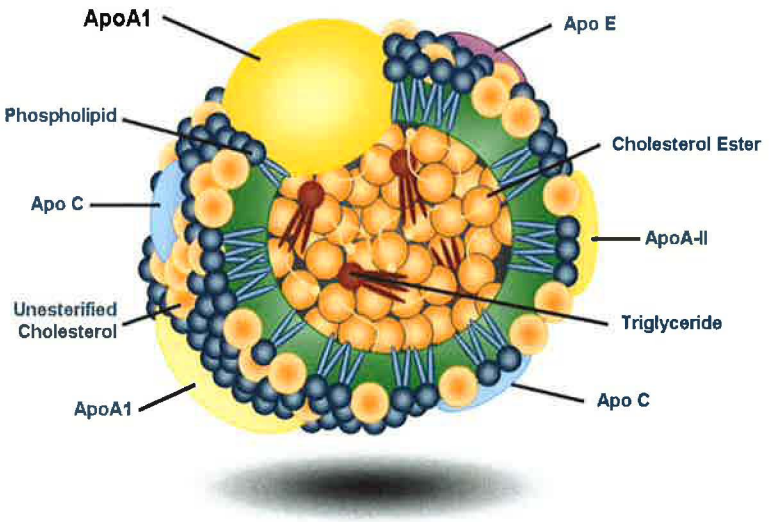
High-Density Lipoprotein - A Paucity of Therapy, A Paucity of Knowledge



Anand Rohatgi, MD
Division of Cardiology
Internal Medicine Grand Rounds
UT Southwestern Medical School
July 9, 2010

This is to acknowledge that Dr. Anand Rohatgi, M.D. has not disclosed any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Anand Rohatgi will not be discussing off-label uses in his/her presentation.

Anand Rohatgi, MD is an Assistant Professor in the Department of Internal Medicine and Division of Cardiology at UT Southwestern. His research interests focus on assessment of novel biomarkers for predicting coronary heart disease and the utility of HDL function as a clinically useful biomarker. He will complete a Master's Degree in Clinical Sciences in 2010. His clinical interests include Preventive Cardiology, General and Non-Invasive Cardiology (Echocardiography). He sees patients in the Preventive Cardiology Program in the Clinical Heart Center.

Anand Rohatgi, MD
Assistant Professor
5323 Harry Hines Blvd.
Dallas, TX 75390-9047
214-645-7500 (Office)
214-645-7501 (Fax)
anand.rohatgi@utsouthwestern.edu

Introduction

Heart disease is the number one cause of death in the U.S., with coronary heart disease (CHD) and stroke accounting for the majority of these deaths. As of 2006, an estimated 17.6 million Americans had CHD and over 425,000 deaths in 2006 were directly attributable to CHD.[1] From 1980 to 2000, there has been a 50% reduction in the death rate from CHD, with about 24% of the reduction attributable to lowering of total cholesterol and consequent low-density lipoprotein cholesterol (LDL-C).[2] From all available epidemiologic evidence and randomized controlled trials of LDL-C lowering, the lower the baseline or achieved LDL-C, the lower the risk of CHD.[3, 4] Current guidelines reflect these associations by targeting LDL-C thresholds for initiating therapy and achieving therapeutic benefit.[3]

Despite these reductions, significant residual risk remains, and CHD remains the leading cause of death in men and women. It has long been known from several large epidemiologic studies *that high-density lipoprotein cholesterol, HDL-C, is inversely associated with CHD.* [5] Four large American studies and one British study had been completed by the 1980s, with varying levels of risk factors but surprisingly similar levels of HDL-C among men and women separately. Taken together, *a 1 mg/dL increase in HDL-C was associated with 1.9-2.3% decrease in CHD risk in men and 3.2% decreased risk in women*. These risks were attenuated for CHD mortality in men except for one study but were magnified for CHD mortality in women. Adjustment for non-HDL-C (total – HDL-C) attenuated all associations between HDL and CHD risk but remained significant in 3 of the 5 studies (Figure 1).

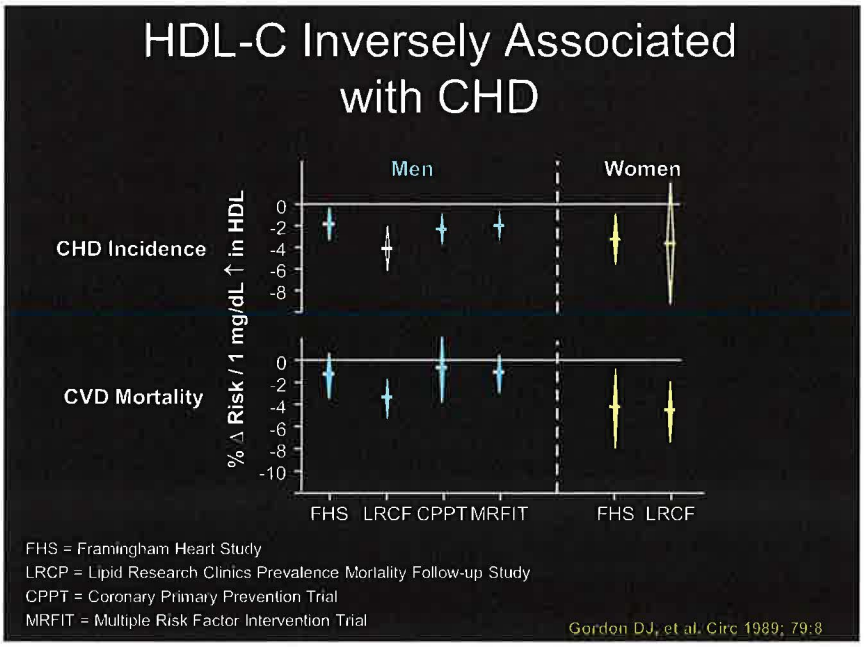


Figure 1

Similarly in patients with established CHD, low HDL-C is highly prevalent (>60%) despite statin use[6] and is associated with increased risk of CHD events despite achievement of extremely low LDL-C levels ≤ 70mg/dL with statin therapy.[7]

These consistent observations have led to the inclusion of low HDL-C (<40mg/dL in men; <50mg/dL in women) as a major risk factor for CHD in most risk prediction algorithms, including the Adult Treatment Panel-III guidelines and the Framingham Risk Score.[8] Unfortunately, whether HDL is causal in the development of or protection from CHD has not been proven in human studies. *Importantly, despite classification of low HDL-C as a major CHD risk factor, evidence-based therapies targeting HDL-C that improve CHD risk are lacking, and improving HDL-C is considered a secondary aim in the ATP-III CHD risk reduction algorithm* (Figure 2).[8]

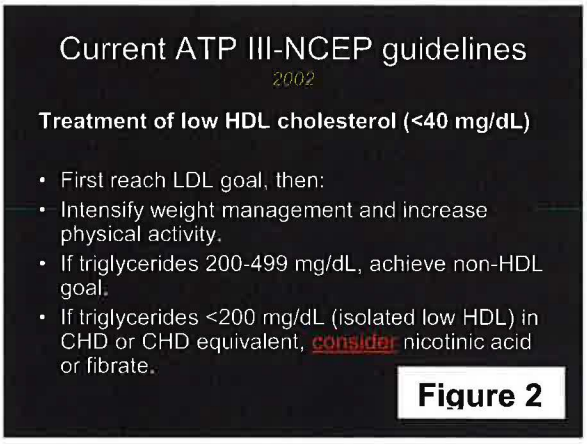


Figure 2

Recently, a novel class of drugs known as cholesteryl ester transfer protein inhibitors (CETP inhibitors) has been developed that markedly increase serum HDL-C by preventing transfer of cholesteryl ester out of HDL to other lipoprotein particles. *In 2007-2008, the findings of several phase III trials of one such compound, torcetrapib, were published, showing increased risk of death and no improvement in progression of carotid and coronary atherosclerosis in patients with CHD or familial hypercholesterolemia (Figure 3).*[9-12] Though there were several adverse effects of torcetrapib, including raised blood pressure and reduced potassium, the results of these studies have cast serious doubt on the strategy of raising HDL-C and have turned attention to measuring other aspects of HDL composition and function as better markers for CHD and targets for therapy.

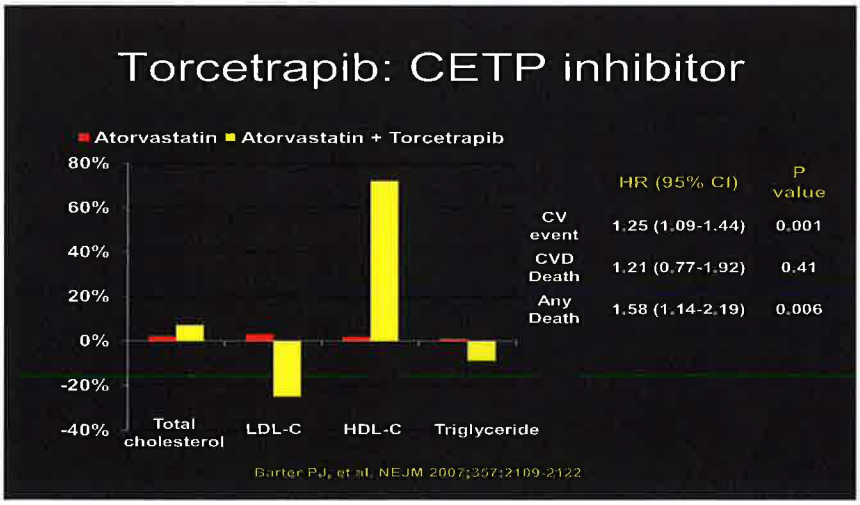


Figure 3

Role of HDL

Cells can produce the appropriate amount of cholesterol needed to maintain membrane and intracellular homeostasis. However, excess intra-cellular free cholesterol is toxic and is rapidly converted to oxysterols, stimulating export out of the cell. Macrophages can harbor oxidized and esterified cholesterol, resulting in the classic "foam cells" that characterize atherosclerotic lesions in arteries. The liver is the only organ that can degrade cholesterol and shuttle it out of the body through bile and feces. *HDL participates as a universal plasma acceptor of cholesterol and phospholipid and directly transports cholesterol from peripheral tissues to the liver. This process, termed reverse cholesterol transport (RCT), is considered to be the main mechanism responsible for preventing the development of atherosclerotic lesions when excess lipid depositing occurs in the arterial walls.*

I. HDL Biogenesis

A. Apolipoprotein A-I (apoA-I)

The HDL life-cycle is highly dynamic, initiating as protein and acquiring cholesterol from multiple acceptors with multiple delivery sites and exchange partners. ***Apolipoprotein A-I (apoA-I)*** is the main protein constituent of HDL (~70% of the apolipoprotein content) and is secreted from the liver and intestinal cells. Lipid-poor apoA-I accepts free cholesterol and phospholipids through transfer from lipoprotein lipase-mediated lipolysis of triglyceride-rich lipoproteins and cellular cholesterol efflux. Although it remains unclear whether HDL-C is causal in CHD, human apoA-I transgenic mice have increased HDL-C and are resistant to atherosclerosis,[13] supporting the concept that apoA-I has a direct role in both HDL-C levels and atherosclerosis. Several studies have shown associations between apoA-I levels and CHD events, but the magnitude of these associations vary by population studied and adjustment for body size and other risk factors.[14, 15] Multiple factors influence transcriptional control of the apoA-I gene, including dietary factors, endocrine effects, and various pharmacotherapies.[16, 17] *In the general population, apoA-I clearance is the most important determinant of plasma HDL-C and apoA-I levels*,[18] though in subjects with similar metabolic profiles apoA-I production rates have a larger effect on HDL concentration.[19] Several other apolipoproteins are found in HDL particles, of which apoA-II is the most abundant (two-thirds of HDL particles). The exact physiologic role of apoA-II and other apolipoproteins, including apoA-IV, apoC (I-III), apoD, apoE, apoJ, apoL-I, and apoM have not been fully elucidated.

