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High Density Lipoprotein (HDL) Modulation Targets

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Abstract

Given the strong genetic determinants of favorable HDL-C levels, the ability to procure the cardiovascular disease and longevity benefits associated with this mediator of the reverse cholesterol transport pathway through pharmaceutical intervention is challenging. Niacin is still the most robust HDL-C raising pharmaceutical agent on the market at its use leads to elevations up to 35%. Cholesteryl ester transfer protein (CETP) and endothelial lipase (EL) are two targets involved in the reverse cholesterol transport pathway that have become therapeutic targets of various investigations for raising HDL. However, the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial was stopped in December 2006 due to excess mortality in the group of patients treated with torcetrapib, a CETP inhibitor. Other CETP inhibitors being studied include anacetrapib and JTT-705. Other CETP inhibitors including TA-8995, DRL-17822, JTT-302, and others are under investigation. Additionally a biologic target CETi-1, an investigational vaccine in phase II development designed to elicit antibodies that bind and inhibit the activity of CETP leading to blocking the ability of the protein to transfer cholesterol from HDL to LDL and thus causing HDL cholesterol levels to rise is under clinical investigation for sometime.

Keywords

High Density lipoprotein (HDL); Low Density lipoprotein (LDL); atherosclerosis; coronary heart disease (CHD); Cholesteryl ester transfer protein (CETP); Endothelial Lipase; Longevity; Stroke; Coronary artery Diseases (CAD); cardiovascular diseases (CVD); Inflammation; genetics; niacin; statins; fibrates

Introduction

Low-density lipoprotein (LDL) has been long known to be atherogenic. More recently, attention is being given to effects of high-density lipoprotein (HDL-C), as it has been shown to be clearly shown to have an inverse relationship with cardiovascular disease (CVD) risk [1]. Scientists, patients and the general public question exactly what effect HDL-C has on health and longevity. As people age, their HDL-C levels and total cholesterol levels decrease. This review highlights different strategies that are under preclinical and clinical investigations for the modulations of HDL-C levels (Table 1).

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HDL Cholesterol in Centenarians

A favorable lipid profile is strongly genetically determined, for example, a subset of Ashkenazi Jews with exceptional longevity has been found to have an inherited phenotype with significantly larger HDL particle sizes than matched controls [2]. Additionally, high levels of total HDL cholesterol have been associated with longevity during healthy aging in very old Japanese-American men [3].

As lipoproteins are diverse molecules with a range of size and density, one issue of interest has been whether this heterogeneity correlates with variable cardiovascular disease (CVD) risk. Whereas epidemiological studies show that levels of low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) predict incident CVD, there is limited evidence relating lipoprotein subfractions and composite measures of subfractions to risk for CVD in prospective cohort studies. HDL subfractions are among the new emerging risk factors for atherosclerosis. In particular, HDL 2b has been shown to be linked to cardiovascular risk. Recent study used a novel micro-fluidics-based method to establish HDL 2b clinical utility using samples from the Prospective Cardiovascular Muenster (PROCAM) Study [4].

The role of genetics in determining HDL

Atzmon et al. [2, 5] sought to find the phenotype and genotype associated with exceptional longevity in a genetically homogenous Ashkenazi Jewish population (ref). These investigators found that genes governing HDL particle size are inherited. Since the controls for the centenarians have passed away, an aged-matched control group for their offspring was created. This control group shared a similar environment, including mostly the spouses of the offspring or their neighbors. Blood tests included lipoproteins, lipoprotein subclasses, and particle sizes determined by proton nuclear magnetic resonance (NMR). Genotyping was performed for the amino acid 405 isoleucine to valine (I405V) substitution variant in the gene for cholesteryl ester transfer protein (CETP), an enzyme involved in the regulation of lipoproteins and their particle sizes. When the lipoprotein size of the centenarian's offspring was compared to the matched control group, the results showed that the offspring of centenarians have both large HDL and LDL particle size compared to the control subjects. On the basis of gender and specific lipoproteins, the lipid profile characteristics were found to be highly heritable (0.4-0.7) [2, 5].

