Epidemiologic studies have identified high-density lipoprotein cholesterol (HDL-C) as a strong, independent, inverse predictor of coronary heart disease risk. This identifies HDL-C as a potential therapeutic target. Compared with low-density lipoprotein cholesterol (LDL-C)-lowering agents, however, currently available HDL-raising drugs are relatively ineffective. Consequently, recent years have seen considerable efforts expended on identifying new drugs that can raise HDL-C. Cholesteryl ester transfer protein (CETP) plays an important role in cholesterol metabolism, being responsible for the transfer of cholesteryl esters from HDL to very low-density lipoproteins and LDLs. The observation that Japanese populations with CETP deficiency exhibited high levels of HDL-C has led to the concept that drugs targeting CETP activity may elevate HDL-C levels and potentially decrease cardiovascular risk. Support of this proposition has been obtained in rabbits where inhibition of CETP activity is markedly antiatherogenic. Two CETP inhibitors—torcetrapib and JTT-705—are currently in the preliminary stages of clinical development. Initial studies with these drugs in humans show that they substantially increase HDL-C levels and modestly decrease LDL-C levels. Larger, long-term, randomized, clinical end point trials are required to determine whether the beneficial effects of CETP inhibitors on lipoprotein metabolism can translate into reductions in cardiovascular events. (J Am Coll Cardiol 2006;47:492–9) 

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CHOLESTEROL METABOLISM

Atherosclerosis develops and the risk for cardiovascular disease (CVD) events increases when modified LDL particles are taken up by macrophages in the artery wall to form foam cells in a process that leads ultimately to the development of plaque. In contrast to LDLs, HDLs are antiatherogenic, partly because of their role in reverse cholesterol transport but also due to a spectrum of documented antioxidative, anti-inflammatory, antiatherosclerotic, and antiapoptotic properties (11–13). The primary pathways involved in cholesterol metabolism and the role that CETP plays in transferring triglycerides and cholesteryl esters (CEs) between lipoproteins are illustrated in Figure 1.
LDLs. Low-density lipoproteins contain a core of mainly CE and a small amount of triglycerides surrounded by surface of phospholipids, free cholesterol, and apolipoprotein (apo) B (14). Cholesterol is secreted from the liver into plasma in very low-density lipoproteins (VLDLs), which in turn are converted to LDLs. Low-density lipoproteins deliver cholesterol to tissues after binding to the LDL receptor.

HDLs. The major protein of HDLs is apo A1, which is synthesized in the liver and secreted into plasma in a lipid-poor form. Lipid-poor apo A1 rapidly acquires free cholesterol from tissues via the adenosine triphosphate (ATP)-binding cassette A1 (ABCA1) transporter to form discoidal HDL particles. Discoidal HDLs interact with lecithin:cholesterol acyltransferase, which converts a proportion of their free cholesterol into CEs that migrate into a hydrophobic core in a process that converts the disc into a mature, spherical HDL particle (Fig. 2). The fact that discoidal HDL particles are normally present at only very low concentration in plasma reflects the rapidity with which they are converted into spheres. The cholesterol in mature HDL particles interacts with the hepatic scavenger receptor class B type 1 (SR-B1) to deliver cholesterol (mainly as free cholesterol) to the liver (Fig. 1) (15). The process of transferring cholesterol from peripheral cells to the liver for removal from the body by biliary secretion is called reverse cholesterol transport. The role of HDLs in facilitating reverse cholesterol transport is one of the mechanisms by which HDLs protect against atherogenesis.

It has recently become clear that ABCA1 is not the only means by which peripheral cells can efflux cholesterol to HDLs. Other mechanisms include interaction of HDLs with SR-B1 (16) and passive diffusion (17). Another transporter, the ATP-binding cassette G1, is expressed in macrophages where it promotes the efflux of cholesterol from the cell to mature, spherical HDLs (Fig. 2) (18,19).