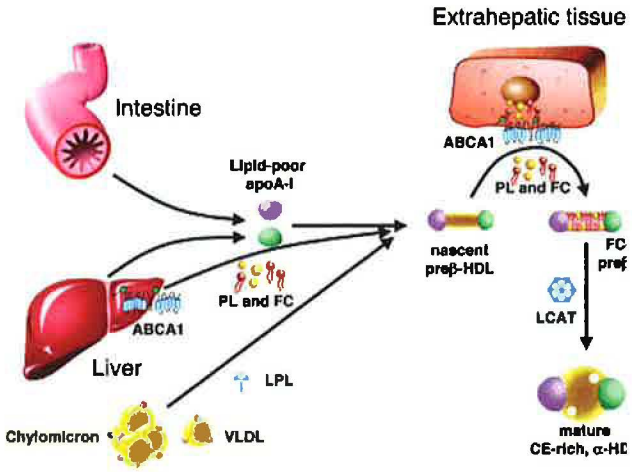
B. Formation of mature HDL and Reverse Cholesterol Transport (RCT)

Lipid-poor apoA-I secreted from the liver and intestinal cells quickly acquire a small amount of lipid in the plasma compartment to form pre-beta HDL particles (pre-beta migration on electrophoresis). Both lipid-poor apoA-I and pre-beta HDL accept cholesterol and phospholipid from peripheral tissues in a step-wise fashion to form mature HDL particles (Figure 4).

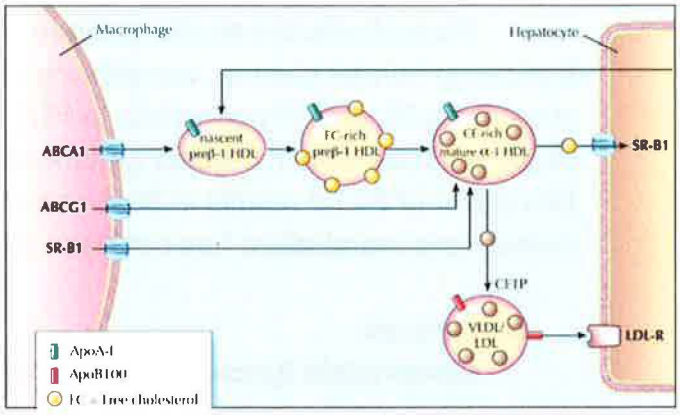
The first and key step of RCT and formation of mature HDL involves ***ATP-binding cassette transporter-1 (ABCA1)***. In the extracellular space, lipid-poor ApoA-I interacts with ABCA1 on cellular membranes (mostly hepatocytes and macrophages) to mediate lipid transfer (cholesterol and phospholipid) from cell membranes to the ApoA-I particles, generating discoidal-shaped nascent pre-beta HDL particles. The critical importance of ABCA1 in HDL formation and cholesterol homeostasis was demonstrated by patients with Tangier disease who have a mutation in the ABCA1 gene, leading to intracellular cholesterol accumulation, profoundly low HDL-C, and increased risk of CHD, organ dysfunction, and characteristic yellow tonsils.[20] ABCA1 animal knockout models have extremely low HDL concentration as well,[21] but the effects of ABCA1 are tissue-specific. Macrophage-specific ABCA1 deficiency has minimal effects on HDL concentration but profound effects on atherosclerosis.[22, 23] Liver-specific ABCA1 deficiency, on the other hand, results in marked reductions in HDL concentration (~80%) and over-expression in the liver is associated with increased HDL concentration and resistance to atherosclerosis.[24, 25]

The cholesterol acquired by HDL is esterified by ***Lecithin:cholesterol acyltransferase (LCAT)***, forming a more spherical HDL particle with an inner hydrophobic core surrounded by ApoA-I molecules. These small alpha HDL particles (HDL3) can continue to accept lipid from macrophages via ***ABCG1 and ABCG4***[26] as well as by aqueous diffusion,[27] generating larger, mature alpha HDL particles (HDL2). Like ABCA1, ***Scavenger Receptor class B, Type I (SR-BI)*** is found on both macrophages and hepatocytes but mediates cholesterol efflux solely to mature HDL instead of nascent HDL particles (Figure 4). *Macrophage-specific RCT in vivo, the process theorized to be central to atherosclerosis, is dependent on the transporters ABCA1 and ABCG1 but not SR-BI.*[28] Interestingly, SR-BI knockout models result in marked increases in HDL concentration but also increased atherosclerosis,[29] and macrophage-specific SR-BI deficient mice have normal HDL levels but increased atherosclerosis,[30] highlighting the discord between the effects of hepatic and macrophage-specific cholesterol efflux, HDL levels, and atherosclerosis.

Figure 4



McPherson R. Mechanisms of Dyslipidemia; Mechanisms in Medicine Inc., 2004



deGoma, et al. JACC 2008;51:2199-2211

II. Remodeling of HDL (Figure 5)

A. Lipid transfer proteins

The composition of circulating HDL particles in the plasma compartment can be altered by bidirectional transport of cholesteryl ester and phospholipid mediated by lipid transfer factors as well as by lipid hydrolysis by lipolytic enzymes. Once nascent HDL particles acquire free cholesterol from peripheral tissue, the majority is esterified by ***lecithin-cholesterol acyltransferase (LCAT)***,[31] forming a hydrophobic core of cholesteryl ester surrounded by apolipoproteins. *This step is critical in the formation of mature HDL particles; humans and mouse models with genetic LCAT deficiency syndromes have marked reduced HDL and apoA-I levels.*[32] Although critical to HDL formation, its effects on RCT are unclear as unesterified cholesterol in humans can be directly transferred to the liver and secreted in bile.[33]

Cholesteryl ester transfer protein (CETP) circulates in the plasma bound to lipoproteins and mediates transfer of cholesteryl ester from HDL to apoB lipoproteins (very low, intermediate, and low-density lipoproteins: VLDL, IDL, LDL; chylomicrons; and remnants) in exchange for triglycerides.[34] The net effect of CETP is depletion of cholesteryl ester and reduction in size of HDL particles and HDL concentration. CETP-mediated transfer is accelerated in hypertriglyceridemia and the post-prandial state.[35, 36] *Observations of genetically CETP-deficient Japanese patients having extremely elevated HDL-C and that pharmacologic CETP inhibition markedly increases HDL-C in humans and reduces atherosclerosis in rabbit models have led to the development of a new class of lipid-modifying drugs*. However, the effects of CETP-inhibition on atherosclerosis remain unclear given the recent failure of torcetrapib[37] and the observation that CETP-mediated transfer of cholesteryl ester from HDL to apoB-containing lipoproteins may serve an important role in RCT (a pathway blocked by CETP inhibition).

Phospholipid transfer protein (PLTP) mediates most of the phospholipid transfer in human plasma, transferring phospholipids from triglyceride-rich lipoproteins such as VLDL and chylomicrons to HDL.[38] In mouse models, PLTP remodels HDL into larger particles with increased generation of lipid-poor apoA-I and pre-beta HDL.[39] Disruption of PLTP results in 60% reduction in HD-C and apoA-I levels,[40] but its role in human lipid metabolism has not been clearly elucidated.

B. Lipases

Lipoprotein lipase (LPL), secreted from adipose and muscle tissue and found on endothelial cells, acts to hydrolyze triglycerides in triglyceride-rich lipoproteins into phospholipids and free cholesterol, which are transferred to HDL, increasing HDL size and concentration.[41] Humans with LPL deficiency have reduced HDL-C levels. Conversely, over expression of LPL in mice results in elevated HDL, but the effect of LPL activity on HDL-C levels appear to be dependent on CETP-mediated transfer of lipids between triglyceride-rich lipoproteins and HDL.[42] Therapeutic upregulation of LPL increases HDL-C and inhibits atherosclerosis in rat models.[43]

Hepatic lipase (HL), on the other hand, acts mainly on HDL, hydrolyzing its lipid contents and generating smaller HDL particles and reduced HDL concentration.[44] The effects of hepatic lipase are enhanced when the triglyceride content of HDL is increased, particularly in states of insulin resistance or hypertriglyceridemia. Humans with loss-of-function HL gene mutations have increased HDL-C as do HL-knockout mice; conversely, HL over-expression results in reduced HDL-C and HDL size.[45]

Endothelial lipase (EL) is a more recently described lipase[46] that is produced by endothelial cells and acts on the endothelial surface with primarily phospholipase A1 activity and little triglyceride lipase activity compared to LPL or HL.[47] EL has been described as having more efficient hydrolyzing effects on HDL than the other lipases.[47] Similar to HL, over-expression of EL leads to reduced HDL concentration whereas loss of function in both mouse and human models leads to increased HDL concentration and increased HDL size.[48-50] In apoE-deficient murine atherosclerosis models, EL-knockout mice have reduced atherosclerosis suggesting a direct role of EL's effects on HDL metabolism and RCT. [51] Interestingly, the effects on HDL-C levels mirror the effects on apoB lipoproteins.[51]

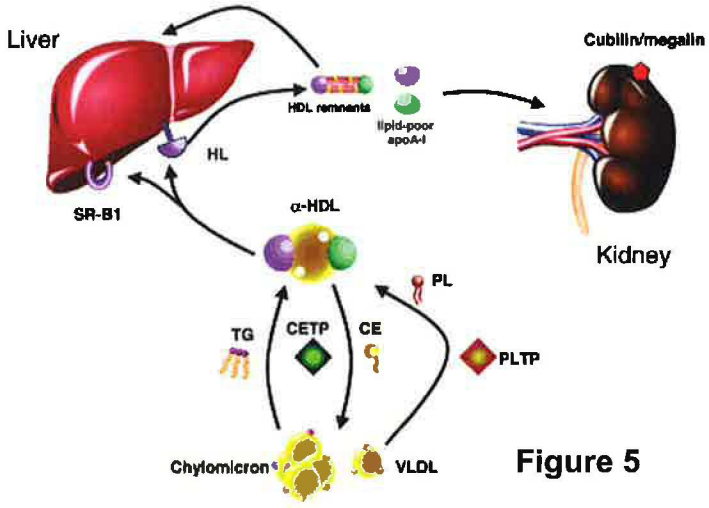


Figure 5

III. HDL-C uptake and HDL apolipoprotein catabolism

A. Scavenger receptor B, Type I (SR-BI)

SR-BI is highly expressed in liver, adrenal gland, and ovary and plays a major role in HDL selective lipid uptake in the liver and steroidogenic tissues.[29] HDL directly binds to SR-BI and lipid diffuses from the hydrophobic core to the plasma membrane, generating smaller, dense HDL particles. SR-BI knockout models result in markedly elevated HDL-C levels but not of plasma apoA-I and increased atherosclerosis. Conversely, SR-BI over-expression leads to reduced HDL-C and apoA-I levels.[52] As with the lipases and transfer proteins mentioned above, HDL size and particle composition affect the interaction between HDL and SR-BI. Degradation and catabolism of HDL and apoA-I occur in the liver and kidney.