Among centenarians in Atzmon et al's study, cognitive capability was tested by minimal mental state test (MMSE) to see if HDL plasma levels served to protect cognitive function. Analysis of the MMSE distribution in comparison to HDL levels showed that subjects with higher HDL plasma levels had higher MMSE test scores, suggesting that HDL has a protective cognitive function [2, 5]. Another study, involving 561 subjects of at least 85 years of age investigated links between serum lipids, cognitive impairment, and coronary artery disease (CAD). In this study a low MMSE score was associated with a low HDL-C level; this association was found even when subjects with CAD and stroke were excluded. Among these centenarians, it may be that the anti-aggregation and anti-inflammatory properties of HDL are responsible for HDL's negative association with cognitive impairment [2, 5].

Study subjects possessing the VV genotype showed lower serum CETP levels and larger lipoprotein particle sizes. The calculated attributable "risk" of an individual carrying the VV genotype to become one of the oldest elderly is 18.1 [1]. In contrast to this study, another study investigating 256 centenarians and 190 controls for the effect of CETP deficiency and Taq1B polymorphisms of CETP gene did not show an association between either of these genetic variations and longevity [6, 7]. A study involving Japanese-American men was

conducted to examine two different CETP gene mutations (D442G, 5.1%; intron 14G: A, 0.5%) found in 3,469 men in the Honolulu Heart Program. Mutations were associated with decreased CETP (-35%) and increased HDL cholesterol levels (+10% for D442G). For Japanese-American men with HDL cholesterol levels >60 mg/dl, with or without mutations, had a low CHD prevalence; this is in accordance with the inverse relationship between HDL and CHD. Men with mutations primarily had increased CHD when their HDL cholesterol values ranged from 41-60 mg/dl. The results of this study indicate CETP deficiency appears to be an independent risk factor for CHD. Mechanistically, CETP may affect HDL speciation and LCAT activity. As CETP traffics atherogenic lipid between HDL and LDL lipoproteins and subsequent return of lipid to the liver for excretion in feces, it is a vital component of the reverse cholesterol transport pathway. Thus, genetic the effects of deficiency of CETP on reverse cholesterol transport may lead to increased CHD risk and may be more influential ultimately than an increase in HDL cholesterol levels [8].

Links between Nutritional Status, Inflammation and HDL in the Elderly

In light of the positive relationships of C-reactive protein (CRP) and interleukin 6 (IL-6) to CVD, many studies have investigated the possible associations between these inflammatory markers and HDL. In studies among general populations of elderly and hospitalized elderly patients, HDL-C levels correlate inversely with CRP (need a reference). However, the results were different for the oldest elderly, who experience age-related immune alteration or activation. A slight increase in CRP and IL-6 occurs in centenarians, even those considered healthy. Additionally, poor nutritional status, associated with hypo-albuminemia, atherosclerosis and dementia, often goes hand and hand with low-grade inflammatory activation. In centenarians, the proteins albumin, pre-albumin and transferrin have been shown to correlate strongly and positively with HDL-C and apo A1, the apolipoprotein present on the surface of HDL molecules. In contrast, CRP and IL-6 values are inversely correlated with HDL and apo A1 [1]. Through multiple regression analysis, the level of albumin was found to be the strongest predictor of HDL-C in centenarians. In the oldest elderly, it can be difficult to determine if the effect of a patient's nutritional status or inflammation on HDL-C is independent of other factors. Albumin does not always reflect nutritional status [1].

Dysfunctional HDL-C

Although HDL-C tends to exhibit strong anti-inflammatory properties, HDL-C can become dysfunctional in individuals with chronic inflammatory conditions [10]. Such conditions include the metabolic syndrome, lupus erythematosus, and rheumatoid arthritis. Increased levels of pro-inflammatory HDL-C have been associated with increased carotid intimal media thickness and an increased risk for adverse clinical outcomes in patients with chronic kidney disease [11]. These increased levels of dysfunctional HDL-C occurred in this population without deviation in LDL, HDL, and triglyceride values compared to those without dysfunction HDL-C. Although dysfunctional HDL-C is not routinely measured outside of research protocols, atorvastatin, LDL-apheresis, and diet along with an aerobic exercise program have been shown to reduce its blood concentrations [10, 12].