Figure 1. Role of cholesteryl ester transfer protein (CETP) in plasma lipid transport. Cholesteryl ester transfer protein promotes bidirectional transfers (shown by the red arrows) of cholesteryl esters (CE) and triglycerides (TG) between high-density lipoproteins (HDLs), very low-density lipoproteins (VLDLs), and low-density lipoproteins (LDLs). Most of the CEs in plasma originate in HDLs in a reaction catalyzed by lecithin:cholesterol acyltransferase (LCAT), while the majority of the TG enters plasma as a component of TG-rich lipoproteins secreted either from the liver as VLDLs or from the intestine as chylomicrons. Very low-density lipoproteins are subsequently converted into LDLs after hydrolysis of a proportion of their TG by lipoprotein lipase (LPL) and hepatic lipase (HL). The overall effect of the CETP-mediated CE exchanges between these lipoproteins is a net mass transfer of CE from the antiatherogenic HDLs to the potentially proatherogenic VLDLs and LDLs. The cholesterol in LDLs is taken up by all cells (both in liver and peripheral tissues) that express the LDL receptor. Modified (oxidized) LDLs are also taken up by macrophages in a scavenger receptor-mediated process that converts the macrophage into a foam cell. Cholesterol, both in its free or unesterified form (FC) and in its esterified form as CE, is returned to the liver by HDLs via the scavenger receptor-B1 (SR-B1) (pathway 1) and by LDLs via the LDL receptor (LDL-R) (pathway 2). See Figure 2 for mechanisms through which peripheral cells may efflux cholesterol to HDL particles.
Role of CETP. Cholesteryl ester transfer protein is secreted by the liver and is a key player in the metabolic interaction between HDLs and the VLDL-LDL fraction. It is a hydrophobic plasma glycoprotein that circulates bound mainly to HDLs (20). The primary function of CETP is to redistribute CEs and triglycerides between lipoproteins (Fig. 1) (12). Because most triglycerides in plasma originate in VLDLs and most CEs are formed in HDL particles in the reaction catalyzed by lecithin:cholesterol acyltransferase (LCAT) generates spherical, mature HDL particles. The lipidation of lipid-poor apo A1 and the conversion of discoidal HDL to spherical particles are rapid as evidenced by the very low levels of lipid-poor apo A1 and discoidal HDL in normal plasma. Free cholesterol may be effused to mature HDL particles by passive diffusion or by a receptor-mediated pathway, including the scavenger receptor B-1 (SR-B1) or the newly identified ATP-binding cassette transporter G1 (ABCG1). PL = phospholipid.

Figure 2. Efflux of cholesterol from peripheral cells. High-density lipoprotein (HDL) particles may accept free cholesterol (FC) from peripheral cells via several mechanisms. The ATP-binding cassette transporter A1 (ABCA1) effluxes FC to lipid-poor apolipoprotein (apo) A1 resulting in the formation of discoidal, nascent HDL. Esterification of the FC in nascent HDL by lecithin:cholesterol acyltransferase (LCAT) generates spherical, mature HDL particles. The lipidation of lipid-poor apo A1 and the conversion of discoidal HDL to spherical particles are rapid as evidenced by the very low levels of lipid-poor apo A1 and discoidal HDL in normal plasma. Free cholesterol may be effused to mature HDL particles by passive diffusion or by a receptor-mediated pathway, including the scavenger receptor B-1 (SR-B1) or the newly identified ATP-binding cassette transporter G1 (ABCG1). PL = phospholipid.

Does CETP Promote Atherogenesis? Theoretical Considerations

Whether CETP activity is atherogenic remains a matter of debate. Although various dyslipidemias have been linked with increased CETP concentrations (22–24), it is possible that elevated CETP is the result of dyslipidemia rather than its cause (25). Indeed, by promoting the transfer of CEs from HDLs to VLDLs and LDLs, activity of CETP may account for a considerable proportion of the peripheral cell cholesterol that is returned to the liver in humans (15,21). In this respect, CETP may be viewed as having antiatherogenic activity. On the other hand, by transferring CEs from HDLs to VLDLs and LDLs, CETP decreases the concentration of antiatherogenic HDLs while increasing the concentration of LDL-C (21).

