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Critical Review

Cholesteryl Ester Transfer Protein: The Controversial Relation to Atherosclerosis and Emerging New Biological Roles

Helena C. F. Oliveira[1] and Eliana C. de Faria[2]
[1]*Department of Physiology and Biophysics, State University of Campinas, Campinas, SP, Brazil*
[2]*Department of Clinical Pathology, State University of Campinas, Campinas, SP, Brazil*

Summary

Cholesteryl ester transfer protein (CETP) exerts a profound impact on high-density lipoprotein (HDL) metabolism and, consequently, on the risk of atherosclerosis development and cardiovascular mortality. Here, we review the complex relationship between CETP and atherosclerosis based upon the experimental, clinical, and epidemiological studies. In addition, we discuss the recent findings that expand the functions of CETP to new areas of interest such as Alzheimer's disease, inflammation, and obesity.
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Keywords CETP; atherosclerosis; Alzheimer disease; inflammation; obesity.

Abbreviations ABCA1/G1, ATP binding cassette transporter A1 or G1; Abeta, amyloid beta; AD, Alzheimer's disease; apo, apolipoprotein; BMI, body mass index; BPI, bactericidal permeability increasing protein; C/EBP, Ccaat-enhancer-binding proteins; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; CSF, cerebrospinal fluid; CVD, cardiovascular disease; HDL, high-density lipoprotein; IL-6, interleucine-6; LBP, lipopolysaccharide binding protein; LCAT, lecithin cholesterol acyl transferase; LDL, low density lipoprotein; LDLr, LDL receptor; LP, lipoprotein; LPL, lipoprotein lipase; LPS, lipopolysaccharide; LRP, LDL receptor related protein; NEFA, non-esterified fatty acids; oxLDL, oxidized LDL; PPAR, peroxisome proliferator-activated receptor; RCT, Reverse cholesterol transport; SNP, single nucleotide polymorphism; SRBI, scavenger receptor class B type I; SREBP, sterol responsive element binding protein; TG, triglycerides; TNF-alpha, tumor necrosis factor – alpha; VLDL, very low density lipoprotein.

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Address correspondence to: Helena C. F. Oliveira, Departamento de Fisiologia e Biofísica, Instituto de Biologia, Universidade Estadual de Campinas, Rua Monteiro Lobato, 255 Campinas, CEP 13083-862, SP, Brasil. Tel: 55-19-3521 6204. Fax: 55-19-35216185.
E-mail: ho98@unicamp.br

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BRIEF HIGHLIGHTS OF CETP HISTORY

A time sequence of the main research findings regarding CETP is shown in Fig. 1. Plasma CETP activity was first discovered to occur *in vitro* by Nichols and Smith (*1*). A few years later, the biochemistry of its activity was characterized by Pattnaik et al. (*2*). Because CETP action results in the net transfer of cholesteryl ester (CE) from HDL to LDL, its potential to modulate both lipoprotein levels, and hence atherosclerosis risk, attracted much interest from the scientific community. Ha and Barter (*3*) reported that CETP is present in fish, reptiles, birds, and mammals, but not in rats and mice. Chemical purification showed that CETP is a plasma glycoprotein consisting of 476 amino acids with an apparent molecular weight of 66–74 KDa (*4*). This discovery led to subsequent cDNA cloning and tissue expression studies (*5*). In humans and primates, CETP has a widespread pattern of tissue expression; however, the liver seems to be the main source of the CETP found in the blood stream. In the late 1980s, the first cases of human CETP deficiency were described in Japanese families. These cases illustrated the fact that the lack of CETP results in markedly high plasma levels of HDL-cholesterol (*6, 7*). In 1989, the production of recombinant CETP allowed structure-function and epitope mapping studies (*8–10*). Next, the human gene was cloned and characterized (*11*), which laid the groundwork for ensuing studies on CETP gene regulation (*12–14*). During the next years, lines of transgenic mice expressing CETP were produced and their susceptibilities to atherosclerosis were determined. As will be discussed in this review, many controversial findings revealing strong influences of other metabolic players in atherosclerosis were obtained. The discovery of inhibitors of CETP and the resolution of its chemical structure have been pursued since early studies showed CETP's ability to decrease HDL-cholesterol. Experimental data using CETP inhibitors in cholesterol-fed animals showed very promising effects for disease treatment. Preliminary human studies were also positive in reducing disease markers (*15*). In the middle of this decade, the first large scale clinical trial using a CETP inhibitor, torcetrapib,