Cholesterol Metabolism

Circulating cholesterol levels are primarily determined by its hepatic production through the HMG-CoA reductase enzyme pathway [13]. It distributes in cells diffusely where it functions as a vital nutrient to support the health of phospholipids cell membranes [13]. However, since a limited amount of cholesterol is required for normal cell function, it is found in excess in atherogenic lipoproteins such as LDL and causes atherosclerosis. As a sophisticated regulator and inhibitor of this process, HDL removes excess cholesterol from

tissues. The major apolipoprotein (apo) of HDLs, apoA-1, leaves the liver without lipids, but once it leaves the plasma it then acquires lipids to be converted into an HDL particle. The membrane transporter, ATP binding cassette transporter A-1 (ABCA1), mediates the transfer of phospholipids and some un-esterified cholesterol from peripheral cells to create a nascent, disc-shaped HDL particle [14, 15]. This particle then accepts un-esterified cholesterol from other plasma lipoproteins and cell membranes. The enzyme lecithin cholesterol acyltransferase (LCAT) is activated by ApoA-1 and esterifies cholesterol so it can become incorporated into HDL molecules, resulting in a spheroidal HDL particle containing a core of cholesterol ester [1, 16, 17]. There are two ways in which HDL particles can dispose of their cholesterol. They can either use the scavenger receptor SR-B1 which binds to hepatocytes triggering excretion into bile or recycling, or they can engage in a process involving cholesteryl ester transfer protein (CETP) where HDL particles are transferred to a VLDL/LDL fraction. The cholesterol that CETP has transferred to other lipoproteins may then be delivered to the tissues. Paradoxically, some investigators have argued that the CETP transfer of cholesterol to the VLDL/LDL fraction may be a pro-atherogenic process [1, 8, 17]. Finally, light is being shed on endothelial lipase as a key component in HDL-cholesterol metabolism. An increase in endothelial lipase expression leads to a decrease in HDL-cholesterol levels. This is because as endothelial lipase catalytic capacity increases, HDL-cholesterol clearance rate increases. Aside from intracellular catabolism of reabsorbed HDL-particles, endothelial lipase may also facilitate the binding and absorption of HDL-particles [6].

Role of HDL-Cholesterol in Reversing Atherogenesis

One way HDL-cholesterol works in an anti-atherogenic fashion is by removing excess lipids from the vascular wall. It is important to note that HDL-cholesterol has also been shown to inhibit the expression of adhesion molecules by vascular endothelial cells exposed to cytokines. Less expression of adhesion molecules is associated with decreased binding of inflammatory cells and inhibition of atherosclerosis. It is known that TNF- α induced increase in the expression of E-selectin and VCAM-1 and HDL by its anti-inflammatory effects might inhibit it. In that regard it has been shown that the anti-TNF- α treatment in patients with chronic inflammatory arthritis induces a modest, but sustained, increase in serum HDL-C levels, which may have a favorable effect in reducing the cardiovascular risk in these patients [18]. To further support this down-regulation of inflammatory cytokine expression, clinical studies show low HDL-cholesterol is correlated with increased levels of adhesion molecules [19, 20]. Modified LDLs (including those that are oxidized) stimulate endothelial cells to express monocyte chemotactic protein (MCP-1), that attracts monocytes into the artery wall [20]. The modified LDLs also help the monocytes to become macrophages that then express scavenger receptors that take up the modified LDL. The result is the creation of foam cells, characteristic of atherosclerosis [21]. Investigation of the signaling pathways involved in the ox-LDL-induced NF- κ B activation and the mechanisms of the anti-inflammatory effect of HDL has also been conducted. Data suggest that the mechanism of ox-LDL-induced NF- κ B activation differs from that mediated by cytokines such as TNF- α . Additionally, it was discovered that HDL counteracts the pro-inflammatory effect of oxLDL by inhibiting the ROS/NF κ B signaling pathway by suppressing the ROS rise at the cellular level. This potent antioxidant-like effect of HDL is may result from antioxidant enzymes such as PAF-acetyl hydrolase or paraoxonase [22].