The CETP-mediated exchange of CEs and triglycerides also alters the size and structure and potential atherogenicity of lipoprotein particles. As triglycerides in VLDLs are exchanged for CEs in HDLs and LDLs, the HDL and LDL particles become triglyceride-enriched. Such particles are substrates for triglyceride lipases, which hydrolyze the triglycerides and in the process generate small, dense LDLs and small, dense HDLs (Fig. 3) (26). Small, dense LDLs are more atherogenic than larger LDLs (27), because they have greater affinity for artery wall proteoglycans (28) and are more readily modified by oxidation before macrophage uptake (29,30). The formation of small, dense LDLs is accompanied by the dissociation of lipid-poor apo A1 (Fig. 3) (31). This lipid-poor apo A1 may then act as an acceptor of cell cholesterol in the process mediated by ABCA1 (31,32).
Alternatively, however, it may be removed by the kidney and lost irreversibly from plasma (33). Not surprisingly, patients with CETP deficiency have an increased proportion of large CE-rich HDL particles (34,35). Conversely, an increase in the CETP-mediated exchanges of triglycerides and CEs between HDLs and VLDLs in individuals with elevated triglyceride levels results not only in a reduced concentration of HDL-C but also a reduction in the HDL particle size (36,37). The impact of CETP on LDL particle size has also been observed in patients with familial hypercholesterolemia (FH). In a study by Hogue et al. (38), patients with heterozygous FH had a higher CETP mass than non-FH controls, and an inverse relationship was observed between LDL peak particle diameter and CETP mass.

To summarize, CETP plays an important role in several key pathways of cholesterol metabolism and theoretically may be associated with both pro- and antiatherogenic activity.

**CETP AND ATHEROSCLEROSIS—EVIDENCE FROM ANIMAL AND HUMAN STUDIES**

**Mouse studies.** Mice are naturally deficient in activity of CETP and relatively resistant to the development of atherosclerosis. Introduction of the CETP gene into mice reduces the level of HDL-C and increases susceptibility to diet-induced atherosclerosis (39,40). A study in APOE*3-Leiden mice expressing CETP and fed a Western-style diet showed a seven-fold increase in the atherosclerotic lesion area after 19 weeks compared with APOE*3-Leiden mice not expressing CETP (41). However, studies in hypertriglyceridemic mice have shown that expression of CETP is antiatherogenic (42,43). Thus, studies in mice have provided inconsistent findings.

**Rabbit studies.** In contrast to mice, rabbits have a high level of CETP activity in plasma and are an ideal model in which to investigate the effects of CETP inhibition. Injection of anti-sense oligodeoxynucleotides against CETP into cholesterol-fed rabbits has been shown to reduce CETP mRNA and mass, to increase in HDL-C levels, and to reduce aortic plaque formation (44). In another study of cholesterol-fed rabbits, an autoimmune response against CETP was induced by a vaccine. This led to a reduction in CETP activity, an increase in HDL-C, and again a reduction in aortic plaque formation (45). There have also been studies of the effects of small molecule CETP inhibitors in rabbits. In cholesterol-fed rabbits, the CETP inhibitor JTT-705 increased plasma HDL-C, decreased non-HDL-C, and decreased aortic arch lesions by 70% (46). However, in a subsequent study of JTT-705, also conducted in rabbits, the difference in the deposition of aortic cholesterol was not significantly different from the control group, despite a similar elevation in HDL-C compared with the original study (47). The CETP inhibitor torcetrapib has
also been shown in cholesterol-fed rabbits to increase the level of HDL-C and to reduce aortic atherosclerosis (48).

**Human studies.** The notion that CETP may be a potential target for reducing CVD originated from reports of a Japanese population of apparently healthy individuals that lacked a functional copy of the CETP gene (34,35). Compared with unaffected individuals, those who were CETP-deficient and who had no measurable CETP activity in plasma exhibited substantial increases in HDL-C (209%) and large decreases in LDL-C (44%). In individuals with heterozygous deficiency who possessed half the normal CETP activity, changes in HDL-C and LDL-C were less dramatic (+25% and −5%, respectively).