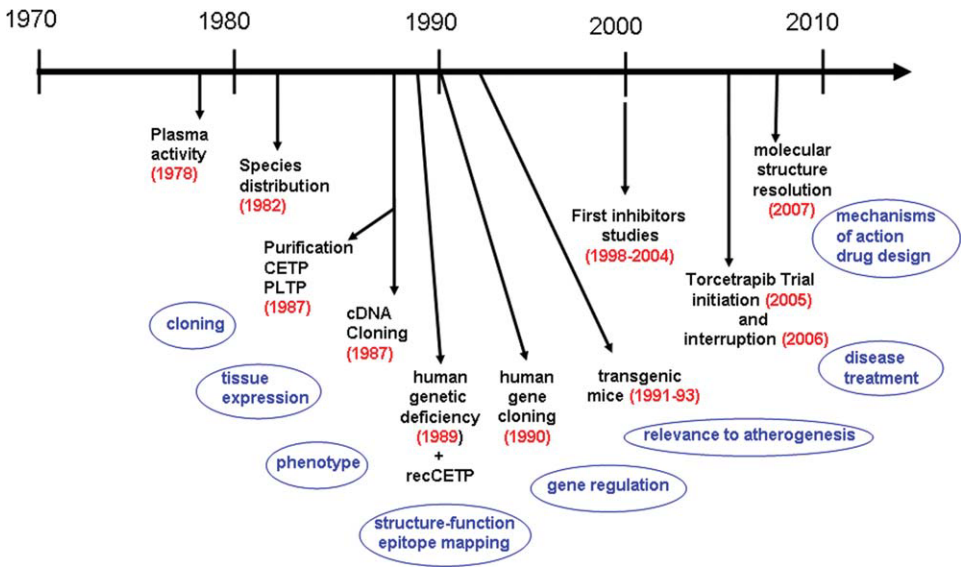

Figure 1. The time-course of major knowledge advances in the biology of CETP: Major findings and immediate scientific contributions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

was interrupted earlier than anticipated due to high mortality rates (*16*). In 2007, about 40 years after the discovery of CETP activity, its molecular structure was finally resolved (*17*). This finding promises to be a milestone since it allows for the elucidation of the CETP mechanism of action and more refined drug design.

CETP AND ATHEROGENESIS

Reverse cholesterol transport (RCT) is a concept originally hypothesized by Glomset (*18*) and is believed to be a critical mechanism by which HDL exerts a protective effect on the development of atherosclerosis. In short, RCT represents an HDL-dependent pathway by which cholesterol is removed from tissues and delivered to the liver for excretion from the body. After decades of research, RCT was shown to occur through a complex sequence of reactions involving many steps and players, including specific cellular transporters, receptors, and extracellular HDL acceptors and enzymes (*19*) (Fig. 2). The first step of RCT is the removal of cholesterol from cell membranes through the interaction of HDL subspecies with ABCA1/G1 membrane transporters. Then, the enzyme lecithin cholesterol acyl transferase (LCAT) esterifies the cholesterol on the surface of HDL, which then enters into the hydrophobic core of the HDL particle and allows for greater unesterified cholesterol efflux from cells to the surface of HDL. The HDL-cholesteryl ester has two fates; it is either directly and selectively delivered to steroidogenic tissues (liver, adrenal, and gonadal) via scavenger receptor class B type I (SRBI), or it is transferred to VLDL and LDL by the action of CETP. These latter lipoproteins are then taken up mainly by the liver through LDL receptors. The pathway that includes CETP is termed indirect RCT. Once in the liver, cholesterol can be secreted into the bile and excreted from the body via feces. Therefore, CETP may play a dual role in RCT. It may represent an additional route for the delivery of cholesterol to the liver or, if LDL receptors are not fully functional, CETP promotes the accumulation of LDL-cholesterol in the plasma, thus favoring the atheroma formation. To attain some insight into the role CETP plays in atherogenesis, transgenic mice expressing CETP in several experimental conditions were tested for their susceptibilities to atherosclerosis. Several studies using CETP transgenic models showed that CETP expression could be either pro- or anti-atherogenic. CETP expression plays a protective role in conditions such as hypertriglyceridemia (*20*), overexpression of LCAT (*21, 22*), excess of apo B combined with lipoprotein lipase deficiency and diabetes (*23*), diabetes and obesity (*24*), castration (*25, 26*), and SRBI deficiency (*27*). Conversely, CETP exacerbates atherosclerosis when expressed in very high levels (seven-fold simian plasma levels) (*28*), in severe hypercholesterolemia due to the deletion of LDL receptors or apo E genes (*29*) and in hypertensive rats (*30*). In addition, cholesterol-fed rabbits exhibited significant reductions (25–70%) in atherosclerotic lesions after vaccination against CETP (*31*), antisense oligonucleotide (*32*), or drug (*33*) inhibition of CETP.