The Antithrombotic Properties of HDL-Cholesterol

Myocardial infarction (MI) or stroke is a result of ischemic death resulting results from the formation of an occlusive intra-arterial thrombus. Thus, reduced blood coagulability may decrease cardiovascular risk for MI. In an observational study of 60 hypercholesterolemic

men, both levels of fibrinogen and an index of platelet aggregability were significantly associated with reduced levels of the anti-atherogenic HDL sub-fraction-2 (HDL₂). Additionally, in a study of 132 men without a history of cardiovascular disease, the level of HDL₂-cholesterol, but not that of total cholesterol or HDL₃-cholesterol, was significantly lower in men with the highest quartile of fibrinogen compared with the other three quartiles [16].

Exercise and Dietary Effects on HDL-C

It is recognized that exercise level-response relationship exists between the level of exercise and lipoprotein levels suggesting that the effects of exercise may not result in significant increases in HDL-C until a certain exercise threshold is met. The threshold is reached based on cross-sectional literature at training volumes of 24 to 32 km/wk (15 to 20 miles/wk) of brisk walking or jogging and an expenditure of between 1200 to 2200 kcal/wk. This range of energy utilization is associated with 2 to 3 mg/dl increases in HDL-C. Results from the National Runner's Health Study, including 8283 men and 1837 women, showed that in men HDL-C increased by 0.135 mg/dl/km (0.218 mg/dl/mile) and the TC: HDL-C ratio decreased by 0.012 per kilometer [23-25]. An extensive lipid profile was evaluated in 60 healthy male sedentary controls, 40 male professional cross-country skiers, and 102 male professional road cyclists. Differences between athletes and sedentary controls with respect to HDL-C (mmol/L) were analyzed by unpaired Student's t-test. The HDL-C results were 1.35 ± 0.27 , $1.66 \pm .28$, 1.74 ± 0.41 , for sedentary controls, professional skiers, and professional cyclists, respectively [7]. Additionally, HDL-C levels are increased by high-saturated-fat diets; and, in contrast, HDL-C levels are decreased by high-polyunsaturated-fat diets [14, 26].

Niacin

Niacin is currently the most effective pharmaceutical agent commercially available for increasing HDL-C. It can increase HDL-C by as much as 35%, in contrast with statins, which robustly lower LDL by >50% [27, 28] but tend to have much less effect on HDL than niacin. Niacin is the only pharmaceutical lipid-lowering product that significantly reduces non-esterified fatty acids (NEFA), resulting in a decrease in plasma VLDL-TG levels. Accordingly, TG, and HDL-C concentrations tend to be strongly negatively correlated. Additionally, through another mechanism via the ABCA1 membrane transporter, niacin can stimulate cholesterol efflux from macrophages to primary HDL acceptors [27]. In a study of how niacin increases HDL-cholesterol in APOE*3Leiden.CETP mice, it was found that CETP activity was reduced. Essentially, the transfer of cholesterol from HDL to VLDL was reduced as related to lower hepatic CETP expression and a reduced plasma VLDL pool. The result of this was an increased lipidation of apoAI. This was shown by an increased HDL particle size and reduced uptake of apoAI by the kidneys [29].

