dl) were added to LDL directly before oxidation on a total mass basis as previously described [31]. Total HDL from each donor was prepared by mixing all five HDL subfractions at their equivalent serum concentrations. The PBS was treated with Chelex 100 ion exchange resin (BioRad, Marnes-

la-Coquette, France) for 1 h to remove contaminating transition metal ions. Accumulation of conjugated dienes was continuously measured as the increment in absorbance at 234 nm [31]; two characteristic phases were identified, the lag phase and the propagation phase. To characterize the oxidation kinetics, average oxidation rate and the duration of propagation phase were calculated for each absorbance curve as described elsewhere [31].

Plasma Markers of Oxidative Stress

Systemic levels of oxidative stress were assessed as plasma levels of oxidised LDL (Mercodia, Uppsala, Sweden) and of 8-isoprostanes, products of non-enzymatic oxidation of arachidonic acid in vivo that represent a robust marker of systemic oxidative stress [32], by a commercial ELISA assay after purification (Cayman Chemical, Ann Arbor, MI, USA; inter- and intra-assay coefficients of variation 8.0 and 11.1%, respectively).

Statistical Analysis

Between-group differences were analysed by Student's two-tailed t-test for independent groups. All results are expressed as means ± SDs. A p value of less than 0.05 was considered statistically significant.

Results

Characteristics of Subjects

Biological and clinical characteristics of subjects are reported in Table 1. The CETP-deficient group did not differ from the non-carrier family control group in terms of age, BMI and plasma levels of total cholesterol, TG, non-HDL-C and LDL-C. As expected, HDL-C levels were increased 1.5 -fold while CETP mass and activity were decreased by $-31%$ and $-38%$ respectively in the CETP-deficient group as compared to controls.

Plasma Levels of HDL Particles

Concentrations of large HDL2 particles tended to be increased (up to 1.8-fold) in CETP-deficient subjects as compared to family controls (Fig. 1). By contrast, concentrations of small HDL3 subpopulations (Fig. 1) and those of total HDL ($343±88$ vs. $315±78$ mg/dl respectively) did not differ between CETP-deficient subjects and controls.

Chemical Composition of HDL Particles

Neutral lipids of the hydrophobic HDL core were strongly affected by CETP deficiency. All HDL particle subpopulations as well as total HDL from CETP-deficient subjects were significantly depleted in TG relative to HDLs from family control subjects (HDL2b, $-49%$, $p<0.05$; HDL2a, $-54%$, $p<0.01$; HDL3a, $-47%$, $p<0.01$; HDL3b, $-53%$, $p<0.01$; HDL3c, $-45%$, $p<0.05$; total HDL, $-48%$, $p<0.01$; Table 2). Moreover, CETP-deficient subjects possessed HDL2a and 3b enriched in CE ($+19%$, $p<0.05$, and $+12%$, $p<0.05$, respectively). As a result, the TG/CE ratio was reduced in each HDL subpopulation and in total HDL in CETP-deficient subjects (Table 2), 2.1-fold in HDL2b and 3a ($p<0.05$), 2.5-fold in HDL2a ($p<0.01$), 2.4-fold in HDL3b ($p<0.05$), 2.2-fold in HDL3c and in total HDL ($p<0.05$), consistent with diminished CETP activity.

In contrast, polar lipids of the HDL surface monolayer were less impacted by CETP deficiency (Table 2). Indeed, no systematic difference in PL content was observed between the groups; by contrast, FC tended to be elevated in HDLs from CETP-deficient subjects relative to family controls.

Finally, no difference in total protein content between the groups was observed (Table 2). Nonetheless, quantification of

Table 1. Biological and clinical characteristics of subjects.

	CETP-deficient subjects (n = 9)	Family control subjects (n = 9)
Sex (m/f)	5/4	5/4
Age (years)	55.0±16.3	54.3±14.0
Body mass index (kg/m^2)	26.1±3.3	26.1±5.6
Total cholesterol (mg/dl)	240±33	220±47
TG (mg/dl)	137±80	158±82
Non-HDL-C (mg/dl)	166±49	170±40
LDL-C (mg/dl)	140±33	141±33
HDL-C (mg/dl)	**75±26* (+50%)**	50±14
CETP mass (µg/ml)	**1.0±0.2*** (−31%)**	1.5±0.2
CETP activity (pmol of substrate transferred per hour and µl of plasma)	**28±5*** (−38%)**	46±8

***p<0.001, *p<0.05 vs controls. Numbers in parentheses denote % differences vs. controls.
doi:10.1371/journal.pone.0049336.t001

apoA-I and apoA-II in the protein moiety revealed a tendency to the elevation of the apoA-I content in HDLs from CETP-deficient subjects relative to the apoA-II content (Table 2). Furthermore, apoA-I content (as wt %) was significantly elevated in HDL2b, 2a and 3a in the CETP-deficient group (up to +22%, p<0.05, in HDL2b; data not shown).