Recent *in vivo* experimental data by Rader's group support the concept that specific macrophage-linked RCT is mechanistically related to atherogenesis (*19*). Early cell-based studies had already raised evidence that CETP might contribute directly to decreased lipid accumulation in arterial foam cells. The addition of CETP to the media of foam cells (*34*) or smooth muscle cells (*35*) stimulated cellular cholesterol efflux rates. We also previously reported that CETP-expressing mouse peritoneal macrophages took up less cholesterol from acetylated LDL and

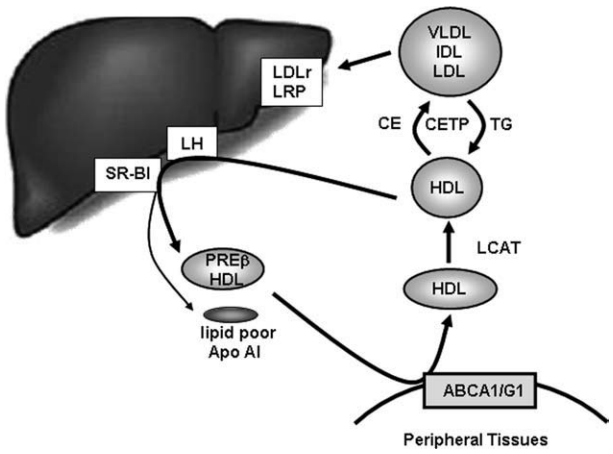

Figure 2. Simplified diagram of reverse cholesterol transport (RCT). The first step of RCT is the removal of cholesterol from cell membranes through the interaction of HDL subspecies with ABCA1/G1 membrane transporters. Then, the enzyme lecithin cholesterol acyl transferase (LCAT) esterifies the cholesterol on the surface of HDL, which then enters into the hydrophobic core of the HDL particle. The HDL-cholesteryl ester (CE) has two fates; it is either directly and selectively delivered to steroidogenic tissues (liver, adrenal, and gonadal) via scavenger receptor class B type I (SRBI), or it is transferred to VLDL and LDL in exchange for triglycerides (TG) by the action of CETP. Hydrolysis of HDL-TG by hepatic lipase (LH) and selective uptake of HDL-CE by SRBI contribute to (re)generation of pre-beta-HDL and lipid poor ApoAI, which re-start the RCT cycle. The V/LDL enriched in CE are then taken up mainly by the liver through LDL receptors (LDLr) and LDL receptor related proteins (LRP). The pathway that includes CETP is termed indirect RCT. Once in the liver, cholesterol can be secreted into the bile and excreted from the body via feces.

released more cell cholesterol to HDL (*25*). However, in other cell types, such as J774 macrophages and Fu5AH hepatoma cells, cholesterol efflux to the serum from CETP-deficient patients was fully preserved (*36*). Thus, further *in vivo* studies are necessary to clarify these cultured cell controversies. By comparing CETP transgenic and wild-type mice, we demonstrated that CETP significantly stimulates the liver uptake of labeled HDL-cholesteryl ester injected into the plasma compartment. However, there were no changes in biliary cholesterol isotopes or mass excretion rates, suggesting that CETP does not alter whole body RCT (*37*). Nevertheless, using an elegant approach for assessing macrophage-specific RCT in mice, Tanigawa et al. (*38*) demonstrated that CETP indeed stimulates RCT until its final mandatory step of cholesterol fecal excretion in a manner that is dependent on the integrity of the low-density lipoprotein receptor pathway. In addition, they showed that CETP expression normalizes impaired RCT in mice deficient in scavenger receptor class B, type I. However, bone marrow transplantation experiments using donor mice expressing CETP suggest that these macrophages make significant contributions to atherogenesis (*39*). Therefore, local events at the level of macrophages are crucial to explain the differences in disease severity. Nonetheless, a clear role for CETP's effect on atherogenesis cannot be drawn from these conflicting results.