While CETP gene mutations are common in Japanese populations (49) and have clearly helped to establish the link between reduced CETP function and elevated HDL-C levels, the effect of decreased CETP activity on the development of atherosclerosis is less clear. For example, in a study of 201 patients with markedly elevated HDL-C levels (≥100 mg/dl), a subgroup of 12 patients (6%) was identified with atherosclerotic CVD. Of these, 10 were observed to be heterozygotes for CETP deficiency (50). Data from the Honolulu Heart Program regarding CETP concentrations and CHD risk are also inconsistent. The study included American men of Japanese ancestry, many of whom were heterozygous for a mutation in the CETP gene and had reduced CETP levels. The data that were originally published suggested an apparent 50% increase in CHD among participants with CETP deficiency and HDL-C levels of 41 to 60 mg/dl (51). However, more recent, seven-year prospective data have now shown the opposite, with the CETP-deficient individuals experiencing fewer CHD events than those without the mutation, although the difference did not achieve statistical significance (52).

In an attempt to elucidate the relationship between CETP activity and CHD risk, numerous studies have investigated polymorphisms of the CETP gene in which only one or two amino acids are changed (53).

One of the more common CETP polymorphisms is Taq1B in intron 1. In one study, this polymorphism accounted for 5.8% of the variance in HDL-C levels (54), and, in the Framingham Offspring study, individuals homozygous for the B1 allele had higher levels of CETP and lower levels of HDL-C compared with either B1B2 or B2B2 subjects. In men, the presence of the B2 allele reduced CETP activity and CHD risk, numerous studies have investigated polymorphisms of the CETP gene in which only one or two amino acids are changed (53).

The beneficial effects of another CETP gene polymorphism have been reported in a study of exceptionally long-lived individuals from a genetically homogeneous population of Ashkenazi Jews (57). Healthy individuals of advanced age (95 to 107 years) in this population had a unique lipoprotein profile consisting of large HDL and LDL particles. Their offspring also had lipoprotein particles of larger size compared with those of an age-matched control group of Ashkenazi Jews and with those of individuals from the Framingham Offspring study. In both the individuals with exceptional longevity and their offspring, the incidence of homozygosity for a CETP polymorphism was significantly higher than in either of the control groups. The CETP polymorphism was associated with reduced CETP levels and CETP activity, and with larger lipoprotein particle size, leading investigators to conclude that it was an important factor in the survival advantage apparent among the long-lived individuals.

Although sparse, there is evidence emerging from clinical trials that elevated CETP levels are associated with increased risk of atherosclerosis. An analysis of baseline CETP levels in 674 men with CHD in the Regression Growth Evaluation Statin Study (REGRESS) revealed that those with baseline CETP concentrations in the highest quartile had significantly greater progression of coronary atherosclerosis after two years than those with baseline CETP concentrations in the lowest quartile (58). Likewise, an analysis of data from 281 patients with FH who participated in the Atorvastatin Simvastatin Atherosclerosis Progression (ASAP) trial showed a positive association between baseline CETP concentration and progression of atherosclerosis as measured by change in carotid intima thickness (22).

Perhaps the most convincing data supporting a link between baseline CETP concentration and CHD risk come from a recent nested case-control study conducted among participants of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Population study (59). This study identified 755 apparently healthy individuals who went on to develop fatal or nonfatal CHD during follow-up and control subjects who remained free of CHD during follow-up. The risk of CHD increased with increasing CETP quintiles, although this relationship was confined to those with elevated triglyceride levels.

**CETP Inhibition as a Therapeutic Strategy for Reducing Cardiovascular Risk**

On balance, the currently available data suggest that decreased CETP activity is antiatherogenic, particularly if associated with an increase in HDL-C levels. Inhibition of CETP is now being investigated as a potential new strategy for the management of CVD. A vaccine, CETi-1, and two small-molecule compounds, JTT-705 and torcetrapib, are currently under investigation in humans.

**CETi-1.** The vaccine CETi-1 induces auto-antibodies that specifically bind and inhibit the activity of endogenous CETP.