Antioxidative Activity of Small, Dense HDL

Protection of normolipidemic LDL by small, dense HDL3b and HDL3c, and by total HDL, from oxidative stress induced by an azo-initiator (AAPH) was evaluated on the basis of total HDL mass. As documented in our earlier studies [31,33,34], the impact of HDL on LDL oxidation is most pronounced during the propagation phase. Indeed, small, dense HDL3 from normolipidemic subjects, and particularly HDL3c, markedly prolong the propagation phase but also greatly decrease oxidation rate in this phase [31,33,34].

In the present study, small, dense HDL3b and 3c particles as well as total HDL from CETP-deficient subjects did not differ from the corresponding fractions isolated from family controls in

Figure 1. Plasma levels of HDL particle subpopulations. Total lipoprotein mass was calculated as the sum of the mass of total protein, CE, FC, PL and TG for each HDL subpopulation isolated from CETP-deficient subjects (n = 9) and from family control subjects (n = 9).
doi:10.1371/journal.pone.0049336.g001

terms of their capacity to protect LDL from free-radical-induced oxidation. Indeed, small, dense HDL3b (Fig. 2A) and 3c (Fig. 2B) particles as well as total HDL (Fig. 2C) from CETP-deficient subjects and from controls were of similar efficacy in prolonging the propagation phase of LDL oxidation and diminishing oxidation rate in the propagation phase of LDL.

Levels of Systemic Oxidative Stress

CETP-deficient subjects did not reveal elevated levels of systemic oxidative stress. Indeed, plasma concentrations of both oxidised LDL (59.0±23.9 U/l for CETP-deficient subjects vs 58.4±11.5 U/l for family controls) and 8-isoprostanes (19±22 for CETP-deficient subjects vs 30±30 ng/l for family controls) as well as HDL-associated paraoxonase activity (4.69±1.00 µmol/min/mg protein for HDL3c from CETP-deficient subjects vs 4.58±1.06 µmol/min/mg protein for HDL3c from family controls) were similar between the groups.

Discussion

We have shown that both small, dense HDL subpopulations and the total HDL fraction derived from hyperalphalipoproteinemic Dutch subjects, deficient in CETP due to a splice-site mutation in the intron 7 and premature protein truncation, display antioxidative activity towards human LDL oxidised by free radicals which is comparable to that in non-affected control members of the families. In support of these data, circulating biomarkers of systemic oxidative stress (oxidised LDL and 8-isoprostanes) did not differ between CETP-deficient subjects and age-matched normolipidemic family controls.

Mechanisms of HDL-mediated protection of LDL from free-radical-induced oxidation involve inactivation of lipid hydroperoxides (LOOHs) via a two-step mechanism which includes transfer of LOOHs to HDL, modulated by physical properties of the surface lipid monolayer, with subsequent reduction to redox-inactive hydroxides by Met residues of apoA-I [35,36,37,38,39]. The content and composition of surface lipids and apoA-I therefore represent key compositional determinants of the antioxidative properties of HDL. In our studies, HDL particles from carriers revealed pronounced compositional changes at the level of their lipid core, including enrichment in CE (up to +19%, p<0.05) and depletion of TG (up to −54%, p<0.01) with a reduced TG/CE ratio (up to 2.5-fold,
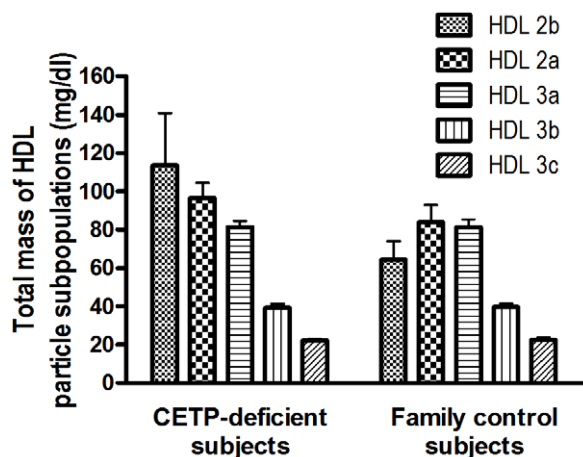
Table 2. Chemical composition of HDL subfractions from CETP-deficient subjects (n = 9) and family control subjects (n = 9).