Lessons from Cell and Animal Studies: A Summary of CETP-Mediated Pro- and Anti-Atherogenic Mechanisms

Table 1 summarizes the mechanisms by which CETP may affect atherogenesis; these mechanisms are mostly based on experimental data. Genetically modified mouse models have been widely used to evaluate the impact of genes and experimental manipulations such as diet and drug therapies on atherosclerosis and lipoprotein metabolism. Despite genetic manipulations intend to "humanize" the animal models, they will never strictly reflect the physiology nor the pathophysiology of human lipid and lipoprotein metabolism; thus, the translational value of the data is limited, which must be taken into account. However, it would be a mistake to ignore strong scientific experimental evidence and prematurely assume that the human disease is categorically different from that seen in experimental animals. The immediate lesson from the variety of pro- and anti-atherogenic effects of CETP, even in the same species (transgenic mice), is that the importance of CETP for disease development depends on the metabolic context. The most powerful metabolic player that definitively alters the role of CETP on atherogenesis is the integrity of the LDL receptor pathway. When this pathway is preserved, CETP seems to be anti-atherogenic. However, when it is altered, either by receptor malfunction or defective ligands, CETP becomes pro-atherogenic. Because the LDL receptor may be regulated by a variety of diets, drugs, and genetic polymorphisms, the repercussions of CETP action vary based on these conditions. In addition, based on experimental evidence, many

Table 1
Mechanisms by which CETP may affect atherogenesis

Pro-atherogenic mechanisms
Increased LDL-cholesterol
Decreased HDL-cholesterol (*16*)
Anti-atherogenic mechanisms
Increased efficiency of the LCAT reaction (Oliveira et al., 1997)
Increased selective uptake of HDL-cholesteryl ester by SRBI (Collet et al., 1999)
Decreased macrophage cholesteryl ester content (*25*)
Decreased circulating oxidized-LDL (*25, 26*)
Increased liver uptake of HDL-cholesterol (*37*)

Oliveira, H. C. F., et al. Cholesteryl ester transfer protein activity enhances plasma cholesteryl ester formation. Studies in CETP transgenic mice and human genetic CETP deficiency. Arterioscler. Thromb. Vasc. Biol. 1997;17(6):1045–52.

Collet, X., et al. Remodeling of HDL by CETP in vivo and by CETP and hepatic lipase in vitro results in enhanced uptake of HDL CE by cells expressing scavenger receptor B-I. J. Lipid Res. 1999;40(7):1185–93.

other specific conditions, such as hormonal status (including insulin and sex hormones) and the presence of hypertriglyceridemia, should be taken into account before considering CETP inhibition in humans. Thus, the selection of patients who might benefit from CETP inhibition remains a major challenge.

Human Studies

Initial studies describing complete human CETP deficiency in Japanese families suggested that this mutation conferred high longevity (*7*). However, a study in the Omagari region of Japan, where CETP deficiency is extremely frequent, showed that, in subjects aged >80 years, the prevalence of severe CETP deficiency and hyperalphalipoproteinemia was significantly lower than in younger subjects. These data suggest that the elevation of HDL caused by the CETP gene mutation is not associated with longevity (*40*).

To verify the impact of CETP on atherosclerosis, genetic variants of the CETP gene have been evaluated in many epidemiological studies. Unfortunately, these data are still inconclusive. Whereas early results from the Honolulu Heart Study suggested that heterozygotes for CETP gene defects had an increased risk for coronary heart disease (*41*), these data were not confirmed in a prospective analysis within the same study (*42*). In another study, a genetic CETP deficiency was found to be extremely frequent in the Omagari area of Japan. Although it conferred hyperalphalipoproteinemia, the frequency of this CETP gene mutation was higher in patients with coronary heart disease than in healthy control subjects (*40*). Notably, other discrepant results were reported in distinct follow-up phases of the same study. In the Framingham Heart Study, both linear (*43*) and inverse (*44*) associations between plasma CETP activity and cardiovascular risk were reported. After a thorough search of the literature, we found incongruent results demonstrating both direct and inverse relationships as well as a lack of correlation between CETP levels or CETP genetic variants and atherosclerosis risk. A recent meta-analysis including 92 studies of healthy subjects (n = 113,833) and 46 studies in coronary cases (n = 27,196) and controls (n = 55,338) evaluated six different single nucleotide polymorphisms (SNP) of the CETP gene. The authors showed a small but significant 5% reduction in coronary heart disease risk for the three CETP SNPs associated with lower CETP and higher HDL levels (*45*). Considering the modest CETP effect, the authors suggested that larger studies should be conducted. Therefore, the issue remains debatable. The Multi-Ethnic Study of Atherosclerosis (MESA) showed that carriers of other variants, the 451Q and 373P alleles, had significantly higher CETP concentrations and activities and lower HDL-C levels. These minor alleles were also associated with the presence of coronary artery calcium, even after adjusting for CVD risk factors and HDL-C. The R451Q polymorphism was also associated with the presence of carotid artery plaque; however, neither allele was correlated with common or internal carotid intima-media thickness. In addition, the CETP polymorphisms -629C/A, Taq1B, and -2505C/A, which have been shown to confer lower CETP and higher HDL levels, were not associated with any subclinical atherosclerotic indicator (*46*). However, a recent genome-wide association study (number of samples >2,700) identified that the most significant single nucleotide polymorphism associated with high-density lipoprotein (HDL)-cholesterol was located just 5′ upstream of the CETP gene. Notably, this minor allele was more prevalent in cases with myocardial infarction than in controls (*47*).