In a double-blind randomized placebo-controlled study (ARBITER 2) of patients with coronary heart disease and low levels of high-density lipoprotein cholesterol (HDL-C < 45mg/dl), patients were given 1000 mg of once-daily extended release niacin in addition to statin therapy [30]. The change in common carotid intima-media thickness (CIMT) is potentially useful surrogate cardiovascular endpoint to monitor the effects of lipid lowering therapy [31]. The change in this cardiovascular endpoint along with the change in HDL-C was studied after one year. All the patients were receiving statin drugs at the start of the study; most of the patients were being treated with simvastatin, and the majority had a daily dose of 20 mg. The subjects were randomized with half receiving extended-release niacin (Niaspan) and half receiving a matching placebo. After one year, HDL-C increased from 39 ± 7 mg/dl to 47 ± 16 mg/dl (21%) in the niacin group and was unchanged in the placebo

group. Triglycerides were also decreased significantly in the niacin group. Mean CIMT increased significantly in the placebo group (0.044 ± 0.100 mm; $P < 0.001$) and was unchanged in the niacin group (0.014 ± 0.104 mm; $P = 0.23$). The conclusions of this study were that adding extended-release niacin to statin therapy counters the progression of atherosclerosis in patients with known CHD and moderately low HDL-C. Therefore, in ARBITER 2, the patients receiving niacin likely benefited from the reduction in triglyceride concentrations along with the observed 21% increase in HDL-C. In this study there was an observed non-significant trend towards a lower cardiovascular event rate in the niacin group, which is consistent with the results of both the Cholesterol Lowering Atherosclerosis Study (CLAS) and HDL-Atherosclerosis Treatment Study (HATS), in which slower rates of atherosclerosis progression were associated with lower cardiovascular event rates when niacin was used along with statin. It should be noted that the lipid and CIMT results of the ARBITER 2 study can only be generalized to the dose and preparation of niacin studied (extended release 1000 mg/d), although a dose of 2000 mg/d has more robust effects on lipids than lower doses [32]. This dose of niacin was selected for the purpose of balancing the drug's adverse effects, primarily flushing, with its nonlinear increase in HDL-C. This 1000 mg niacin dosage is much lower than the 2 to 4 g per day used in the HDL-Atherosclerosis Treatment Study (HATS) [33].

Statins

Statins elevate high-density lipoprotein (HDL)-cholesterol levels by 5-15%. This effect depending on the statin is observed at a low dose, however; higher doses do not elevate HDL levels more than the lower 20mg/day dosage [34]. Atorvastatin and pravastatin increase levels of HDL particles of large and medium size. Evidence from type IIa hyperlipidemic patients treated with atorvastatin showed how statins have anti-oxidative activities through the function of enzymes associated with HDL. Enhanced HDL-functionality leading to enhanced anti-inflammatory characteristics was demonstrated in a study when CHD patients were treated with simvastatin 40mg/day for 4 weeks [27].

In type 2 diabetic subjects carrying the CETP TaqIB polymorphism, the increase in HDL-cholesterol (+7.2%) after atorvastatin treatment was correlated with a reduction in CETP mass (-32%). A study was conducted on mice to determine if the increase in HDL depends on CETP expression. APOE*3-Leiden (E3L) mice were crossbred with mice expressing human CETP resulting in E3L.CETP mice. E3L and E3L.CETP mice were fed a diet either with or without atorvastatin. Atorvastatin, 0.01% in the diet, reduced plasma cholesterol in both E3L and E3L.CETP mice (-26 and -33%, $P < 0.05$). However, HDL-cholesterol was only increased in E3L.CETP mice (+52%). In E3L.CETP mice, atorvastatin decreased the hepatic CETP mRNA expression (-57%; $P < 0.01$), total CETP level (-29%) and cholesteryl esters (CE) transfer activity (-36%; $P < 0.05$) in plasma. Essentially, in E3L.CETP mice HDL-cholesterol is increased in atorvastatin by the decrease in hepatic CETP expression and thus, the reduction in CETP-dependent transfer of cholesterol from HDL to (V) LDL [34].