	Group	HDL2b	HDL2a	HDL3a	HDL3b	HDL3c	Total HDL
TG	CETP-deficient	4.3±3.0*	2.2±1.2**	2.0±1.2**	1.8±1.1**	1.8±1.2*	2.5±1.5**
	Family control	8.5±3.8	4.8±1.8	3.8±1.1	3.8±1.6	3.3±1.8	4.9±1.7
CE	CETP-deficient	28.7±3,8	26.1±4.5*	23.3±2.8	21.8±1.6*	17.8±1.0	25.3±4.0
	Family control	26.0±3.6	21.9±3.4	21.0±2.9	19.4±2.2	16.7±2.0	21.8±3.1
FC	CETP-deficient	6.4±1.4	4.1±1.1	3.1±0.6	2.5±0.4	1.7±0.5	4.3±1.5
	Family control	5.8±1.1	3.6±0.7	2.8±0.4	2.3±0.3	1.6±0.5	3.5±0.7
PL	CETP-deficient	31.8±2.5	30.2±4.2	28.8±2.0	25.6±1.1	20.5±0.9	29.1±2.2
	Family control	30.3±3.7	31.8±2.4	29.4±1.9	26.4±1.5	20.9±1.8	29.3±2.3
Protein	CETP-deficient	28.8±2.6	37.4±4.4	42.8±3.0	48.2±0.8	58.2±1.8	38.8±4.8
	Family control	29.4±1.9	37.9±3.1	43.0±2.5	48.1±1.6	57.4±2.4	40.5±3.2
A-I/A-II	CETP-deficient	29.7±4.4*	38.7±1.9 *	41.6±1.2*	47.5±3.1	47.1±1.8	3.8±0.9
	Family control	24.3±4.0	36.6±1.0	40.3±1.0	45.8±3.1	44.8±3.0	3.2±0.3
TG/CE	CETP-deficient	0.16±0.13*	0.09±0.06**	0.09±0.06*	0.09±0.06*	0.10±0.07*	0.11±0.07*
	Family control	0.35±0.19	0.23±0.12	0.19±0.08	0.21±0.11	0.21±0.15	0.24±0.12

TC, FC, PL and TG concentrations were measured using commercially available kits (CHOP-PAP, Biomerieux, France). Cholesteryl ester (CE) content was calculated by multiplying the difference between TC and FC by 1.67. Total protein was measured using the BCA assay. Apolipoprotein A-I and apoA-II were determined by immunonephelometry. All parameters, except the ratios, are expressed as percentage of total HDL mass. *$p < 0.05$, **$p < 0.01$, vs. corresponding HDL from controls.
doi:10.1371/journal.pone.0049336.t002

$p < 0.01$); surface lipids (PL, FC) however remained unaffected. In parallel, HDL content of apoA-I was increased in HDLs from carriers (up to +22% vs. controls, $p < 0.05$). In clear contrast, our earlier studies revealed that HDL particles were depleted of CE and of apoA-I and enriched in TG in atherogenic dyslipidemia of insulin-resistant states associated with elevated CETP activity, such as metabolic syndrome and Type 2 diabetes; such compositional alterations were paralleled by reduced antioxidative activity of HDL [40]. Similar structure-functional relationships were documented in normo-triglyceridemic low HDL-C dyslipidemia [41]. Compositional modifications of HDLs observed in CETP-deficient subjects in our present study are therefore inconsistent with defective antioxidative activities.

Metabolically, reduced CETP activity in heterozygous CETP deficiency modifies core lipid composition of HDL particles, delaying their catabolism and increasing their apoA-I content. Functionality of HDL which is largely determined by the content of apoA-I, the key component underlying antioxidative activities of HDL [42], can therefore be potentially improved by CETP deficiency. On the other hand, prolonged HDL lifespan in CETP-deficient subjects suggests the possibility of enhanced apoA-I oxidation [43] which may result in reduced HDL capacity to protect LDL from free radical-induced oxidation secondary to the reduced apoA-I content of redox-active Met residues [42]. The relevance of the latter pathway is however in disagreement with our present data which support the contention that HDL exhibits antioxidative activity within the normal range in heterozygous CETP deficiency. Consistent with the data derived from studies focussing on extreme genetics and common variants [13,16,44,45], HDL-C levels were increased 1.5-fold, and CETP mass and activity were decreased by −31% and −38% respectively, in carriers versus non-carriers in our present study. Such consistency in biomarkers of lipid metabolism between earlier and present studies suggests the pertinence of our present findings to the general context of heterozygous CETP deficiency.

Our conclusion regarding the normal antioxidative function of HDL in heterozygous CETP deficiency is further supported by published data on antioxidative activity of HDL particles in CETP-deficient states. Indeed, the capacity of HDL to protect LDL against oxidative modification is not affected by CETP overexpression in a transgenic mice model [46]. Furthermore, the CETP inhibitor dalcetrapib decreased circulating levels of oxidised LDL in familial hypoalphalipoproteinemia [47], whereas CETP inhibition in vitro by a monoclonal antibody renders LDL more resistant to oxidation [48], observations which are consistent with the absence of a deficiency in the HDL-mediated protection of LDL from oxidation in vivo. Importantly, the normal capacity of HDL to protect LDL from oxidative modification by free radicals in the arterial intima can contribute to protection from atherogenesis. Indeed, despite the fact that the pathophysiological importance of LDL oxidation by free radicals remains to be demonstrated, this pathway is operative in human atherosclerosis and can be efficiently inhibited by HDL [49,50,51].