Disconnect between HDL cholesterol and CHD

The inverse relationship between HDL-C and CHD risk in the general population has not been consistently observed in individuals and families with rare monogenic disorders of HDL-C. Part of the reason for this discordance is the remarkable phenotypic variability in HDL-C, atherosclerosis, and CHD among these individuals. Rare mutations in ABCA1, apoA-I, and LCAT lead to markedly low HDL-C levels, whereas mutations in endothelial lipase (LIPG) and CETP lead to increased HDL-C.

I. Mutations associated with low HDL-C

A. ABCA1 mutations

Individuals homozygous (Tangier disease) or heterozygous (familial hypoalphalipoproteinemia) for ABCA1 mutations have extremely low levels of HDL-C, apoA-I, and impaired cellular cholesterol efflux. Though multiple reports suggest increased incidence of CHD,[53] the majority of patients do not develop clinical CHD over the same time periods of follow-up as unaffected family members.[54-56] Patients with Tangier disease also have lower total cholesterol, LDL-C, and apoB, which may attenuate the atherogenic profile associated with low HDL-C.

Among those who do develop CHD, ABCA1 heterozygotes and homozygotes develop clinical CHD decades earlier with a dose-response allelic effect.[54, 55] Surrogate measures of CHD, namely carotid intima media thickness (CIMT), are increased at an earlier age in subjects with heterozygous ABCA1 mutations.[57] However, consistently higher triglyceride levels associated with ABCA1 mutations may also mediate some of the increased risk of CHD in these individuals, diluting the role of isolated low HDL-C in CHD. In the largest cohort of ABCA1 heterozygotes to date, involving 109 ABCA1 heterozygotes in a total sample size of over 40,000 Danish subjects with over 6500

CHD cases, **ABCA1 heterozygotes had reduced HDL-C by 17 mg/dL but no increased risk of CHD (OR 0.93 95%CI [0.53-1.62]).**[58] In the overall population studied, a 17-mg/dL decrease in HDL-C was associated with a significantly increased odds ratio of 1.7, *emphasizing the discordant effects of genetically determined low HDL-C and population-based low HDL-C*.

B. ApoA-I mutations

ApoA-I is the major apolipoprotein on HDL and accounts for the majority of the effects of HDL on RCT. Plasma levels of apoA-I inversely correlate with CHD, and, when combined with apoB, outperform all other traditional measures of lipids in predicting CHD risk.[14, 15] Multiple rare functional mutations in the apoA-I gene have been described that lead to decreased HDL-C, apoA-I, and cholesterol efflux. These mutations have been associated with premature CAD.[59, 60]

In contrast to these apoA-I mutations, carriers of the apoA-I_{Milano} mutation have greater cholesterol efflux[61] and protective effects against lipid oxidation[62] than normal apoA-I despite significantly reduced HDL-C and apoA-I levels. The originally described kindred in Italy had a lack of clinically evident CHD despite an atherogenic profile of low HDL-C and high triglyceride levels.[63, 64] Using CIMT, it was found that carriers of apoA-I_{Milano} have paradoxically similar atherosclerosis to controls,[65] but carriers of other apoA-I mutations had increased CIMT, as expected from life-long low HDL-C and apoA-I.[65, 66]

C. LCAT mutations

Mutations in the LCAT gene lead to two distinct syndromes, familial LCAT deficiency with complete loss of LCAT activity and fish-eye disease characterized by loss of LCAT activity on HDL alone. Both syndromes lead to markedly reduced HDL-C, smaller, dense HDL particles, and corneal opacification. Studies of LCAT mutations and CHD in humans have been conflicting. Prior reports suggested no increased incidence of premature CHD in familial LCAT deficiency but increased risk in fish-eye disease.[32] Several studies have shown increased CIMT in patients with genetically determined LCAT deficiency,[67, 68] while other recent studies have not.[69, 70] In the most recent and comprehensive study o13 Italian families with LCAT deficiency, increasing copy number of LCAT mutations was associated with paradoxically decreased CIMT,[69, 70] in stark contrast to prior reports but consistent with recent findings that LCAT deficiency does not affect cellular cholesterol efflux.[71] Furthermore, in a nested case-control study, high plasma LCAT activity was not associated with decreased events but was associated with increased risk among subjects with high HDL-C.[72]

II. Mutations associated with high HDL-C

A. Endothelial lipase mutations (LIPG)

The most frequently studied LIPG mutation in humans has been the common variant, Thr111Le. Studies have reported conflicting results on its association with HDL-C levels and no association with CHD.[73, 74] On the other hand, in several large case-control cohorts, the rare mutation Asn396Ser was found to be associated with a 8 mg/dL increase in HDL-C, increased HDL particle size and number of large particles, and increased apoA-I levels.[73] Unlike other mutations that affect HDL-C, the Asn396Ser variant was not associated with any other lipids, including LDL, triglyceride, apoB, or other lipoprotein particle sizes. An analysis of the association between this mutation and CHD in a large pooled data set will be forthcoming to shed light on the causal role of HDL-C in CHD.

B. CETP mutations

In 1985 a 58 year-old Japanese man with an HDL-C of 301 mg/dL and his sister with HDL-C of 174 mg/dL were reported to have absence of CETP activity.[75] Several rare (Japanese) and common mutations in CETP are associated with increased HDL-C levels, but studies reporting association with CHD risk have been conflicting. In a recent meta-analysis involving 27,196 CHD cases and 55,338 controls, OR per allele for three common mutations were associated with modest increases in HDL-C and apoA-I (3-5%) and were modestly protective for CHD: OR 0.95 (95%CI 0.92-0.99) for TaqIb, 0.94 (95%CI 0.89-1.00) for I405V, and 0.95 (95%CI 0.91-1.00) for -629C>A.[76] Similarly in the Women's Genome Health Study, a genome wide scan revealed that the CETP mutation, 16q3, was the only mutation associated with both HDL-C and CHD: a per-allele increase in HDL of 3.1 mg/dL and a modest non-significant protective effect on incident CHD, HDL-adjusted HR of 0.84 (95%CI 0.68-1.03).[77] Interestingly half the 20 mutations in CETP in this study were significantly associated with higher HDL-C, as expected with loss of CETP function, and half were associated with significant paradoxical decreases in HDL-C.

In contrast to findings in genetically determined CETP deficiency states, pharmacologic CETP inhibition with torcetrapib had a much greater effect on increasing HDL-C (↑ 72%) and lowering LDL(↓ 24%) but was associated with a significant 25% increased risk of CV events and 58% increased risk of death.[37] Similarly contrasting findings were seen when plasma CETP activity was measured in the Framingham Heart Study; higher CETP activity was associated with reduced risk of CHD (OR per SD 0.86, 95%CI 0.76-0.97; HDL-adjusted),[78] calling into question the presumed cardiac benefits of CETP inhibition.

Summarizing findings from human subjects with genetically-determined low or high HDL-C, the effects on CHD are not uniform and sometimes paradoxical to the inverse associations seen between HDL-C and CHD in population studies. Except for the Asn396Ser endothelial lipase variant, most other variants affecting HDL-C levels

also affect other lipid parameters that presumably may either explain or attenuate associations with CHD.

III. Discordances between therapies that alter HDL-C and CHD

Most of the risk reduction in CHD due to lipid-modifying therapies has been attributed to LDL lowering, with statins being the most consistent and potent drug class showing this effect. As shown before, statins overall are associated with a 6-8% increase in HDL, and patients with significant LDL lowering as well as increases in HDL seem to benefit most when individual trials have been examined. Interestingly, the highest statin doses are associated with a slight reduction in HDL despite more CHD risk reduction than medium dose statins.[79] However, some therapies such as fibrates and the glitazones have shown variable effects on CHD despite consistent and more potent increases in HDL-C.[80, 81] In addition, estrogens and torcetrapib are examples of drugs that significantly increase HDL but are associated with increased CHD risk.

A meta-regression study analyzed over 108 RCTs of lipid-modifying therapies to assess whether changes in HDL on therapy were associated with CHD events, independent of LDL and drug class.[79] The study found that change in LDL was associated with CHD risk reduction regardless of HDL level or drug class and explained a significant portion of the benefit. *On the other hand, change in HDL did not significantly correlate with CHD risk adjusted for LDL and drug class.*

When analyses were restricted to those therapies that are known to significantly raise HDL and excluded those associated with CHD harm, change in HDL was significantly associated with risk reduction but that association was completely abolished when adjusted for LDL and drug class, and, in fact, trended to increased harm. Restricting to studies of drugs specifically chosen to raise HDL (niacin combinations and fibrates) eliminated the trend to harm but still showed no association between change in HDL on therapy and CHD, unadjusted or adjusted for LDL and drug class.

The conclusion from this meta-regression of over 100 RCTs of lipid-modifying therapies was that change in HDL on treatment did not account for the CHD reduction in RCTs, when adjusted for LDL. Limitations include the omission of the Coronary Drug Project[82] (niacin associated with CHD benefit but no HDL-C measured) and the Jupiter study[83] (rosuvastatin) as well as combining niacin and fibrate studies in the sensitivity analyses. *The signal for increased harm with increased HDL adjusted for LDL will have to be further corroborated but does suggest again a limitation of using HDL-C as a measure of response to therapy.*