Fibrates

Fibrates have been shown to increase HDL-C levels by mean of 10% in 53 randomized, controlled trials (ref). This increase in HDL-C is less than what is produced by niacin. Fibrates decrease plasma levels of triglyceride lipoprotein by up to 48% as well. Thus, fibrates effect HDL lipid composition by decreasing HDL-TG and increasing CE. In patients with type 2 diabetes and in hypertriglyceridemic patients, fenofibrate, bezafibrate and gemfibrozil all preferentially increased small and/or medium HDL particle numbers. In hypertriglyceridemic patients, the increase in small HDL caused by fibrates may reach +168%. A link was found between plasma levels of small HDL3-C and CV risk in insulin-

resistant patients in the VA-HIT trial, where patients were treated with gemfibrozil. Thus, fibrates may help to improve the function of small, dense HDL particles, as well as increase HDL levels within the plasma [27].

Like with statins, fenofibrate was studied using APOE*3-Leiden (E3L) transgenic mice without and with the human CETP transgene. Fenofibrate (0.04% in the diet) did not affect HDL-cholesterol in E3L mice. However, fenofibrate dose-dependently increased HDL-cholesterol in E3L.CETP mice (as high as +91%). IN E3L.CETP mice, fenofibrate reduced hepatic CETP mRNA (−72%; $P < 0.01$) and CE transfer activity in plasma (−73%; $P < 0.01$). Like with statins, fenofibrate increases HDL-cholesterol by reducing CETP-dependent transfer of cholesterol from HDL to LDL [35].

Cholesteryl Ester Transfer Protein (CETP)

CETP inhibitors represent interesting potential therapeutic agents in the modification of CVD risk as they can produce dose-dependent HDL-cholesterol increases of up to 100%. Torcetrapib was the first CETP inhibitor to be involved in a large-scale, prospective, placebo-controlled interventional trial called the Investigation of Lipid Level Management to Understand its Impact in Atherosclerosis Events (ILLUMINATE). However, the trial was stopped before completion in December 2006 due to excess mortality in the group of patients treated with torcetrapib [36]. In the Rating Atherosclerotic Disease by Imaging with a New CETP Inhibitor (RADIANCE) 1 study, the common carotid intima-media thickness (cIMT) of the common carotid artery increased in the group treated with torcetrapib plus atorvastatin compared to the group treated with atorvastatin alone (0.0076 ± 0.0011 versus 0.0025 ± 0.0011 mm/y; $P = 0.0014$) [37, 38]. Patients treated with torcetrapib experienced a 72% increase in HDL cholesterol and a 25% drop in LDL cholesterol. Off-target toxic effects led to a 40% increase in cardiovascular mortality, 25% increase in the rate of fatal and non-fatal cardiovascular events and 100% increase in non-cardiovascular death in the group treated with torcetrapib [39]. After the ILLUMINATE study was terminated, new data indicated that torcetrapib treatment was linked to an increase in aldosterone levels, changes in serum electrolytes, suggesting mineralocorticoid excess, and elevated blood pressure (mean 5.4 mmHg rise in systolic blood pressure) [36, 40, 41]. Mineralocorticoid receptors on the principal cells of the distal tubule of the nephrons are activated by aldosterone produced in the adrenal gland. Activation of these receptors activates an increase in sodium and bicarbonate re-absorption, along with an increase in potassium excretion and finally an increase in plasma volume. This mineralocorticoid-based explanation for the adverse effects of torcetrapib is supported by lower potassium levels correlating with the SBP increase after torcetrapib administration. Moreover, mineralocorticoid hormones have pro-atherogenic effects that are not entirely due to effects on blood pressure. For example, aldosterone causes arterial stiffening through collagen deposition in the extracellular matrix, which may explain the increase in the cIMT found in the torcetrapib group [14, 15].

Another theory to explain the increase in the cIMT observed in the torcetrapid arm is the regulation of HDL cholesterol by torcetrapib that may be dysfunctional. Data regarding the relationship of CETP deficiency due to genetic polymorphisms to atherosclerosis are not clear. Some studies have demonstrated a decrease in atherosclerotic disease, while others have demonstrated an increased propensity. In contrast, partial inhibition should result in functional HDL particles [16].

Anacetrapib, a newer CETP inhibitor has increased HDL-cholesterol levels up to 129% in patients with dyslipidemia when administered at a dose of 300mg/day. Based on a clinical phase I study, anacetrapib reduced LDL-cholesterol levels by up to 38%, without the harmful effects on blood pressure seen with torcetrapib [36].