Therefore, remarkable discrepancies in the results from population studies preclude the clarification of interpretations regarding the manner in which CETP impacts an extremely complex disease such as atherosclerosis.

Clinical Trials with CETP Inhibitors

Despite uncertainty regarding the impact of CETP on atherosclerosis, its inhibition has long been envisioned as a promising therapy to increase HDL and decrease cardiovascular diseases. Torcetrapib was the first small molecule inhibitor of CETP to reach a large-scale phase 3 end point trial, named the ILLUMINATE (Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events). Male and female patients between the ages of 45 and 75 years with a history of cardiovascular disease (n = 15,067) were randomly assigned to receive atorvastatin plus torcetrapib or atorvastatin plus placebo. However, the study was interrupted earlier than anticipated because of an excess of deaths and cardiovascular events in the group receiving torcetrapib. Cardiovascular disease, cancer, and infection were the main causes of death, and their rates were 40%, 70%, and 100% higher, respectively, in the torcetrapib group (*16*). Some adverse effects of torcetrapib, such as hypertension and increased aldosterone levels, are thought to be molecular off-target effects (*48*). Nonetheless, others have hypothesized that CETP inhibition would lead to a defective interaction between HDL and SRB1 (*49*), which is important for the activation of endothelial nitric oxide synthase and the control of vasorelaxation (*50*). Furthermore, other adverse effects may be directly connected to CETP inhibition, such as the blockage of an anti-inflammatory action of CETP, which may lead to death by sepsis.

Aside from the unexpected undesirable effects of torcetrapib, in the ILLUSTRATE study (Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis), a global analysis of patients taking torcetrapib showed that the majority of them demonstrated no regressions of coronary atherosclerosis (*51*). However, in a *post hoc* analysis, a significant regression of coronary atherosclerosis was observed in patients in the highest HDL-C quartile (*52*).

The failure of the torcetrapib clinical trial was related to unpredicted adverse effects. Additional clinical trials with other CETP inhibitors that lack compound-specific off-target effects are necessary to clarify whether inhibiting CETP can be a successful strategy for the treatment of atherosclerosis. Two other small molecule inhibitors of CETP, dalcetrapib (formerly called JTT705), and anacetrapib, are currently in the clinical stages of development.

NOVEL BIOLOGICAL FUNCTIONS OF CETP

The great interest that led to important advances in CETP biology was mainly motivated by its potential as a therapeutic target against cardiovascular disease. Although the failure of the torcetrapib trials seems to be related to off-target toxicity, it raised the possibility that alternative, overlooked functions of CETP may exist. Some of these non-canonical functions of CETP may be related to its ability to transfer neutral lipids and reduce HDL-cholesterol, but others may be related to novel aspects of its function. Next, we discuss some of these new aspects of CETP functions.

Protection Against Lipoprotein Oxidation

HDL has antioxidant properties that are mainly due to its antioxidant constituents, which include Apo AI and the enzymes paroxonase, PAF acetyl hydrolase, and glutathione peroxidase (*53*). It bears the ability to protect LDL and cells from oxidation (*54*). In addition, we have proposed that CETP, in conjunction with HDL, could function as a mechanism to reduce circulating levels of oxidized LDL (oxLDL). We have shown that CETP transfers esterified cholesterol from oxLDL to HDL more efficiently than from native LDL (*55*). In doing so, CETP can facilitate the removal of oxidized lipids via HDL and diminish the levels of oxLDL in the plasma. This role of CETP seems to be relevant since CETP deficient patients have higher levels of oxLDL (*56*). In addition, ovariectomized CETP transgenic mice present levels of anti-oxLDL antibodies that are lower than their CETP non-expressing littermates (*22*). Protection against lipoprotein oxidation is a well-known anti-atherogenic action of estrogen (*57*). Thus, another potential protective mechanism mediated by CETP is the transfer of esterified 17b-estradiol from HDL to LDL particles (*58*), conferring protection against oxidation of LDL.