Heterogenous HDL particle composition and function

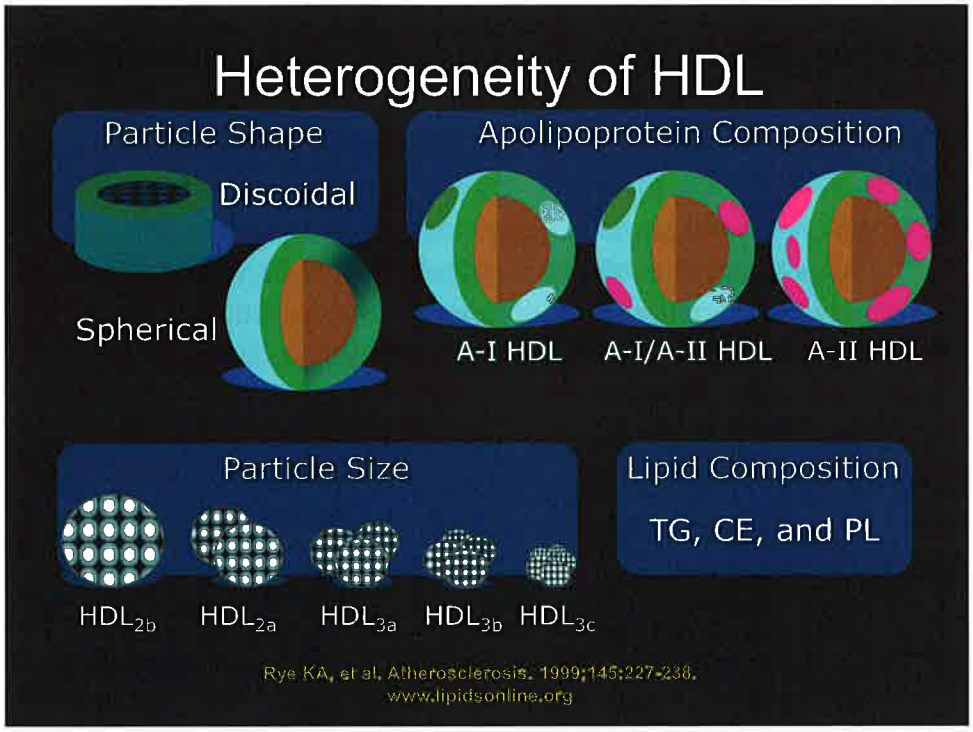


Figure 6

As mentioned above, HDL particles vary significantly according to shape, size, protein, and lipid composition (Figure 6).[84] Several methods exist for determining various HDL subspecies. Ultracentrifugation has long been the gold standard for determining lipoprotein subspecies by density (HDL: 1.063 – 1.21 g/mL). Gradient-gel electrophoresis discerns subspecies by size and NMR by proton signal, giving rise to 3-5 HDL species.[85]

2-dimensional gel electrophoresis is a more recently developed method that allows more quantitative characterization of HDL subspecies. In this method, plasma or serum is electrophoresed in 2 phases, resulting in 12 distinct Apo-AI containing HDL subspecies that vary by size and charge (alpha and beta migrating).[86] In individuals with normal total and LDL levels, those with low HDL have significantly lower levels of apoA-I in the large mature alphaA1 particles and higher levels of apoA-I in the small, nascent pre-beta1 particles.[87] In the Framingham offspring cohort study, 2D-gel electrophoresis was performed in 169 CHD male cases and 1277 matched controls.[88] Despite matching by HDL-C, levels of HDL subspecies markedly differed, with less large mature HDL particles and more nascent pre-beta1 and small, dense mature HDL particles ion subjects with CHD. The strongest HDL particle association was with large alpha1, showing an inverse correlation with prevalent CHD, adjusted for traditional risk factors and lipids levels, including triglyceride levels. However, there was some discordance in

subspecies measurements, where pre-alpha 1 was lower in pts w/ CHD but conferred increased risk with increasing levels when adjusted for risk factors and other lipids.

The protein make-up of HDL particles is also quite heterogeneous. Most of the apolipoprotein on the surface of HDL is apolipoprotein A-I (apoA-I). Various other apolipoproteins make up a smaller fraction of the remaining apolipoprotein component and have diverse biological activities as well. The recent advent of protein analysis by mass spectroscopy, or proteomics, has allowed remarkable discrimination in detecting proteins on the surface of HDL. The HDL proteome appears to have as many as 30 different proteins, many of which are not standard apolipoproteins known to be associated with HDL.[89, 90] These types of analyses suggest that the heterogeneous protein makeup of HDL reflects heterogeneous HDL functionality, including lipid metabolism, protease action, and immunity. Investigations into these diverse HDL functions are yielding new insights into how HDL may exert its anti-atherogenic effects and how therapeutic modulation can alter these functions in relation to HDL-C levels.

HDL Function

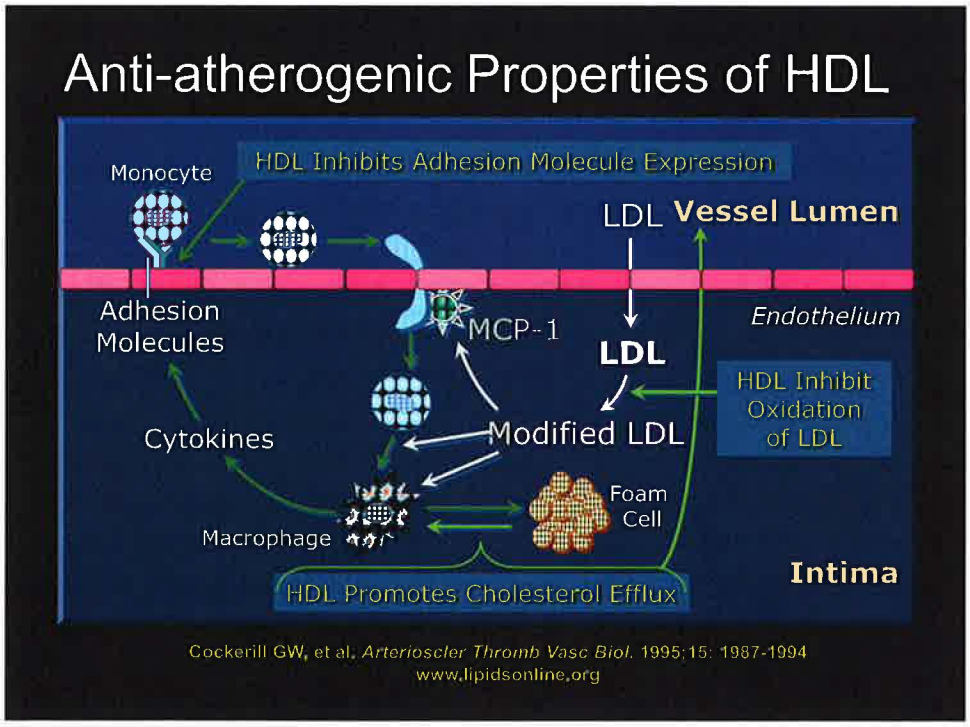


Figure 7

The process of atherosclerotic plaque development in the arterial wall involves multiple steps, including uptake of oxidized LDL by macrophages in the intima, increased cytokine production leading to endothelial cell adhesion molecule expression and recruitment of monocytes to the endothelial surface, and transmigration into the intimal space and foam cell formation. The effects of HDL on these steps are

considered to be anti-atherogenic but can vary significantly in various disease states and in response to therapy (Figure 7).

A. Cholesterol Efflux (Reverse Cholesterol Transport – RCT)

The concept of reverse cholesterol transport involves movement of cholesterol from peripheral tissue into the plasma compartment, delivered to the liver and excreted through bile and feces. Because this is a highly dynamic process, quantifying cholesterol efflux may give better insight into the functional activity of HDL. There are several ways of quantifying cholesterol efflux, and only recently have systems been developed and optimized to study human HDL. By loading or incubating cells with radiolabeled cholesterol, movement of this labeled pool into various compartments can be measured, including an extracellular medium, the plasma compartment, and feces. In terms of testing human blood and HDL, the ex vivo cellular efflux models have developed scientific momentum in assessing the RCT transport properties of HDL.

Ex vivo cellular cholesterol efflux models have used 3 main cell lines, macrophages, hepatoma, and fibroblast cells, to quantify the movement of radiolabeled cholesterol from within the cells to an extracellular acceptor (Figure 8).[27] Acceptors that have been studied include purified or human isolated HDL or apoA-I, whole serum or plasma. Since the predominant mechanism related to atherosclerosis is dependent on macrophage-specific cholesterol efflux, that cellular model has potential promise for human investigation.

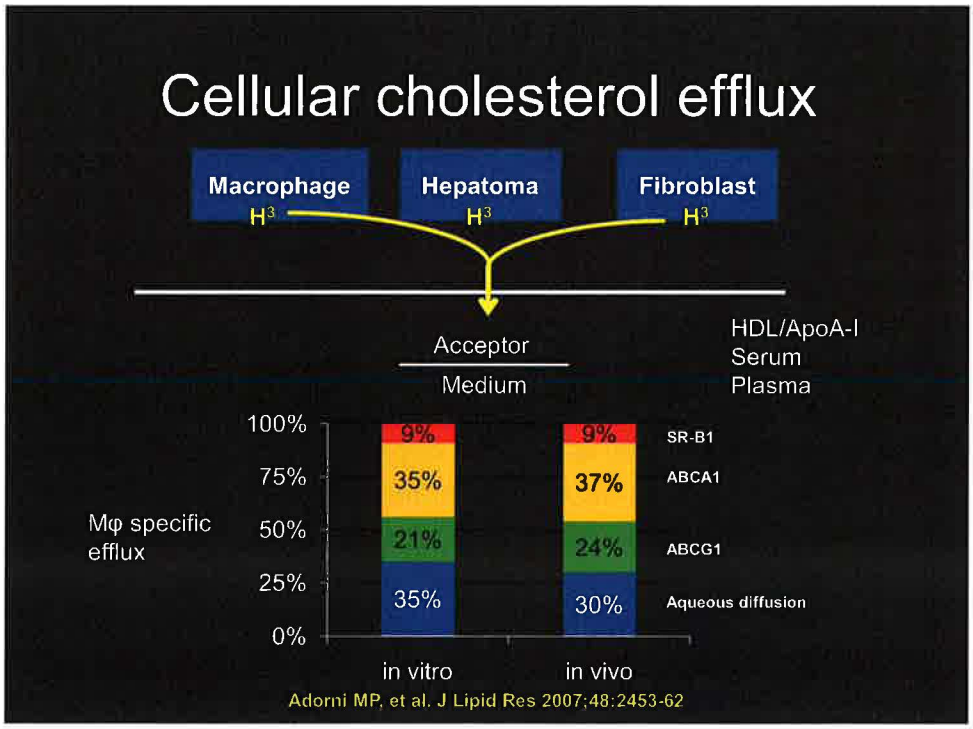


Figure 8

As discussed before, macrophages contain several different transporters that can mediate cholesterol efflux, including ABCA1, the predominant transporter of intracellular free cholesterol to nascent HDL, and ABCG1 and SRB1, which transport cholesterol to mature HDL particles. Reassuringly, the ex vivo system correlates well with in vivo murine models of macrophage cholesterol efflux, indicating that ABCA1 and ABCG1 contribute significantly to macrophage-specific efflux, but not SR-B1. In addition, unmeasured efflux, presumably reflecting passive aqueous diffusion, also contributes to efflux when cells are loaded with cholesterol, and is responsible for the majority of macrophage efflux in cholesterol normal cells. *The advantage of measuring macrophage RCT is that it better correlates with atherosclerosis than HDL-C levels in animal models.* In mouse studies, genetic manipulations and pharmacologic interventions leading to increased macrophage RCT led to decreased atherosclerosis and vice versa, except for ACCG1 KO models.[91] In these same mice, the relationship between changes in HDL-C levels and RCT was not as robust, and only the Apo-A1 models showed concordant relationships between HDL-C levels and RCT. Studies using HDL from human samples show that there is a wide distribution of macrophage-specific cholesterol efflux in subjects with similar HDL-C levels,[92] corroborating the discordance seen in mouse models. Whether macrophage-specific cholesterol efflux is a better correlate with atherosclerosis in humans has yet to be determined.