Normal antioxidative activity of HDL in CETP-deficient subjects as observed by us is also important in the context of the role of CETP for the production of small HDL particles. Indeed, recycling of lipid-free/lipid-poor apoA-I from mature HDL combined with the subsequent actions of hepatic lipase and scavenger receptor class B type I (SR-BI) constitutes an important aspect of the lipoprotein-remodelling action of CETP [52]. As small, dense HDL particles display potent anti-atherogenic activities [31,53,54], CETP deficiency might theoretically impair atheroprotective properties of the total plasma HDL pool via reduction in plasma levels of small HDL; however, our present data do not support such a hypothesis. The observation of normal antioxidative properties of total HDL is further consistent with the minor contribution of large, light HDL2 to this biological activity as reported earlier [31]. Indeed, although the composition of large, light HDL2 particles was strongly affected by CETP deficiency in our studies, the antioxidative properties of total HDL remained unchanged.
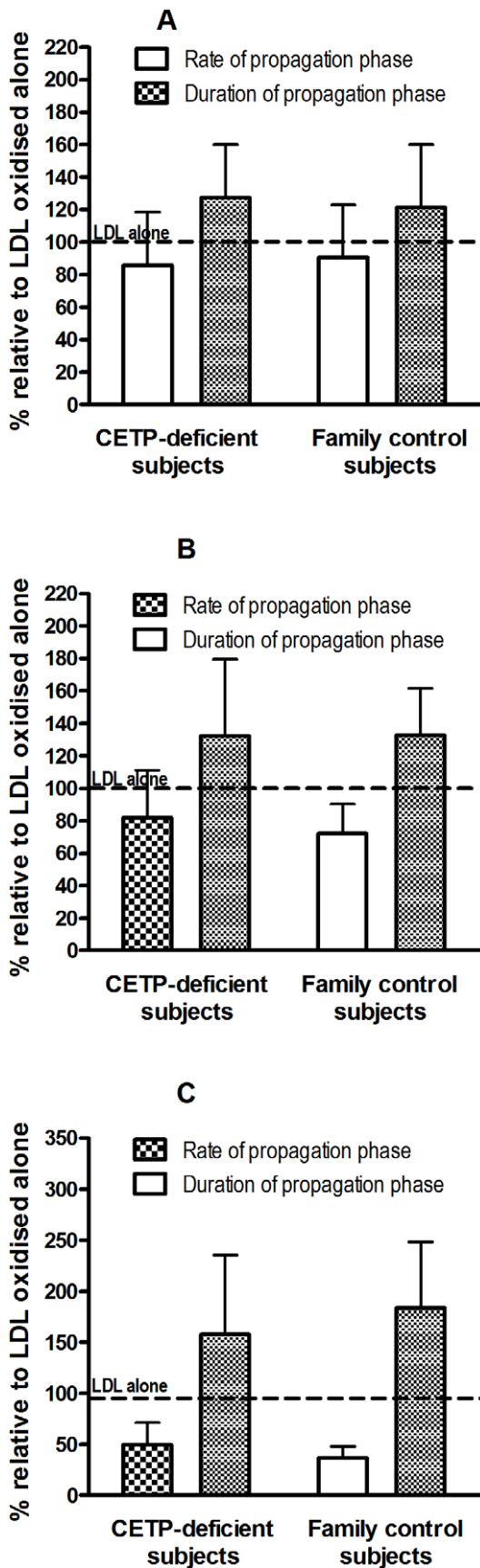
Figure 2. Antioxidative activity of HDL particles from CETP-deficient (n = 9) and family control (n = 9) subjects. Reference LDL (10 mg TC/dl) was incubated at 37°C in PBS in the presence of AAPH (1 mmol/l). Small, dense HDL3b (A), HDL3c (B) or total HDL (C) particles were added to LDL directly before oxidation at 10 mg total mass/dl (A, B) or 40 mg total mass/dl (C). Accumulation of conjugated dienes was continuously measured as the increment in absorbance at 234 nm; two characteristic phases were identified, the lag phase and the propagation phase. To characterise the oxidation kinetics, oxidation rate within the propagation phase and duration of this phase were calculated for each absorbance curve.
doi:10.1371/journal.pone.0049336.g002

Importantly, CETP deficiency does not compromise the capacity of HDL to efflux cellular cholesterol, another key atheroprotective activity of HDL. Indeed, homozygous CETP deficiency features accumulation of large HDL2 particles with elevated content of apoE and lecithin:cholesterol acyltransferase (LCAT); such particles display elevated cholesterol efflux capacity on THP-1 cells [21]. In addition, HDLs isolated from a compound heterozygote for a known D459G variant and a novel 18-bp deletion mutation in the CETP promoter display elevated cholesterol efflux capacity via SR-BI [22]. Together, these considerations further support the concept that partial CETP deficiency does not impair functionality of plasma HDL [55].