Anti-Inflammatory Activity of CETP

Nearly half of the non-cardiovascular deaths that occurred in the ILLUMINATE trial of torcetrapib involved patients with infections. Because CETP belongs to a protein family that contains two other anti-inflammatory proteins, LBP (lipopolysaccharide binding protein) and BPI (bactericidal permeability increasing protein), it is reasonable to think that torcetrapib also inhibited these proteins or that CETP itself plays an important anti-inflammatory role. In fact, impressive recent *in vivo* experiments and human studies have revealed that CETP may have a potent beneficial action during acute inflammatory states. Increased survival rates after a challenge of lipopolysaccharide (LPS), a major constituent of gram-negative bacteria, were reported for CETP-expressing mice compared to CETP non-expressing control mice. The protection against death was shown to be dependent on the CETP gene copy number because homozygous mice were more resistant than their heterozygous and control counterparts (*59*). The plasma concentrations of TNF-alpha and IL-6 increased less in LPS-treated CETP transgenic mice than in control mice. In addition, it was shown that CETP induced increases in the binding of LPS to HDL and LDL and that the liver uptake of LPS was greater in CETP transgenic mice than in control mice. Thus, the authors concluded that CETP is an endogenous component involved in the first line of defense against an exacerbated production of proinflammatory mediators (*59*). LPS has been previously reported as an inhibitor of CETP mRNA and plasma activity (*60, 61*). Because HDL is known to modulate the acute inflammatory response through the binding and neutralization of bacterial toxins, these results were viewed as an adaptive response during infection and inflammation that helps to maintain an elevated HDL plasma concentration. However, this may not to be the case, since during acute inflammation in humans after cardiac surgery, both CETP and HDL levels were significantly decreased (*62*). More recently, a prospective cohort study and case–control analysis from hospitalized patients revealed that plasma CETP levels diminished between hospital admission and third day of sepsis. The magnitude of this decrease was significantly more pronounced in non-survivors than in survivors. The authors demonstrated that HDL cholesterol level at admission was a risk factor for severe sepsis, and the reduction of plasma CETP was directly associated with mortality (*63*). Clark et al. (*64*) reported *in vitro* studies showing that torcetrapib did not interfere with the ability of LBP, BPI, and CETP to bind LPS. However, the mechanisms by which CETP might protect against sepsis-related mortality probably do not involve directly binding to LPS, as suggested by the results of Cazita and co-works (*59*).

In summary, the sepsis related deaths in torcetrapib-treated patients (*16*), hospitalized patients with reduced CETP (*63*), and LPS-injected CETP deficient mice (*59*) comprise a strong set of evidence for the protective action of CETP on acute inflammation that cannot be ignored any longer and must be taken into account in future studies of CETP inhibition.

Alzheimer's Disease Risk

CETP is expressed in the brain, and the disturbance of cholesterol homeostasis appears to be an important factor in the development of Alzheimer's disease (AD). Cholesterol regulates the production of amyloid beta (Abeta), which is central to the pathogenesis of AD. High cellular cholesterol levels promote and low cellular cholesterol levels reduce Abeta production both *in vitro* and *in vivo*. Because HDL plays a central role in the removal of excess cholesterol from cells and CETP modulates HDL levels, several studies have investigated whether sequence variants of the CETP gene might be of importance in mediating AD susceptibility, either independently or in concert with the apolipoprotein E (APOE) epsilon4 allele, a known risk factor for AD. Yamada et al. (*65*) examined CETP-like immunolabeling in non-neurological and in AD liver and brain tissues. In the brains of non-neurological cases, positively stained astrocytes were preferentially distributed in the white matter. In AD tissue, many reactive astrocytes in the gray matter and in the astrocytes of the white matter displayed CETP-like immuno-

reactivity. The authors proposed that CETP-positive astrocytes might play a role in the tissue repair of AD patients.

In the last 5 years, several studies investigating potential associations between CETP gene polymorphisms and AD risk have been published. A study with a Northern Han-Chinese population showed that the frequency of the DG genotype or the G allele of the CETP D442G polymorphism was greater in control subjects than in AD patients. These results suggest that the G allele may have a potential protective effect against the development of AD, especially in APOE epsilon4 carriers (*66*).