B. Vascular Inflammation - Cell-Adhesion Molecules

The increased expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin on endothelial cells is a necessary component of the chronic inflammatory process of atherosclerosis.[93] Adhesion molecules tether monocytes in the circulation, causing them to roll along the luminal surface of endothelial cells and transmigrate into the sub-endothelial space where they can acquire free cholesterol and become resident foam cells. The foam cells and oxidized lipids stimulate cytokines that contribute to further increased expression of adhesion molecules, setting up a vicious cycle of inflammation in the arterial wall.

The effects of HDL on the endothelium and vascular inflammation have been recently characterized. In a pivotal study by Philip Barter's group in Australia, human umbilical vein endothelial cells were incubated with HDL isolated from healthy individuals, and adhesion molecule expression was assessed in response to TNF-alpha activation.[94] They found that the cytokine-induced expression of endothelial cell adhesion molecules was reduced in a dose-dependent manner with increasing concentrations of HDL, and, at physiologic levels, was almost completely inhibited. The same group went on to show that the in vitro protective effects of HDL on endothelial adhesion molecule expression were also seen in an vivo model of vascular inflammation by inserting compressive collar rings around the carotid arteries of anesthetized rabbits and then measuring the amount of adhesion molecule expression

after sacrifice.[95] Insertion of the collar induced a robust neutrophil response in the carotid vessel wall that was abolished by infusion of recombinant HDL (rHDL). Similarly, the markedly increased expression of VCAM-1 and ICAM-1 was also abolished with infusion of rHDL.

Further confirming the role of HDL in inhibiting vascular inflammation, Fogelman's group in UCLA developed a monocyte chemotaxis assay assessing the migration of human-derived monocytes across human aortic endothelial cells.[96] They found that the increased monocyte migration in response to LDL was inhibited in a dose-dependent fashion by HDL from normal subjects but was unaffected by HDL from patients with CHD. They went on to show that even patients with CHD with high HDL had impaired inhibition of monocyte chemotactic activity. A cell-free assay has been developed assessing the effects of HDL on lipid oxidation which shows similar results to the monocyte chemotaxis assay and may be amenable for large-scale clinical testing if replicated and validated in larger cohorts.[96]

C. Endothelial Nitric Oxide (NO)

Nitric oxide (NO) produced by the endothelium is a potent vasodilator and has multiple effects on endothelium and vascular smooth muscle. In atherosclerotic vascular disease, levels of endothelium-derived NO are significantly reduced, leading to increased neutrophil adherence, enhanced smooth muscle cell proliferation, and platelet aggregation and adhesion.[97] NO is produced by endothelial nitric oxide synthase (eNOS), and mouse models of NOS antagonism or genetic NOS deficiency lead to accelerated atherosclerosis, supporting the concept that NO is protective and that NO deficiency serves a critical role in atherosclerosis.[97] Much of the biology of the effects of HDL on endothelium and eNOS has been worked out here at UTSW in the labs of Philip Shaul, Chieko Mineo and others.

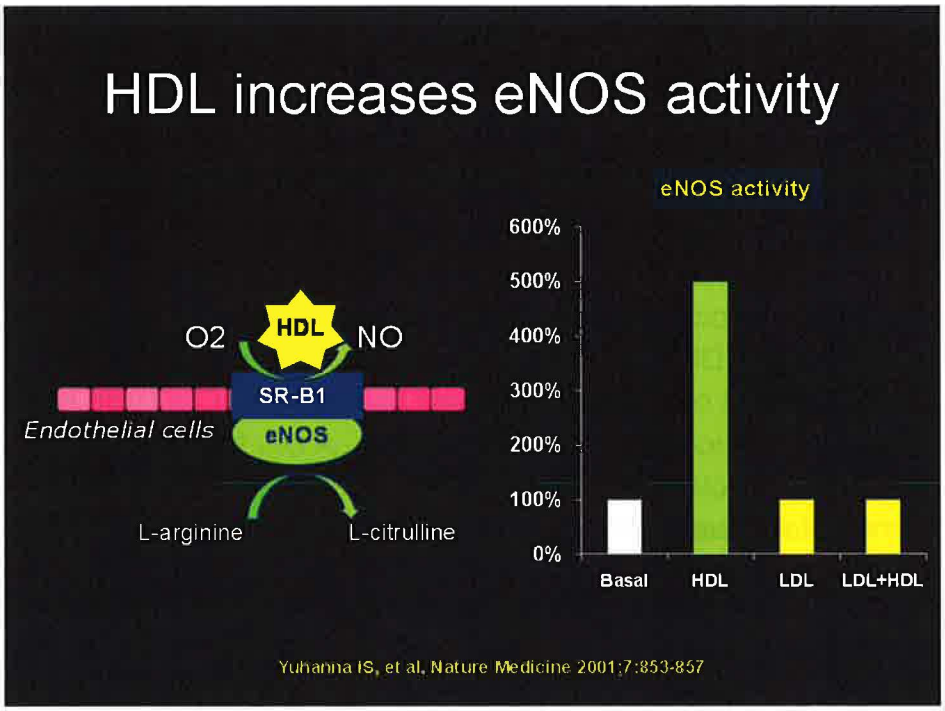


Figure 9

In a cell-based system, endothelial production of nitrite and nitrate is generated by incubating endothelial cells with L-arginine, a substrate of nitric oxide synthase (NOS). NO synthase activity can be characterized by measuring the conversion of labeled L-arginine to L-citrulline. Increased eNOS activity in this cell-based system would suggest protective effects on the endothelium and atherosclerosis and may serve as a way of testing the effects of agents on the capacity of human HDL to promote eNOS activity. When HDL from healthy subjects was tested, there was a 5-fold increase in eNOS activity compared to the basal state, and this effect was completely blunted by either LDL alone or adding LDL to HDL (Figure 9).[98]

Shaul and colleagues also assessed the endothelial effects of HDL in an in vivo system and found that HDL caused direct relaxation of phenlyephrine precontracted thoracic aorta rings from mice, an effect that was blunted by a NOS antagonist and by denuding the endothelium.[98] They also determined that the effects of HDL on NOS activation and endothelium-dependent vasorelaxation were mediated by SR-B1 in endothelial cells, the same receptor in liver cells that accepts free cholesterol from mature HDL particles and delivers cholesterol to mature HDL particles from macrophages as well. In mice, this cell-based system has been used to test the effects of novel HDL therapeutics such as apoA-1 mimetic peptides and has been correlated with vasodilatory response in intact animal models.

As an example of how measuring NO production may be used in humans, a recent small study of about 40 mostly male subjects tested the effects of HDL on endothelial NO production (by a different method) in subjects with diabetes compared to healthy controls.[99] They found that the HDL from patients with diabetes produced

much less endothelial NO and resulted in reduced relaxation of mouse aortic rings (Figure 10).

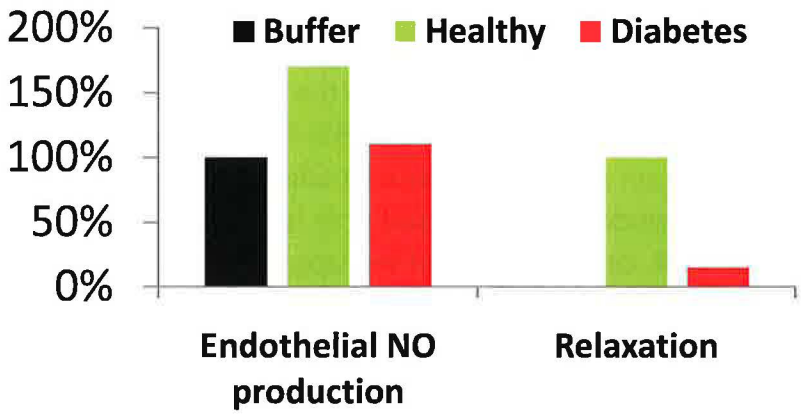


Figure 10

In summary, HDL has significant effects on cholesterol transport and vascular health, including inhibiting inflammation and oxidation and directly promoting endothelial nitric oxide activity, all of which are purported to exert anti-atherogenic effects in healthy individuals and are diminished in diseased states. Quantifying these HDL functions may serve as better biomarkers for predicting CHD and predicting response to therapies that modify HDL. Other effects of HDL include anti-apoptic and antithrombotic effects, and an increasing understanding of the role of HDL in innate immunity and protection from infection.[100]

Novel HDL-modifying therapies

Given the lack of drugs that specifically target HDL metabolism (except for niacin), and the recent failure of the first CETP-inhibitor, torcetrapib, multiple novel compounds are being studied to test the hypothesis that improving HDL-C levels and HDL function will reduce the risk of CHD. Two classes of drugs will be briefly reviewed here, HDL mimetics and CETP-inhibitors, with a concluding summary of the current level of evidence for niacin.

A. HDL Mimetics

Recombinant HDL (rHDL)

Recombinant HDL has been formulated using human apoA-1 complexed with phospholipid at fixed molar ratios, usually infused via peripheral IV at a dose of 80mg/kg over four hours. Several studies have shown beneficial effects of rHDL in humans. In one study of patients with hypercholesterolemia, infusion of rHDL led to restoration of endothelial function as measured by forearm blood flow.[101] In another study of 20 patients presenting for peripheral arterial revascularization, patients were randomized to a single 4-hour infusion of 80mg/kg rHDL and then underwent intervention 1 week later.[102] HDL from patients randomized to rHDL exhibited significantly greater

cholesterol efflux, which correlated with markedly reduced cholesterol content and macrophage accumulation in excised arterial plaques. The Melbourne group studied the effects of rHDL in 13 male patients with diabetes.[103] A single 4-hour infusion resulted in reduced endothelial cell expression of adhesion molecules ex-vivo and reduced platelet aggregation. The infusion resulted in increased capacity of HDL from patients to promote cholesterol efflux from platelets. In contrast to the receptor-dependent effects of HDL on cholesterol efflux via ABCA1, ACBG1, and SR-B1 and on endothelial NOS activation via SR-B1, the effects on platelet cholesterol efflux and platelet activation were found to be receptor-independent and affected only by the phospholipid and cholesterol concentration of rHDL or HDL, and not the apoA-I content. These studies highlight the diverse effects of HDL and rHDL through multiple different pathways.

ApoA-I_{Milano}

Based on the finding of a rare ApoA-I mutant in Italy that led to reduced HDL but no CHD based on a single cysteine to arginine susbstitution,[63] a recombinant form was developed and showed marked reductions in atherosclerosis in animal models, even after a single infusion. In the first human study of ApoA-I_{Milano}, patients who recently had acute coronary syndromes were randomized to receive recombinant ApoA-I_{Milano} or placebo for 5 weekly infusions. The primary endpoint, change in percent atheroma volume by intravascular ultrasound during coronary angiography, was modestly but significantly reduced from baseline in the intervention arm, but the difference was not significant compared to placebo, perhaps because of the small numbers of patients.[104]

In a similar study design of patients following acute coronary syndromes, a recombinant form of HDL using apo-A1 derived from wild-type human plasma complexed to soybean-derived phospholipid was tested using intravascular ultrasound derived characteristics as the primary endpoints. In this study 4 weekly infusions were administered, but the high dose arm (80mg/kg) was discontinued due to asymptomatic increases in liver enzymes. Though there were improvements from baseline in coronary plaque characteristics in those randomized to rHDL, these changes were not consistently different from placebo.[105]

Autologous HDL delipidation

Another interesting IV HDL therapy is autologous HDL delipidation, whereby the patient's blood is plasmapheresed and returned to the patient after the cholesterol from HDL is stripped using various solvents. In a pilot study of ACS patients assessing tolerability, 7 weekly delipidation sessions led to non-significant reductions in coronary plaque characteristics, but similar in magnitude to the Apo-$A1_{milano}$ trial. The authors suggested that because these effects are larger than those seen with high-dose statins, larger clinical trials investigating clinical endpoints may show potential benefit of such therapies in the acute setting.