In order to evaluate the potential physiopathological and therapeutic relevance of this conclusion, the relationship between CETP deficiency and cardiovascular risk should be taken into account. Mutations in the CETP gene frequently cause familial hyperalphalipoproteinemia [56]; most CETP polymorphisms, such as three common CETP genotypes Taq1B (+279G>A), Ile405Val (+16A>G) and −629C>A, are equally associated with elevated HDL-C levels [13,16,44,45]. Potentially reflecting the atheroprotective role of HDL, the TaqIB, I405V and −629C>A genotypes associated with lower CETP activity and higher HDL-C levels are inversely associated with coronary risk [13,14,16]. Moreover, individuals with longevity syndrome display an elevated frequency of the B2 allele of TaqIB polymorphism and of the Int 14A variant in the CETP gene, both associated with high levels of HDL-C [57,58].

These results support the hypothesis of a causal relationship between low CETP activity, high HDL-C and reduced cardiovascular risk, which forms a basis for therapeutic strategies involving partial CETP inhibition in humans [6]. Indeed, metabolic conditions of CETP inhibition share some similar characteristics to those of CETP deficiency, the latter involving reduced levels of CETP protein; however, it is essential to emphasise that the two metabolic conditions cannot be directly compared. Studies in rabbits, a species with naturally high levels of CETP, support the therapeutic potential of partial CETP inhibition as an approach to retarding atherogenesis [59,60]. CETP inhibitors are presently the most potent HDL-raising agents, with dose-dependent HDL-C elevation of up to +100% or more for some agents [6]. HDL particles which are functional in terms of their capacity to efflux cellular cholesterol are formed following either potent or moderate levels of CETP inhibition by anacetrapib [61] or dalcetrapib [62,63]. As a result, the net action of partial CETP inhibition on reverse cholesterol transport from peripheral tissues to the liver is not deleterious in CETP-expressing species. Detrimental effects of CETP inhibition on atheroprotective functions of HDL other than cholesterol efflux cannot however be excluded [64] and remain in the focus of ongoing discussions, in view of further clinical development of other CETP inhibitors [43,65,66]. Our present results in partial genetic CETP deficiency shed a new

light on this controversial area, suggesting that moderate CETP inhibition should not exert deleterious impact on the antioxidative properties of HDL.

Author Contributions

Conceived and designed the experiments: MJC GKH AK. Performed the experiments: SC AB. Analyzed the data: SC GKH AK. Wrote the paper: SC AB MJC GKH AK.

References

1. Camont L, Chapman MJ, Kontush A (2011) Biological activities of HDL subpopulations and their relevance to cardiovascular disease. Trends Mol Med 17: 594–603.

2. Kronenberg F, Coon H, Ellison RC, Borecki I, Arnett DK, et al. (2002) Segregation analysis of HDL cholesterol in the NHLBI Family Heart Study and in Utah pedigrees. Eur J Hum Genet 10: 367–374.

3. Perusse L, Rice T, Despres JP, Bergeron J, Province MA, et al. (1997) Familial resemblance of plasma lipids, lipoproteins and postheparin lipoprotein and hepatic lipases in the HERITAGE Family Study. Arterioscler Thromb Vasc Biol 17: 3263–3269.

4. Pietilainen KH, Soderlund S, Rissanen A, Nakanishi S, Jauhiainen M, et al. (2009) HDL subspecies in young adult twins: heritability and impact of overweight. Obesity (Silver Spring) 17: 1208–1214.

5. Weissglas-Volkov D, Pajukanta P (2010) Genetic causes of high and low serum HDL-cholesterol. J Lipid Res 51: 2032–2057.

6. Chapman MJ, Le Goff W, Guerin M, Kontush A (2010) Cholesteryl ester transfer protein: at the heart of the action of lipid-modulating therapy with statins, fibrates, niacin, and cholesteryl ester transfer protein inhibitors. Eur Heart J 31: 149–164.

7. Miller M, Rhyne J, Hamlette S, Birnbaum J, Rodriguez A (2003) Genetics of HDL regulation in humans. Curr Opin Lipidol 14: 273–279.

8. Plengpanich W, Siriwong S, Khovidhunkit W (2009) Two novel mutations and functional analyses of the CETP and LIPC genes underlying severe hyperalphalipoproteinemia. Metabolism 58: 1178–1184.