In a randomized double-blind, and placebo controlled phase II dose-response trial, the efficacy and safety of daily treatment with 300mg, 600mg, 900mg of JTT-705 (dalcetrapib) in 198 patients with mild hyperlipidemia was assessed. Treatment with 900mg JTT-705 for 4 weeks resulted in a 37% decrease in CETP activity ($P<0.0001$), a 34% increase in HDL cholesterol ($P<0.0001$), and a 7% decrease in LDL cholesterol ($P=0.017$). For dosages up to 900mg of JTT-705 there were no changes in body mass index, waist circumference, blood pressure, or signs of hepatotoxic or nephrotoxic effects. JTT-705 may have an effect on the gastrointestinal system that is mild. Diarrhea occurred in 5, 4, 3, 2 individuals in the 900-, 600-, 300-mg and placebo groups respectively. Flatulence occurred in 2, 2, 3, and 1 individuals in the 900-, 600-, 300-mg and placebo groups respectively. After 4 weeks of treatment with JTT-705, the 900mg dose was associated with a non-significant higher frequency of complaints relating to the gastrointestinal tract [42, 43].

JTT-705 in combination with pravastatin has been studied in a randomized, double-blind, placebo-controlled trial involving 152 subjects with LDL-C >160 mg/dl. Patients were either given pravastatin 40 mg and a placebo, or pravastatin 40mg and JTT-705 300mg, or pravastatin 40mg and JTT-705 600mg. After 4 weeks, JTT-705 600mg plus pravastatin 40mg resulted in a 30% decrease from baseline in CETP activity and a 28% increase from baseline in HDL-C. In this study arm, LDL-C decreased by 5% from baseline. The JTT-705 300mg dose plus pravastatin 40mg resulted in a decrease in CETP activity by about 16% and an increase in HDL-C by about 14% after 4 weeks [8, 44]. The issue of whether CETP inhibition in humans down-regulates atherosclerosis and reduces risk for CVD events is far from being settled while we await results from phase III clinical trials using dalcetrapib and anacetrapib. The fact that the mechanism of HDL elevation due to CETP occurs through the blocking of a vital element in reverse cholesterol transport remains a concern for these therapeutic agents. Since niacin also possesses a robust ability to increase HDL-C and has other beneficial effects on fatty acid metabolism, it remains a safe and useful agent for individuals with suboptimal HDL-C, particularly in those with insulin resistance or at high CVD risk and are already taking a statin or other lipid-lowering drug [45].

Other CEPT inhibitors including TA-8995, DRL-17822 (Table 2), and others are under investigation. DRL 17822, a selective inhibitor of CETP, for the treatment of dyslipidemia, atherosclerosis and associated cardiovascular diseases. The compound shows potent elevation in HDL-C and reduction of atherosclerotic plaques in animals, and has a clean safety profile in preclinical studies [45].

CETP inhibitors (e.g. JTT-705 and torcetrapib) seem to be the most promising regimen to increase HDL-C levels. Torcetrapib can substantially increase HDL-C levels (up to 106%), alone or in combination with atorvastatin. HDL-C strategies, in combination with effective statins, are a new drug target aimed at a further reduction in CVD morbidity and mortality compared with statin monotherapy [45]. However, safety profiles did not favor advancing torcetrapib resulting in the discontinuation of the trials [45]. Additionally a biologic target CETi-1, an investigational vaccine in phase II development designed to elicit antibodies that bind and inhibit the activity of CETP leading to blocking the ability of the protein to transfer cholesterol from HDL to LDL and thus causing HDL cholesterol levels to rise is under clinical investigation for sometime (Table 2) [46].

Endothelial Lipase

The HDL degrading enzyme, endothelial lipase becomes much more active in the presence of inflammation and Inflammatory cytokines induce endothelial lipase expression [17, 47, 48]. Additionally, an antibody against endothelial lipase showed that both of these increases were caused by endothelial lipase [6].