The results from Rotterdam study (*67*) suggest that the VV genotype of the I405V CETP polymorphism (increased HDL and decreased CETP levels) increases the risk of AD in the absence of the APOE4 allele. However, a prospective cohort study of subjects from the Einstein Aging Study showed that the VV genotype is associated with a lower rate of memory decline and a lower risk of incident dementia, including AD (*68*).

A study in a Spanish population evaluated two common CETP polymorphisms, in the promoter region (C-629A) and in the exon 14 (I405V). APOE epsilon4 carriers that were homozygous for the CETP -629A allele had approximately a threefold lower risk of developing AD than the carriers of the -629C allele (*69*). These data suggest that CETP behaves as a modifier gene for AD risk associated with the APOE epsilon4 allele.

The TaqI B polymorphism of the human CETP gene results in decreased CETP mass and increased HDL-cholesterol. The distribution of the TaqI B polymorphism in an independent population of AD patients and spousal control group showed no significant differences with respect to either genotype or allele frequency (*70*). In addition, no significant association between CETP genotypes or haplotypes related to three single nucleotide polymorphism, including the Taq1B, and late onset AD was detected in two study cohorts, the Religious Orders Study and the University of Kentucky series (*71*).

A previous study suggested that CETP gene variations influence cerebral and peripheral cholesterol metabolism but do not influence AD risk (*72*). The investigators studied the effects of three CETP polymorphisms (-1946 VNTR, C-629A, and I405V) on the risk of AD and cholesterol, lathosterol, and 24S-hydroxycholesterol levels in cerebrospinal fluid (CSF) and plasma of AD patients and controls. None of the investigated CETP polymorphisms or haplotypes had any effect on the risk of AD, but a three-marker CETP haplotype influenced CSF levels of lathosterol and 24S-hydroxycholesterol and plasma levels of total cholesterol in controls but not AD patients.

Therefore, most of the investigations showed that CETP SNPs have no effect on AD risk, whereas three studies identified three SNPs (405V, 442G, and -629A) that were positively associated with AD risk reduction. In addition, one study showed that the 405V SNP was significantly associated with an increased risk of AD. Thus, although relevant, as in the case of atherosclerosis, studies of variants in any single gene are limited in their ability to explain complex, multigenic diseases that probably reflect the integrated effects of multiple gene polymorphisms within the same pathway. This must be the case for CETP, which may be associated with Alzheimer's disease along with many other cholesterol-related genes, including apolipoproteins, lipoprotein receptors, cholesterol metabolizing enzymes, and transporters.

Anti-Adipogenic Action of CETP

CETP is abundantly expressed in human adipose tissue and in the early stages of adipogenesis, even before the expression of transcription factors such as PPAR gamma, SREBP, and C/EBP (*73*). The expression of CETP mRNA is greater in small, subcutaneous adipocytes containing less lipid drops (*74*) and decreases with age (*75*).

Human adipose tissue stores a relatively large amount of body cholesterol preferentially in the non-esterified form because of a high cholesterol esterase activity. The adipocytes are dependent on plasma lipoproteins as cholesterol sources because their cholesterol synthesis is limited (*76*). Previous studies have shown that CETP promotes HDL-cholesteryl ester (CE) selective uptake by adipocytes in culture (*77*). We have shown that the expression of CETP significantly increases the *in vivo* uptake of HDL-CE by the visceral adipose tissue of transgenic mice when compared to CETP non-expressing littermates (*37*). Therefore, it seems that CETP plays a local role in adipose tissue, perhaps in the maintenance of a free cholesterol/triglyceride constant ratio. Gauthier et al. (*78*) presented evidence of a novel role for cell-associated CETP in stimulating the selective uptake of cholesteryl ester by liver cells. They demonstrated that this cholesteryl ester selective uptake is independent of any other known lipoprotein receptors (LDL receptor, SRBI, and LRP). These findings were confirmed in *in vivo* studies (*79*). Thus, the same might also be true for adipose tissue.

A few human studies have positively correlated circulating CETP levels and obesity. Accordingly, weight loss induced by bariatric surgery reduced CETP in 19 severely obese female subjects (*80*). However, insulin resistance and diabetes are common confounding covariants in obese subjects and lead to uncertainty regarding data interpretation. We have previously shown that CETP is negatively regulated by insulin and is upregulated in experimental models of diabetes (*81*). Therefore, CETP may be elevated in obese people secondarily to diabetes or insulin resistance states. Thus, studies in a normal weight population are necessary to clarify this matter. In fact, Teran-Garcia et al. (*82*) reported that a CETP gene polymorphism I405V contribute to differential changes in adiposity after overfeeding normal weight subjects. Individuals with the VV genotype (low CETP) had the highest percentage increase in visceral fat after overfeeding (130%). These results suggest an inverse correlation between visceral fat gain and CETP levels in a normal-weight population.