9. Thompson JF, Wood LS, Pickering EH, DeChairo B, Hyde CL (2007) High-density genotyping and functional SNP localization in the CETP gene. J Lipid Res 48: 434–443.

10. Calabresi L, Nilsson P, Pinotti E, Gomaraschi M, Favari E, et al. (2009) A novel homozygous mutation in CETP gene as a cause of CETP deficiency in a Caucasian kindred. Atherosclerosis 205: 506–511.

11. Weissglas-Volkov D, Aguilar-Salinas CA, Sinsheimer JS, Riba L, Huertas-Vazquez A, et al. (2010) Investigation of Variants Identified in Caucasian Genome-Wide Association Studies for Plasma High-Density Lipoprotein Cholesterol and Triglycerides Levels in Mexican Dyslipidemic Study Samples. Circ Cardiovasc Genet 3: 31–38.

12. Klos KL, Kullo IJ (2007) Genetic determinants of HDL: monogenic disorders and contributions to variation. Curr Opin Cardiol 22: 344–351.

13. Thompson A, Di Angelantonio E, Sarwar N, Erqou S, Saleheen D, et al. (2008) Association of cholesteryl ester transfer protein genotypes with CETP mass and activity, lipid levels, and coronary risk. JAMA 299: 2777–2788.

14. Regieli JJ, Jukema JW, Grobbee DE, Kastelein JJ, Kuivenhoven JA, et al. (2008) CETP genotype predicts increased mortality in statin-treated men with proven cardiovascular disease: an adverse pharmacogenetic interaction. Eur Heart J 29: 2792–2799.

15. Wilson PW (2008) CETP genes, metabolic effects, and coronary disease risk. JAMA 299: 2795–2796.

16. Boekholdt SM, Sacks FM, Jukema JW, Shepherd J, Freeman DJ, et al. (2005) Cholesteryl ester transfer protein TaqIB variant, high-density lipoprotein cholesterol levels, cardiovascular risk, and efficacy of pravastatin treatment: individual patient meta-analysis of 13,677 subjects. Circulation 111: 278–287.

17. Curb JD, Abbott RD, Rodriguez BL, Masaki K, Chen R, et al. (2004) A prospective study of HDL-C and cholesteryl ester transfer protein gene mutations and the risk of coronary heart disease in the elderly. J Lipid Res 45: 948–953.

18. Ridker PM, Pare G, Parker AN, Zee RYL, Miletich JP, et al. (2009) Polymorphism in the CETP Gene Region, HDL Cholesterol, and Risk of Future Myocardial Infarction: Genomewide Analysis Among 18 245 Initially Healthy Women From the Women's Genome Health Study. Circ Cardiovasc Genet 2: 26–33.

19. Anand SS, Xie C, Pare G, Montpetit A, Rangarajan S, et al. (2009) Genetic Variants Associated With Myocardial Infarction Risk Factors in Over 8000 Individuals From Five Ethnic Groups: The INTERHEART Genetics Study. Circ Cardiovasc Genet 2: 16–25.

20. Agerholm-Larsen B, Nordestgaard BG, Steffensen R, Jensen G, Tybjaerg-Hansen A (2000) Elevated HDL cholesterol is a risk factor for ischemic heart disease in white women when caused by a common mutation in the cholesteryl ester transfer protein gene. Circulation 101: 1907–1912.

21. Tall AR (2009) The Effects of Cholesterol Ester Transfer Protein Inhibition on Cholesterol Efflux. Am J Cardiol 104: 39E–45E.

22. Plengpanich W, Le Goff W, Poolsuk S, Julia Z, Guerin M, et al. (2011) CETP deficiency due to a novel mutation in the CETP gene promoter and its effect on cholesterol efflux and selective uptake into hepatocytes. Atherosclerosis 216: 370–373.

23. Miwa K, Inazu A, Kawashiri M, Nohara A, Higashikata T, et al. (2009) Cholesterol efflux from J774 macrophages and Fu5AH hepatoma cells to serum is preserved in CETP-deficient patients. Clin Chim Acta 402: 19–24.

24. van der Steeg WA, Hovingh GK, Klerkx AHEM, Hutten BA, Nootenboom IC, et al. (2007) Cholesteryl ester transfer protein and hyperalphalipoproteinemia in Caucasians. J Lipid Res 48: 674–682.

25. Rumsey SC, Stucchi AF, Nicolosi RJ, Ginsberg HN, Ramakrishnan R, et al. (1994) Human plasma LDL cryopreserved with sucrose maintains in vivo kinetics indistinguishable from freshly isolated human LDL in cynomolgus monkeys. J Lipid Res 35: 1592–1598.

26. Egloff M, Leglise D, Duvillard L, Steinmetz J, Boyer MJ, et al. (1999) [Multicenter evaluation on different analyzers of three methods for direct HDL-cholesterol assay]. Ann Biol Clin (Paris) 57: 561–572.

27. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18: 499–502.

28. Dullaart RP, Sluiter WJ, Dikkeschei LD, Hoogenberg K, Van Tol A (1994) Effect of adiposity on plasma lipid transfer protein activities: a possible link between insulin resistance and high density lipoprotein metabolism. Eur J Clin Invest 24: 188–194.

29. Niemeijer-Kanters SD, Dallinga-Thie GM, de Ruijter-Heijstek FC, Algra A, Erkelens DW, et al. (2001) Effect of intensive lipid-lowering strategy on low-density lipoprotein particle size in patients with type 2 diabetes mellitus. Atherosclerosis 156: 209–216.

30. Chapman MJ, Goldstein S, Lagrange D, Laplaud PM (1981) A density gradient ultracentrifugal procedure for the isolation of the major lipoprotein classes from human serum. J Lipid Res 22: 339–358.

31. Kontush A, Chantepie S, Chapman MJ (2003) Small, dense HDL particles exert potent protection of atherogenic LDL against oxidative stress. Arterioscler Thromb Vasc Biol 23: 1881–1888.

32. Mezzetti A, Cipollone F, Cuccurullo F (2000) Oxidative stress and cardiovascular complications in diabetes: isoprostanes as new markers on an old paradigm. Cardiovasc Res 47: 475–488.

33. Hansel B, Giral P, Nobecourt E, Chantepie S, Bruckert E, et al. (2004) Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. J Clin Endocrinol Metab 89: 4963–4971.

34. Kontush A, de Faria EC, Chantepie S, Chapman MJ (2004) Antioxidative activity of HDL particle subspecies is impaired in hyperalphalipoproteinemia: relevance of enzymatic and physicochemical properties. Arterioscler Thromb Vasc Biol 24: 526–533.

35. Sattler W, Christison J, Stocker R (1995) Cholesterylester hydroperoxide reducing activity associated with isolated high- and low-density lipoproteins. Free RadicBiolMed 18: 421–429.

36. Christison JK, Rye KA, Stocker R (1995) Exchange of oxidized cholesteryl linoleate between LDL and HDL mediated by cholesteryl ester transfer protein. JLipid Res 36: 2017–2026.

37. Garner B, Witting PK, Waldeck AR, Christison JK, Raftery M, et al. (1998) Oxidation of high density lipoproteins. I. Formation of methionine sulfoxide in apolipoproteins AI and AII is an early event that accompanies lipid peroxidation and can be enhanced by alpha-tocopherol. JBiolChem 273: 6080–6087.

38. Garner B, Waldeck AR, Witting PK, Rye KA, Stocker R (1998) Oxidation of high density lipoproteins. II. Evidence for direct reduction of lipid hydroperoxides by methionine residues of apolipoproteins AI and AII. JBiolChem 273: 6088–6095.

39. Zerrad-Saadi A, Therond P, Chantepie S, Couturier M, Rye K-A, et al. (2009) HDL3-Mediated Inactivation of LDL-Associated Phospholipid Hydroperoxides Is Determined by the Redox Status of Apolipoprotein A-I and HDL Particle Surface Lipid Rigidity: Relevance to Inflammation and Atherogenesis. Arterioscler Thromb Vasc Biol 29: 2169–2175.

40. Kontush A, Chapman MJ (2006) Functionally defective HDL: A new therapeutic target at the crossroads of dyslipidemia, inflammation and atherosclerosis. Pharmacol Rev 3: 342–374.

41. Kontush A, de Faria EC, Chantepie S, Chapman MJ (2005) A normotriglyceridemic, low HDL-cholesterol phenotype is characterised by elevated oxidative stress and HDL particles with attenuated antioxidative activity. Atherosclerosis 182: 277–285.

42. Kontush A, Chapman MJ (2010) Antiatherogenic function of HDL particle subpopulations: focus on antioxidative activities. Curr Opin Lipidol 21: 312–318.

43. Duriez P (2007) CETP inhibition. The Lancet 370: 1882–1883.

44. Boekholdt SM, Thompson JF (2003) Natural genetic variation as a tool in understanding the role of CETP in lipid levels and disease. J Lipid Res 44: 1080–1093.

45. Tsai MY, Johnson C, Kao WHL, Sharrett AR, Arends VL, et al. (2008) Cholesteryl ester transfer protein genetic polymorphisms, HDL cholesterol, and subclinical cardiovascular disease in the Multi-Ethnic Study of Atherosclerosis. Atherosclerosis 200: 359–367.

46. Rotllan N, Calpe-Berdiel L, Guillaumet-Adkins A, Suren-Castillo S, Blanco-Vaca F, et al. (2008) CETP activity variation in mice does not affect two major HDL antiatherogenic properties: macrophage-specific reverse cholesterol transport and LDL antioxidant protection. Atherosclerosis 196: 505–513.