In summary, IV infusions of recombinant HDL or autologous HDL delipidation have led to non-significant trends toward improved coronary plaque volume and other characteristics in high-risk patients. Larger trials investigating clinical endpoints will determine whether these resource- and cost-intensive therapies will translate into clinical benefit.

Oral ApoA-I mimetic: D4F

The large size of ApoA-I at 243 amino acids has made it expensive and difficult to synthesize, necessitating IV administration and severely restricting its use to acute and inpatient settings. The development of an 18aa peptide that mimics the helical structure of apoA-I has led to the development of several oral apo-AI mimetics, of which the D4F version has been tested in humans.[106] In animal models, D4F leads to reduced atherosclerosis synergistically with statins and reduced inflammation. In the first human study, a single dose given to patients with CHD or otherwise high-risk patients was tolerated well without significant side effects and led to improvements in inflammatory indices, such as monocyte chemotaxis, compared to baseline.[107] Whether these effects of oral or IV mimetic peptides will translate into true clinical benefit will have to await larger clinical trials.

B. CETP Inhibitors

Torcetrapib was the first CETP-inhibitor to be tested in Phase III clinical trials. As described above, randomization to torcetrapib was associated with increased all-cause and coronary death and no significant change in coronary or carotid atherosclerosis.[9-11, 37] Despite these disappointing findings, post-hoc analyses suggested that the subjects who achieved the highest HDL-C levels on torcetrapib actually had coronary regression and that other CETP inhibitors without such deleterious adverse events would have potential cardiac benefits.[108] Dalcetrapib is another CETP inhibitor without the deleterious effects on blood pressure and the rennin-angiotensin-aldosterone system associated with torcetrapib. Enrollment has completed in a phase III, double-blind, placebo-controlled multi-center trial of 15,600 patients with acute coronary syndromes. Patients were randomized to dalcetrapib 600mg once daily vs. placebo on top of statin therapy (clinicaltrials.gov). Hard clinical endpoints will be assessed as the primary endpoint, with results projected in 2013. Other CETP inhibitors such as anacetrapib and JTT-705 (vaccine) are in smaller phase II-III clinical studies and also do not have the adverse side effect profile of torcetrapib. All of the CETP inhibitors markedly increase HDL-C levels by 60-100%, increase large HDL particles, and improve cholesterol efflux. Whether these changes in HDL metabolism will lead to clinical benefit will be determined with the results of large clinical trials.

C. Niacin

Clinical Trials

Niacin is a B vitamin and available over the counter. It also comes as an extended release prescription (ER-Niacin, Abbott). The most common side effect is flushing which has been reported in over 60% of subjects with the immediate-release formulation, leading to high rates of discontinuation.

Niacin is the most potent HDL-raising drug currently available for use. On background statin therapy, niacin raises HDL by about 20% and lowers total and LDL by 10-15% and triglycerides by 20-30%.[109] Very few randomized controlled trials have studied the effect of niacin on cardiovascular endpoints. *The only study reported in the literature to date that was designed to study actual clinical events was the Coronary Drug Project, completed in 1975*.[82] Several lipid agents were studied in men with existing CHD, including niacin at a dose of 3 grams/d. After 5 years, there was a significant 27% reduction in non-fatal CHD events compared to placebo but no difference in mortality. *After 15 years follow-up, after the study had terminated almost a decade before, there was a significant 11% reduction in mortality in those who had been originally randomized to niacin compared to placebo*.[110]

Several other studies have randomized patients to niacin (HATS, ARBITER-2, ARBITER-6),[109, 111, 112] assessing changes in atherosclerosis. These studies have also been in patients with existing CHD but enrolled relatively small numbers compared to the CDP. Reported coronary events in the niacin group were lower than the comparator groups with borderline significance. It is difficult to extrapolate too much from these data given the low numbers of absolute events. However, of note, both ARBITER studies showed a reduction in events in those randomized to niacin in addition to background low to medium-dose background statin therapy.

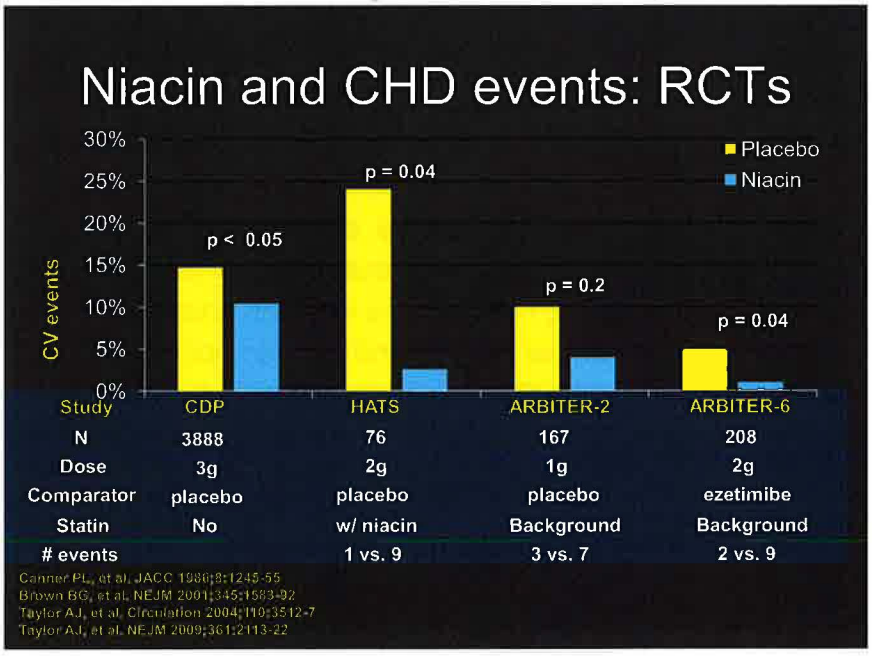


Figure 11

The ARBITER trials assessed changes in CIMT by ultrasound in patients randomized to niacin vs. a comparator in patients with CHD. A true placebo arm without statin showed progression,[109] ezetimibe with statin showed no significant change, [112] and niacin with statin showed regression of CIMT.[109, 112] Another small study of patients with CHD using MRI instead of ultrasound showed similar regression of carotid wall area with niacin compared to placebo on top of statin.[113] The HATS study showed significant regression in coronary stenosis in patients randomized to simvastatin plus niacin compared to placebo.[111]

Mechanisms of Niacin's benefits

The effects of niacin on apolipoprotein B containing lipoproteins are believed to be primarily mediated through inhibition of triglyceride synthesis in the liver, leading to decreased production of all apoB-containing compounds, including LDL, VLDL, and Lp(a).[114] The mechanism of action leading to increased HDL is less well worked out, but is believed to be due to decreased catabolic rate of HDL and ApoA-1, possibly by blocking whole particle uptake by the liver and extending the half-life of circulating HDL and apoA-1.[114]

Not only does niacin reduce atherogenic lipoprotein levels and increase HDL-C, it also improves multiple indices of HDL function. Niacin has been shown to increase ABCA1 expression in vitro in the liver and human-derived monocytes and reduce cellular cholesterol content.[115] *Niacin has also been shown to increase cholesterol efflux* by as much as 28% compared to placebo, likely due primarily to increased HDL-C.[116] A recent study extensively evaluated the effects of niacin on endothelial function in patients with diabetes. In this study, 30 subjects with diabetes were randomized to placebo or ER-niacin titrated up to 1500mg/d for 3 months. Compared to placebo, *niacin was associated with a 22% increase in HDL-C and a significant 50% increase in the effect of patient HDL to promote endothelial NO production*. These findings were correlated with a significant increase in flow-mediated dilation of the radial artery in these same subjects as measured by a non-invasive ultrasound device pre- and post-arterial occlusion.[99]

Niacin also directly inhibits acute vascular inflammation. In another recently published study, the carotid collar model in rabbits was used to test the effect of orally administered niacin on acute vascular inflammation.[117] Niacin markedly decreased expression of endothelial cell adhesion molecules and inhibited intima-media neutrophil recruitment and myeloperoxidase accumulation. These changes were independent of plasma lipids and HDL levels and were similar when cells were directly incubated with niacin, suggesting a lipid-independent effect. These studies in aggregate support the hypothesis that niacin's beneficial role in atherosclerosis is likely due to both direct HDL

raising and improved HDL function. Whether these changes will translate into consistent clinical benefits on top of statin therapy will be determined by on-going clinical trials.

D. Active Clinical Trials involving Niacin and CETP inhibitors

	AIM-HIGH	HPS2-THRIVE	DAL-Outcomes
# pts	**3300**	**25000**	**15600**
Age	**>45**	**50-80**	**>45**
Pts	**Vasc Dx; metsyn**	**Vasc Dz**	**ACS**
Dose	**ER-niacin 2g**	**ER-niacin 2g**	**Dalcetrapib 600mg**
Statin	**Simva**	**Simva 40 ± zetia**	**Yes - Standard care**
Outcomes	**MACE**	**MACE**	**MACE**
Results	**2011??**	**2013**	**2013**

Summary and Future directions

Despite inverse associations between HDL-C levels and CHD in population-based studies, genetic syndromes in humans leading to either low or high HDL-C levels do not support a causal role of HDL in CHD. Furthermore, several therapies that raise HDL do not translate into clinical benefit, including torcetrapib (CETP inhibitor), fibrates, and estrogen. These discordances may be explained by the lack of HDL-C levels to capture the heterogeneous composition and function of HDL. Efforts to elucidate the diverse functions of HDL and correlate them to CHD and responses to therapy will facilitate understanding of HDL metabolism and perhaps lead to better therapeutic targets than HDL-C. Ultimately, active randomized clinical trials of HDL-modifying therapies will determine the clinical benefit of modulating HDL.