We report here the original data relating CETP plasma levels and adiposity markers in control subjects. We measured plasma CETP activity by an exogenous substrate isotopic assay (indica-

tive of CETP mass) in 288 normo-weighted recruited subjects (CETP: 11% ± 8% of CE transfer, BMI: 25 ± 5 Kg/m^2, age: 48 ± 15 years, sex: F/M = 188/100). We found a significant negative correlation between plasma CETP and body mass index (Pearson correlation coefficient = −0.18, $P < 0.0035$) and waist circumference (−0.15, $P < 0.015$). In addition, analyses in another set of 295 normo-weighted subjects showed that for the I405V CETP polymorphism, the genotype IV had the lowest CETP activity (11% ± 7% of CE transfer vs. 14% ± 9% in II and 12% ± 7% in VV, $P < 0.008$), higher BMI (25 ± 7 Kg/m^2 vs. 25 ± 7 in II and 22 ± 8 in VV), and higher waist circumference (82 ± 13 cm vs. 78 ± 9 in II and 78 ± 11 in VV, $P < 0.02$). Thus, in both studies, plasma CETP levels seem to be inversely related to adiposity markers.

Experimental data reinforce these statistical correlations. We have recently demonstrated that the expression of a natural promoter-driven CETP gene in hypertriglyceridemic mice fed a high fat diet resulted in (i) the reduction of adipose tissue mass, (ii) decreased adipocyte size, and (iii) decreased plasma leptin levels when compared to CETP non-expressing hypertriglyceridemic mice (*83*). Additional evidence for a CETP-mediated reduction in adipocyte size was provided by Zhou et al. (*84*) using an adipose tissue-specific promoter (aP2)-driven CETP transgenic mice. These mice have physiological plasma concentrations of CETP, smaller adipocytes, and reduced mRNA expression of the adipogenic genes LPL, PPARg, and SREBP-1c when compared with controls. Thus, two independent CETP-expressing mouse models show the same adiposity-reducing effect of CETP.

The mechanisms by which CETP could modulate fat stores are presently unknown. We postulate two non-excluding hypotheses to explain the anti-adipogenic role of CETP. The first hypothesis is based on a putative influence of CETP on fuel partitioning between adipose tissue and other tissues. Considering that (i) CETP mediates TG transfer from TG-rich LP to HDL, (ii) HDL-TG are preferentially hydrolyzed by hepatic lipase, and (iii) HDL-released non-esterified fatty acids (NEFA) are likely to be taken up locally by liver cells, CETP would be promoting a shift in the fate of NEFA from adipose to liver and contributing to decreased fat stores in adipocytes. The second mechanism by which CETP may modulate fat depots is related to the ability of CETP to increase adipocyte cholesterol content and indirectly modulate the expression of genes involved in fat accumulation. In support of these hypotheses, we have recently described that CETP expression in transgenic mice delays the plasma clearance of TG-rich lipoproteins after an oral fat load by two mechanisms: the transfer of TG to HDL, which presents a longer plasma half-life, and the decrease of LPL activity and expression (*85*).

In summary, according to studies in human adipocytes (*74, 75*), independent CETP transgenic lines of mice (*83, 84*) and normal weight populations (*82* and new data presented here), the expression of CETP and its levels are negatively correlated with the amount of body fat depots.

CONCLUDING REMARKS

In this review, we presented experimental evidence and evidence based on human populations of the controversial role of CETP on atherogenesis and the potential biochemical mechanisms that are considered pro- or anti-atherogenic. On the basis of a selection of these evidences, CETP inhibition was chosen as a therapeutic strategy to reduce atherosclerosis. Surprisingly, the first large-scale clinical trial using the CETP inhibitor torcetrapib resulted in increased cardiovascular and non-cardiovascular mortalities of treated patients. However, the current view is that a compound-specific toxicity must be ruled out before abandoning this strategy. Unexpected sepsis-related deaths in torcetrapib treated subjects and other recent work in mice and men clearly point to an anti-inflammatory role for CETP. Other new lines of research are currently beginning to expand the functions of CETP to new areas of interest such as Alzheimer's disease and obesity.

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