In exploring the interaction of HDL with the endothelium, it has been established that HDL hydrolysis by endothelial lipase is a pathway for PPAR α activation and indicates a potential mechanism contributing to HDL effects on limiting leukocyte adhesion to the endothelial and vascular inflammation. Characterization of this pathway shows selectivity with PPAR α activation by endothelial lipase most potent with the HDL substrate, as compared to LDL and VLDL [17].

The metabolic syndrome may be characterized as a chronic inflammatory condition. Elevated plasma endothelial lipase mass has been found in patients with the metabolic syndrome compared to normal controls. Secondly, increased plasma concentrations of endothelial lipase were found in individuals with high inflammatory markers [49, 50].

Endothelial lipase has also been linked to atherosclerosis. An increase in endothelial lipase mass has been associated with coronary artery calcification, a measure of subclinical atherosclerosis in humans [51]. Pathological sectioning of human coronary arteries has revealed endothelial lipase in atheromatous plaques. Endothelial lipase expression has been observed in macrophages and smooth muscle cells as well. In rats with hypertension, there was a greatly reduced HDL-cholesterol level with respect to the controls; endothelial lipase expression was higher in the aorta, heart and lungs. Importantly, all NCEP ATP III-defined metabolic syndrome factors (which are all risk factors for atherosclerosis) were correlated with endothelial lipase concentration in both pre- and post-heparin plasma.

Two studies have examined the role of endothelial lipase in atherosclerosis in mouse models. However, the results of these studies were conflicting. In one study, endothelial lipase knockout mice were crossed onto an apoE deficient background (double knockout) and they were then compared to apoE knockout mice. Atherosclerotic development was assessed by measuring lesions at the root of the aortic arch. After 12 weeks on a high fat diet, decreased atherosclerotic lesions were found in endothelial lipase knockout mice compared to the controls. This difference was only found in the males fed a high-fat diet. In contrast, another study was conducted on endothelial lipase deficient mice that were either on the apoE knockout or the LDL receptor knockout background. Endothelial lipase and LDL receptor double knockout mice and their LDL receptor deficient controls were fed a high-fat diet for 18 weeks. No significant difference in the level of atherosclerosis between the endothelial lipase knockout mice and the control animals was found [6].

Conclusion

HDL-C modulates lipid metabolism and the process of atherosclerosis through a variety of pathways. These include reverse cholesterol transport, anti-inflammatory and antioxidant effects. HDL-C has been found to be a more important risk predictor for CVD than LDL-C, total cholesterol, or triglycerides in the Framingham Heart Study. CETP inhibitors in clinical trials include anacetrapib, JTT-705, and others where their ability to down-regulate atherosclerosis and reduce CVD risk while being safe is not known. These trials follow the failure of first CETP torcetrapib in combination with statin in the large-scale trial (ILLUMINATE trial) due to off-target toxicity that may be linked to a mineralcorticoid excess. Finally, scientific attention is being given to the enzyme endothelial lipase, linked to atherosclerosis.

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Table 1

Targets and Approached for the Modulation of HDL Levels

Strategies	Targets
■ Increase Apo A-1 production	■ Apo A-1 Expression ■ Apo A-1 Milano
■ Promote reverse cholesterol transport	■ LXR ■ ABCA1 agonists
■ Niacin Receptor	■ HM74α agonist
■ Delay catabolism of HDL	■ Endothelial Lipase

Table 2
CETP inhibitors in development

Drug	Company	CETP-Related Phase	Comments
Torcetrapib atorvastatin	Pfizer	CETP inhibitor [*] III statin	Discontinued; concerning pro-hypertensive side-effect seen in Phase II, III trials
JTT-705 [*]	Roche	CETP inhibitor II	Lower potency compared to torcetrapib
CETi-1	Avant	Anti-CETP vaccine II	Biologic therapy; lack of potency may limit its market. It is going back and forth between preclinical and clinical
BAY-60-5521	Bayer	CETP inhibitor I	Limited information

^{*} Discontinued after adverse events in Phase III