47. Bisoendial RJ, Hovingh GK, El Harchaoui K, Levels JHM, Tsimikas S, et al. (2005) Consequences of Cholesteryl Ester Transfer Protein Inhibition in Patients With Familial Hypoalphalipoproteinemia. Arterioscler Thromb Vasc Biol 25: e133–134.

48. Sugano M, Sawada S, Tsuchida K, Makino N, Kamada M (2000) Low density lipoproteins develop resistance to oxidative modification due to inhibition of cholesteryl ester transfer protein by a monoclonal antibody. J Lipid Res 41: 126–133.

49. Gaut JP, Heinecke JW (2001) Mechanisms for oxidizing low-density lipoprotein. Insights from patterns of oxidation products in the artery wall and from mouse models of atherosclerosis. Trends Cardiovasc Med 11: 103–112.

50. Yoshida H, Kisugi R (2010) Mechanisms of LDL oxidation. Clin Chim Acta 411: 1875–1882.

51. Tsimikas S, Miller YI (2011) Oxidative modification of lipoproteins: mechanisms, role in inflammation and potential clinical applications in cardiovascular disease. Curr Pharm Des 17: 27–37.

52. Joy T, Hegele RA (2008) Is raising HDL a futile strategy for atheroprotection? Nat Rev Drug Discov 7: 143–155.

53. Yoshikawa M, Sakuma N, Hibino T, Sato T, Fujinami T (1997) HDL3 exerts more powerful anti-oxidative, protective effects against copper-catalyzed LDL oxidation than HDL2. Clin Biochem 30: 221–225.

54. Huang JM, Huang ZX, Zhu W (1998) Mechanism of high-density lipoprotein subfractions inhibiting copper-catalyzed oxidation of low-density lipoprotein. Clin Biochem 31: 537–543.

55. Orloff DG (2007) Regulatory considerations in the development of high-density lipoprotein therapies. Am J Cardiol 100: S10–14.

56. Cefalu AB, Noto D, Magnolo L, Pinotti E, Gomaraschi M, et al. (2009) Novel mutations of CETP gene in Italian subjects with hyperalphalipoproteinemia. Atherosclerosis 204: 202–207.

57. Kolovou G, Stamatelatou M, Anagnostopoulou K, Kostakou P, Kolovou V, et al. (2010) Cholesteryl ester transfer protein gene polymorphisms and longevity syndrome. Open Cardiovasc Med J 4: 14–19.

58. Koropatnick TA, Kimbell J, Chen R, Grove JS, Donlon TA, et al. (2008) A prospective study of high-density lipoprotein cholesterol, cholesteryl ester transfer protein gene variants, and healthy aging in very old Japanese-american men. J Gerontol A Biol Sci Med Sci 63: 1235–1240.

59. Barter PJ, Brewer HB Jr, Chapman MJ, Hennekens CH, Rader DJ, et al. (2003) Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. Arterioscler Thromb Vasc Biol 23: 160–167.

60. Gaofu Q, Jun L, Xiuyun Z, Wentao L, Jie W, et al. (2005) Antibody against cholesteryl ester transfer protein (CETP) elicited by a recombinant chimeric enzyme vaccine attenuated atherosclerosis in a rabbit model. Life Sci 77: 2690–2702.

61. Yvan-Charvet L, Kling J, Pagler T, Li H, Hubbard B, et al. (2010) Cholesterol Efflux Potential and Antiinflammatory Properties of High-Density Lipoprotein After Treatment With Niacin or Anacetrapib. Arterioscler Thromb Vasc Biol 30: 1430–1438.

62. Niesor EJ, Okamoto H, Maugeais C, von der Mark E, Brousse M, et al. (2009) Abstract 1271: The Effects of Dalcetrapib on Macrophage Reverse Cholesterol Transport in a Rodent Model. Circulation 120: S468-a-.

63. Niesor EJ, Magg C, Ogawa N, Okamoto H, von der Mark E, et al. (2010) Modulating cholesteryl ester transfer protein activity maintains efficient pre-beta-HDL formation and increases reverse cholesterol transport. J Lipid Res 51: 3443–3454.

64. Kontush A, Chapman MJ (2012) High-Density Lipoproteins: Structure, Metabolism, Function and Therapeutics. New York: Wiley & Sons. 648 p.

65. Kontush A, Guerin M, Chapman MJ (2008) Spotlight on HDL-raising therapies: insights from the torcetrapib trials. Nat Clin Pract Cardiovasc Med 5: 329–336.

66. Masson D, Jiang X-C, Lagrost L, Tall AR (2009) The role of plasma lipid transfer proteins in lipoprotein metabolism and atherogenesis. J Lipid Res 50: S201–206.