Literature Cited

1. Lloyd-Jones D, Adams RJ, Brown TM, et al. Heart disease and stroke statistics--2010 update: a report from the American Heart Association. Circulation 2010;121:e46-e215.
2. Ford ES, Ajani UA, Croft JB, et al. Explaining the decrease in U.S. deaths from coronary disease, 1980-2000. N Engl J Med 2007;356:2388-98.
3. Grundy SM, Cleeman JI, Merz CN, et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Circulation 2004;110:227-39.
4. Baigent C, Keech A, Kearney PM, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet 2005;366:1267-78.
5. Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation 1989;79:8-15.
6. Alsheikh-Ali AA, Lin JL, Abourjaily P, Ahearn D, Kuvin JT, Karas RH. Prevalence of low high-density lipoprotein cholesterol in patients with documented coronary heart disease or risk equivalent and controlled low-density lipoprotein cholesterol. Am J Cardiol 2007;100:1499-501.
7. Barter P, Gotto AM, LaRosa JC, et al. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. N Engl J Med 2007;357:1301-10.
8. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143-421.
9. Nissen SE, Tardif JC, Nicholls SJ, et al. Effect of torcetrapib on the progression of coronary atherosclerosis.[see comment]. N Engl J Med 2007;356:1304-16.
10. Kastelein JJ, van Leuven SI, Burgess L, et al. Effect of torcetrapib on carotid atherosclerosis in familial hypercholesterolemia. N Engl J Med 2007;356:1620-30.
11. Bots ML, Visseren FL, Evans GW, et al. Torcetrapib and carotid intima-media thickness in mixed dyslipidaemia (RADIANCE 2 study): a randomised, double-blind trial.[see comment]. Lancet 2007;370:153-60.
12. Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med 2007;357:2109-22.
13. Rubin EM, Krauss RM, Spangler EA, Verstuyft JG, Clift SM. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. Nature 1991;353:265-7.
14. Ingelsson E, Schaefer EJ, Contois JH, et al. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. Jama 2007;298:776-85.
15. Sierra-Johnson J, Fisher RM, Romero-Corral A, et al. Concentration of apolipoprotein B is comparable with the apolipoprotein B/apolipoprotein A-I ratio and better than routine clinical lipid measurements in predicting coronary heart disease mortality: findings from a multi-ethnic US population. Eur Heart J 2009;30:710-7.
16. Srivastava RA, Srivastava N. High density lipoprotein, apolipoprotein A-I, and coronary artery disease. Mol Cell Biochem 2000;209:131-44.
17. Malik S. Transcriptional regulation of the apolipoprotein AI gene. Front Biosci 2003;8:d360-8.
18. Brinton EA, Eisenberg S, Breslow JL. Elevated high density lipoprotein cholesterol levels correlate with decreased apolipoprotein A-I and A-II fractional catabolic rate in women. J Clin Invest 1989;84:262-9.
19. Velez-Carrasco W, Lichtenstein AH, Welty FK, et al. Dietary restriction of saturated fat and cholesterol decreases HDL ApoA-I secretion. Arterioscler Thromb Vasc Biol 1999;19:918-24.
20. Brooks-Wilson A, Marcil M, Clee SM, et al. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet 1999;22:336-45.

21. Aiello RJ, Brees D, Francone OL. ABCA1-deficient mice: insights into the role of monocyte lipid efflux in HDL formation and inflammation. Arterioscler Thromb Vasc Biol 2003;23:972-80.
22. Aiello RJ, Brees D, Bourassa PA, et al. Increased atherosclerosis in hyperlipidemic mice with inactivation of ABCA1 in macrophages. Arterioscler Thromb Vasc Biol 2002;22:630-7.
23. Haghpassand M, Bourassa PA, Francone OL, Aiello RJ. Monocyte/macrophage expression of ABCA1 has minimal contribution to plasma HDL levels. J Clin Invest 2001;108:1315-20.
24. Basso F, Freeman L, Knapper CL, et al. Role of the hepatic ABCA1 transporter in modulating intrahepatic cholesterol and plasma HDL cholesterol concentrations. J Lipid Res 2003;44:296-302.
25. Singaraja RR, Fievet C, Castro G, et al. Increased ABCA1 activity protects against atherosclerosis. J Clin Invest 2002;110:35-42.
26. Wang N, Lan D, Chen W, Matsuura F, Tall AR. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. Proc Natl Acad Sci U S A 2004;101:9774-9.
27. Adorni MP, Zimetti F, Billheimer JT, et al. The roles of different pathways in the release of cholesterol from macrophages. J Lipid Res 2007;48:2453-62.
28. Wang X, Collins HL, Ranalletta M, et al. Macrophage ABCA1 and ABCG1, but not SR-BI, promote macrophage reverse cholesterol transport in vivo. J Clin Invest 2007;117:2216-24.
29. Trigatti B, Covey S, Rizvi A. Scavenger receptor class B type I in high-density lipoprotein metabolism, atherosclerosis and heart disease: lessons from gene-targeted mice. Biochem Soc Trans 2004;32:116-20.
30. Van Eck M, Bos IS, Hildebrand RB, Van Rij BT, Van Berkel TJ. Dual role for scavenger receptor class B, type I on bone marrow-derived cells in atherosclerotic lesion development. Am J Pathol 2004;165:785-94.
31. Jonas A. Lecithin cholesterol acyltransferase. Biochim Biophys Acta 2000;1529:245-56.
32. Kuivenhoven JA, Pritchard H, Hill J, Frohlich J, Assmann G, Kastelein J. The molecular pathology of lecithin:cholesterol acyltransferase (LCAT) deficiency syndromes. J Lipid Res 1997;38:191-205.
33. Schwartz CC, VandenBroek JM, Cooper PS. Lipoprotein cholesteryl ester production, transfer, and output in vivo in humans. J Lipid Res 2004;45:1594-607.
34. Barter PJ, Brewer HB, Jr., Chapman MJ, Hennekens CH, Rader DJ, Tall AR. Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. Arterioscler Thromb Vasc Biol 2003;23:160-7.
35. Guerin M, Egger P, Soudant C, et al. Cholesteryl ester flux from HDL to VLDL-1 is preferentially enhanced in type IIB hyperlipidemia in the postprandial state. J Lipid Res 2002;43:1652-60.
36. Guerin M, Le Goff W, Lassel TS, Van Tol A, Steiner G, Chapman MJ. Atherogenic role of elevated CE transfer from HDL to VLDL(1) and dense LDL in type 2 diabetes : impact of the degree of triglyceridemia. Arterioscler Thromb Vasc Biol 2001;21:282-8.
37. Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med 2007;357:2109-22.
38. Huuskonen J, Olkkonen VM, Jauhiainen M, Ehnholm C. The impact of phospholipid transfer protein (PLTP) on HDL metabolism. Atherosclerosis 2001;155:269-81.
39. Jiang X, Francone OL, Bruce C, et al. Increased prebeta-high density lipoprotein, apolipoprotein AI, and phospholipid in mice expressing the human phospholipid transfer protein and human apolipoprotein AI transgenes. J Clin Invest 1996;98:2373-80.
40. Jiang XC, Bruce C, Mar J, et al. Targeted mutation of plasma phospholipid transfer protein gene markedly reduces high-density lipoprotein levels. J Clin Invest 1999;103:907-14.
41. Merkel M, Eckel RH, Goldberg IJ. Lipoprotein lipase: genetics, lipid uptake, and regulation. J Lipid Res 2002;43:1997-2006.

42. Clee SM, Zhang H, Bissada N, et al. Relationship between lipoprotein lipase and high density lipoprotein cholesterol in mice: modulation by cholesteryl ester transfer protein and dietary status. J Lipid Res 1997;38:2079-89.
43. Tsutsumi K, Inoue Y, Shima A, Iwasaki K, Kawamura M, Murase T. The novel compound NO-1886 increases lipoprotein lipase activity with resulting elevation of high density lipoprotein cholesterol, and long-term administration inhibits atherogenesis in the coronary arteries of rats with experimental atherosclerosis. J Clin Invest 1993;92:411-7.
44. Perret B, Mabile L, Martinez L, Terce F, Barbaras R, Collet X. Hepatic lipase: structure/function relationship, synthesis, and regulation. J Lipid Res 2002;43:1163-9.
45. Santamarina-Fojo S, Gonzalez-Navarro H, Freeman L, Wagner E, Nong Z. Hepatic lipase, lipoprotein metabolism, and atherogenesis. Arterioscler Thromb Vasc Biol 2004;24:1750-4.
46. Jaye M, Lynch KJ, Krawiec J, et al. A novel endothelial-derived lipase that modulates HDL metabolism. Nat Genet 1999;21:424-8.
47. McCoy MG, Sun GS, Marchadier D, Maugeais C, Glick JM, Rader DJ. Characterization of the lipolytic activity of endothelial lipase. J Lipid Res 2002;43:921-9.
48. deLemos AS, Wolfe ML, Long CJ, Sivapackianathan R, Rader DJ. Identification of genetic variants in endothelial lipase in persons with elevated high-density lipoprotein cholesterol. Circulation 2002;106:1321-6.
49. Ishida T, Choi S, Kundu RK, et al. Endothelial lipase is a major determinant of HDL level. J Clin Invest 2003;111:347-55.
50. Paradis ME, Couture P, Bosse Y, et al. The T111I mutation in the EL gene modulates the impact of dietary fat on the HDL profile in women. J Lipid Res 2003;44:1902-8.
51. Ishida T, Choi SY, Kundu RK, et al. Endothelial lipase modulates susceptibility to atherosclerosis in apolipoprotein-E-deficient mice. J Biol Chem 2004;279:45085-92.
52. Kozarsky KF, Donahee MH, Rigotti A, Iqbal SN, Edelman ER, Krieger M. Overexpression of the HDL receptor SR-BI alters plasma HDL and bile cholesterol levels. Nature 1997;387:414-7.
53. Schaefer EJ, Zech LA, Schwartz DE, Brewer HB, Jr. Coronary heart disease prevalence and other clinical features in familial high-density lipoprotein deficiency (Tangier disease). Ann Intern Med 1980;93:261-6.
54. Clee SM, Kastelein JJ, van Dam M, et al. Age and residual cholesterol efflux affect HDL cholesterol levels and coronary artery disease in ABCA1 heterozygotes. J Clin Invest 2000;106:1263-70.
55. Kyriakou T, Pontefract DE, Viturro E, et al. Functional polymorphism in ABCA1 influences age of symptom onset in coronary artery disease patients. Hum Mol Genet 2007;16:1412-22.
56. Hovingh GK, Kuivenhoven JA, Bisoendial RJ, et al. HDL deficiency and atherosclerosis: lessons from Tangier disease. J Intern Med 2004;255:299-301.
57. van Dam MJ, de Groot E, Clee SM, et al. Association between increased arterial-wall thickness and impairment in ABCA1-driven cholesterol efflux: an observational study. Lancet 2002;359:37-42.
58. Frikke-Schmidt R, Nordestgaard BG, Stene MC, et al. Association of loss-of-function mutations in the ABCA1 gene with high-density lipoprotein cholesterol levels and risk of ischemic heart disease. Jama 2008;299:2524-32.
59. Schaefer EJ, Genest JJ, Jr., Ordovas JM, Salem DN, Wilson PW. Familial lipoprotein disorders and premature coronary artery disease. Atherosclerosis 1994;108 Suppl:S41-54.
60. Pisciotta L, Miccoli R, Cantafora A, et al. Recurrent mutations of the apolipoprotein A-I gene in three kindreds with severe HDL deficiency. Atherosclerosis 2003;167:335-45.
61. Favari E, Gomaraschi M, Zanotti I, et al. A unique protease-sensitive high density lipoprotein particle containing the apolipoprotein A-I(Milano) dimer effectively promotes ATP-binding Cassette A1-mediated cell cholesterol efflux. J Biol Chem 2007;282:5125-32.