In a phase I human study with CETi-1, one patient at the highest dose (250 mg) of a total of 36 patients who received a single injection developed anti-CETP antibodies. In an
extension study of 23 patients, 53% (8 of 15) who received a second injection of the active vaccine developed anti-CETP antibodies compared with 0% (0 of 8) in the placebo group (60). The vaccine was well tolerated, and no significant laboratory abnormalities occurred. The effect of the vaccine on the concentration of HDL-C in humans has not yet been reported.

**JTT-705.** JTT-705 inhibits CETP activity by forming a disulfide bond that causes irreversible binding to the protein.

In a phase II, randomized, double-blind, placebo-controlled study of 198 healthy individuals with mild dyslipidemia, JTT-705 was evaluated at doses of 300, 600, and 900 mg/day (61). A dose-dependent decrease in CETP activity was measured, reaching a maximum decrease of 37% from baseline after four weeks of treatment at the 900 mg/day dose. At this dose, HDL-C was increased by 34%. Decreases in LDL-C levels were minimal.

JTT-705 in combination with pravastatin has been assessed in a randomized, double-blind, placebo-controlled trial conducted in 152 individuals with LDL-C >160 mg/dl. Patients were randomized into three study arms: placebo and pravastatin 40 mg, JTT-705 300 mg with pravastatin 40 mg, and JTT-705 600 mg with pravastatin 40 mg. After four weeks, JTT-705 600 mg plus pravastatin led to a 30% decrease from baseline in CETP activity and a 28% increase from baseline in HDL-C (p < 0.001 for both parameters vs. placebo), while LDL-C decreased by 5% from baseline. JTT-705 300 mg plus pravastatin was about half as effective as the higher dose, decreasing CETP activity by approximately 16% and increasing HDL-C by approximately 14% (62).

**Torcetrapib.** Torcetrapib is a potent and selective inhibitor of CETP. It enhances the association between CETP and HDLs, forming a complex that inhibits the transfer of lipids between HDLs and other lipoproteins.

Torcetrapib has been evaluated in two small, placebo-controlled studies (63,64).

In one study, 40 healthy young subjects were randomized to receive placebo or increasing doses of torcetrapib from 10 to 240 mg daily for 14 days (63). Activity of CETP was reduced by 12% to 80%. The concentration of HDL-C increased by 16% to 91% and that of LDL-C decreased by 21% to 42%. These changes were associated with elevations in apo A1 and apoE and reductions in apoB. In another report of the effects of torcetrapib (64), 19 subjects with concentrations of HDL-C less than 40 mg/dl were treated with torcetrapib at doses up to 120 mg twice daily. Some subjects also received atorvastatin. Treatment with torcetrapib at a daily dose of 120 mg increased HDL-C by 61% and 46% in the presence and absence of concomitant atorvastatin therapy, respectively. At a dose of 120 mg twice daily, torcetrapib increased HDL-C by 106%. The mean size of both HDL and LDL particles increased with torcetrapib therapy (64), an observation consistent with previous reports of large HDL particles in individuals with a partial or complete genetic deficiency of CETP.

It has been reported recently that torcetrapib does not increase overall reverse cholesterol transport in humans (as measured by fecal sterol excretion) (65), although it should be emphasized that this technique tells us nothing about the impact of the drug on the efflux of cholesterol from macrophages in the artery wall. The true test of whether CETP inhibition is cardioprotective in humans will have to await the results of the ongoing clinical trials, the first of which should report some time in 2007.

**CONCLUSIONS**

While epidemiologic evidence has demonstrated a clear link between low levels of HDL-C and increased CHD risk, there are limited data on the benefits of elevating HDL-C through pharmacologic intervention. This may be because existing therapies achieve only moderate increases in HDL-C.

Cholesteryl ester transfer protein appears to have a number of potentially proatherogenic effects, including decreasing HDL-C levels, increasing LDL-C levels, and reducing HDL and LDL particle size. Inhibitors of CETP are in clinical development and have been shown to have a favorable impact on the lipoprotein profile and may potentially be a new strategy for reducing CVD. Large-scale, randomized trials evaluating the impact of these inhibitors on atherosclerotic progression using vascular imaging and on the incidence of cardiovascular events are now required.

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CETP Inhibition to Prevent CVD