62. Bielicki JK, Oda MN. Apolipoprotein A-I(Milano) and apolipoprotein A-I(Paris) exhibit an antioxidant activity distinct from that of wild-type apolipoprotein A-I. Biochemistry 2002;41:2089-96.
63. Franceschini G, Sirtori CR, Bosisio E, et al. Relationship of the phenotypic expression of the A-IMilano apoprotein with plasma lipid and lipoprotein patterns. Atherosclerosis 1985;58:159-74.
64. Gualandri V, Franceschini G, Sirtori CR, et al. AIMilano apoprotein identification of the complete kindred and evidence of a dominant genetic transmission. Am J Hum Genet 1985;37:1083-97.
65. Sirtori CR, Calabresi L, Franceschini G, et al. Cardiovascular status of carriers of the apolipoprotein A-I(Milano) mutant: the Limone sul Garda study. Circulation 2001;103:1949-54.
66. Hovingh GK, Brownlie A, Bisoendial RJ, et al. A novel apoA-I mutation (L178P) leads to endothelial dysfunction, increased arterial wall thickness, and premature coronary artery disease. J Am Coll Cardiol 2004;44:1429-35.
67. Hovingh GK, Hutten BA, Holleboom AG, et al. Compromised LCAT function is associated with increased atherosclerosis. Circulation 2005;112:879-84.
68. Ayyobi AF, McGladdery SH, Chan S, John Mancini GB, Hill JS, Frohlich JJ. Lecithin: cholesterol acyltransferase (LCAT) deficiency and risk of vascular disease: 25 year follow-up. Atherosclerosis 2004;177:361-6.
69. Calabresi L, Baldassarre D, Castelnuovo S, et al. Functional lecithin: cholesterol acyltransferase is not required for efficient atheroprotection in humans. Circulation 2009;120:628-35.
70. Miller M, Rhyne J, Hong SH, Friel G, Dolinar C, Riley W. Do mutations causing low HDL-C promote increased carotid intima-media thickness? Clin Chim Acta 2007;377:273-5.
71. Calabresi L, Favari E, Moleri E, et al. Functional LCAT is not required for macrophage cholesterol efflux to human serum. Atherosclerosis 2009;204:141-6.
72. Dullaart RP, Perton F, van der Klauw MM, Hillege HL, Sluiter WJ. High plasma lecithin:cholesterol acyltransferase activity does not predict low incidence of cardiovascular events: possible attenuation of cardioprotection associated with high HDL cholesterol. Atherosclerosis 2010;208:537-42.
73. Edmondson AC, Brown RJ, Kathiresan S, et al. Loss-of-function variants in endothelial lipase are a cause of elevated HDL cholesterol in humans. J Clin Invest 2009;119:1042-50.
74. Jensen MK, Rimm EB, Mukamal KJ, et al. The T111I variant in the endothelial lipase gene and risk of coronary heart disease in three independent populations. Eur Heart J 2009;30:1584-9.
75. Koizumi J, Mabuchi H, Yoshimura A, et al. Deficiency of serum cholesteryl-ester transfer activity in patients with familial hyperalphalipoproteinaemia. Atherosclerosis 1985;58:175-86.
76. Thompson A, Di Angelantonio E, Sarwar N, et al. Association of cholesteryl ester transfer protein genotypes with CETP mass and activity, lipid levels, and coronary risk. Jama 2008;299:2777-88.
77. Ridker PM, Pare G, Parker AN, Zee RY, Miletich JP, Chasman DI. Polymorphism in the CETP gene region, HDL cholesterol, and risk of future myocardial infarction: Genomewide analysis among 18 245 initially healthy women from the Women's Genome Health Study. Circ Cardiovasc Genet 2009;2:26-33.
78. Vasan RS, Pencina MJ, Robins SJ, et al. Association of circulating cholesteryl ester transfer protein activity with incidence of cardiovascular disease in the community. Circulation 2009;120:2414-20.
79. Briel M, Ferreira-Gonzalez I, You JJ, et al. Association between change in high density lipoprotein cholesterol and cardiovascular disease morbidity and mortality: systematic review and meta-regression analysis. BMJ 2009;338:b92.
80. Robins SJ, Collins D, Wittes JT, et al. Relation of gemfibrozil treatment and lipid levels with major coronary events: VA-HIT: a randomized controlled trial. Jama 2001;285:1585-91.
81. Keech A, Simes RJ, Barter P, et al. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. Lancet 2005;366:1849-61.
82. Clofibrate and niacin in coronary heart disease. Jama 1975;231:360-81.

83. Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008;359:2195-207.
84. Rye KA, Clay MA, Barter PJ. Remodelling of high density lipoproteins by plasma factors. Atherosclerosis 1999;145:227-38.
85. Otvos JD. Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. Clin Lab 2002;48:171-80.
86. Asztalos BF, Sloop CH, Wong L, Roheim PS. Two-dimensional electrophoresis of plasma lipoproteins: recognition of new apo A-I-containing subpopulations. Biochim Biophys Acta 1993;1169:291-300.
87. Asztalos BF, Lefevre M, Foster TA, et al. Normolipidemic subjects with low HDL cholesterol levels have altered HDL subpopulations. Arterioscler Thromb Vasc Biol 1997;17:1885-93.
88. Asztalos BF, Cupples LA, Demissie S, et al. High-density lipoprotein subpopulation profile and coronary heart disease prevalence in male participants of the Framingham Offspring Study. Arterioscler Thromb Vasc Biol 2004;24:2181-7.
89. Vaisar T, Pennathur S, Green PS, et al. Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. J Clin Invest 2007;117:746-56.
90. Davidson WS, Silva RA, Chantepie S, Lagor WR, Chapman MJ, Kontush A. Proteomic analysis of defined HDL subpopulations reveals particle-specific protein clusters: relevance to antioxidative function. Arterioscler Thromb Vasc Biol 2009;29:870-6.
91. Rader DJ, Alexander ET, Weibel GL, Billheimer J, Rothblat GH. The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis. J Lipid Res 2009;50 Suppl:S189-94.
92. de la Llera-Moya M, Drazul-Schrader D, Asztalos BF, Cuchel M, Rader DJ, Rothblat GH. The ability to promote efflux via ABCA1 determines the capacity of serum specimens with similar high-density lipoprotein cholesterol to remove cholesterol from macrophages. Arterioscler Thromb Vasc Biol 2010;30:796-801.
93. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999;340:115-26.
94. Cockerill GW, Rye KA, Gamble JR, Vadas MA, Barter PJ. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. Arterioscler Thromb Vasc Biol 1995;15:1987-94.
95. Nicholls SJ, Dusting GJ, Cutri B, et al. Reconstituted high-density lipoproteins inhibit the acute pro-oxidant and proinflammatory vascular changes induced by a periarterial collar in normocholesterolemic rabbits. Circulation 2005;111:1543-50.
96. Navab M, Hama SY, Hough GP, Subbanagounder G, Reddy ST, Fogelman AM. A cell-free assay for detecting HDL that is dysfunctional in preventing the formation of or inactivating oxidized phospholipids. J Lipid Res 2001;42:1308-17.
97. Mineo C, Deguchi H, Griffin JH, Shaul PW. Endothelial and antithrombotic actions of HDL. Circ Res 2006;98:1352-64.
98. Yuhanna IS, Zhu Y, Cox BE, et al. High-density lipoprotein binding to scavenger receptor-BI activates endothelial nitric oxide synthase. Nat Med 2001;7:853-7.
99. Sorrentino SA, Besler C, Rohrer L, et al. Endothelial-vasoprotective effects of high-density lipoprotein are impaired in patients with type 2 diabetes mellitus but are improved after extended-release niacin therapy. Circulation 2010;121:110-22.
100. Movva R, Rader DJ. Laboratory Assessment of HDL Heterogeneity and Function. Clin Chem 2008;54:788-800.
101. Spieker LE, Sudano I, Hurlimann D, et al. High-density lipoprotein restores endothelial function in hypercholesterolemic men. Circulation 2002;105:1399-402.
102. Shaw JA, Bobik A, Murphy A, et al. Infusion of reconstituted high-density lipoprotein leads to acute changes in human atherosclerotic plaque. Circ Res 2008;103:1084-91.

103. Patel S, Drew BG, Nakhla S, et al. Reconstituted high-density lipoprotein increases plasma high-density lipoprotein anti-inflammatory properties and cholesterol efflux capacity in patients with type 2 diabetes. J Am Coll Cardiol 2009;53:962-71.
104. Nissen SE, Tsunoda T, Tuzcu EM, et al. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. Jama 2003;290:2292-300.
105. Tardif JC, Gregoire J, L'Allier PL, et al. Effects of reconstituted high-density lipoprotein infusions on coronary atherosclerosis: a randomized controlled trial. Jama 2007;297:1675-82.
106. Meyer P, Nigam A, Marcil M, Tardif JC. The therapeutic potential of high-density lipoprotein mimetic agents in coronary artery disease. Curr Atheroscler Rep 2009;11:329-33.
107. Bloedon LT, Dunbar R, Duffy D, et al. Safety, pharmacokinetics, and pharmacodynamics of oral apoA-I mimetic peptide D-4F in high-risk cardiovascular patients. J Lipid Res 2008;49:1344-52.
108. Nicholls SJ, Tuzcu EM, Brennan DM, Tardif JC, Nissen SE. Cholesteryl ester transfer protein inhibition, high-density lipoprotein raising, and progression of coronary atherosclerosis: insights from ILLUSTRATE (Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation). Circulation 2008;118:2506-14.
109. Taylor AJ, Villines TC, Stanek EJ, et al. Extended-release niacin or ezetimibe and carotid intima-media thickness. N Engl J Med 2009;361:2113-22.
110. Canner PL, Berge KG, Wenger NK, et al. Fifteen year mortality in Coronary Drug Project patients: long-term benefit with niacin. J Am Coll Cardiol 1986;8:1245-55.
111. Brown BG, Zhao XQ, Chait A, et al. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. N Engl J Med 2001;345:1583-92.
112. Taylor AJ, Sullenberger LE, Lee HJ, Lee JK, Grace KA. Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) 2: a double-blind, placebo-controlled study of extended-release niacin on atherosclerosis progression in secondary prevention patients treated with statins. Circulation 2004;110:3512-7.
113. Lee JM, Robson MD, Yu LM, et al. Effects of high-dose modified-release nicotinic acid on atherosclerosis and vascular function: a randomized, placebo-controlled, magnetic resonance imaging study. J Am Coll Cardiol 2009;54:1787-94.
114. Kamanna VS, Kashyap ML. Mechanism of action of niacin. Am J Cardiol 2008;101:20B-6B.
115. Rubic T, Trottmann M, Lorenz RL. Stimulation of CD36 and the key effector of reverse cholesterol transport ATP-binding cassette A1 in monocytoid cells by niacin. Biochem Pharmacol 2004;67:411-9.
116. Yvan-Charvet L, Kling J, Pagler T, et al. Cholesterol efflux potential and antiinflammatory properties of high-density lipoprotein after treatment with niacin or anacetrapib. Arterioscler Thromb Vasc Biol 2010;30:1430-8.
117. Wu BJ, Yan L, Charlton F, Witting P, Barter PJ, Rye KA. Evidence that niacin inhibits acute vascular inflammation and improves endothelial dysfunction independent of changes in plasma lipids. Arterioscler Thromb Vasc Biol 2010;30:968-75.