Numerous prospective epidemiological studies have shown a strong inverse relationship between HDL cholesterol (HDL-C) levels and coronary heart disease (CHD). Many controlled clinical trials also demonstrate that treating patients who have low HDL-C with various lipid-modifying therapies, including statins, fibrates, niacin, or combination therapy, can reduce major coronary events. The National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III guidelines recognize low HDL-C (<40 mg/dL) as an independent major risk factor for CHD, as a component of the metabolic syndrome, and as a potential target for therapeutic intervention. However, the primary target of the NCEP ATP III guidelines is to lower LDL cholesterol (LDL-C), given the strong and abundant clinical trial data for both primary and secondary prevention of adverse coronary events by lowering LDL-C. The recent update to the ATP III guidelines recommends even more aggressive treatment of LDL-C given new clinical trial data. However, even in patients treated to aggressive LDL-C goals, coronary events still occur, and low HDL-C is a major risk factor in this group. Therefore, a natural next step in the search for therapies to further reduce cardiovascular morbidity and mortality leads to raising HDL-C levels and/or improving HDL function. An important concept with regard to HDL-based therapies is that the plasma HDL-C level per se may not reflect the functionality of HDL or the impact of a specific HDL-targeted therapy on atherosclerosis or cardiovascular risk. In this review we focus on the concepts underlying the therapeutic targeting of HDL metabolism and the specific areas and compounds in clinical and preclinical development.

Current Approaches to Patients With Low HDL-C

Therapeutic lifestyle changes are currently recommended as first-line therapy in patients with low HDL-C levels. Smoking depresses HDL-C levels, and cessation is associated with modest increases in HDL-C. Aerobic exercise can raise HDL-C levels, but in the absence of weight loss its effects on HDL are generally very modest. There is a strong correlation between visceral adiposity and low HDL-C, the mechanisms of which are not fully understood. Weight loss can be associated with moderate to occasionally substantial increases in HDL-C levels. The impact of dietary composition itself on HDL metabolism is complex. High-saturated-fat diets raise HDL-C levels, high-polyunsaturated-fat diets reduce HDL-C levels, and monounsaturated fats tend to be neutral with regard to HDL-C. Finally, alcohol use has substantial dose-dependent effects in raising HDL-C levels but is not generally or routinely recommended as an HDL-raising strategy.

Currently available lipid-modifying drugs have generally modest effects on HDL-C levels. Statins are first-line drug therapy for treatment of elevated LDL-C as well as for most high-risk patients with low HDL-C but raise HDL-C by only 5% to 10%. Whether the modest HDL-raising effect of statins contributes to their cardiovascular benefits is unknown. In fact, the Cholesterol Treatment Trialist Collaborators’ recent meta-analysis of 14 statin trials showed no difference in benefit from statin therapy by HDL-C subgroup. Fibrates, agonists of peroxisome proliferator-activated receptor (PPAR)-α, raise HDL-C by 5% to 20%, the magnitude dependent to an extent on the baseline level of triglycerides. Several clinical trials with fibrates in both primary and secondary prevention have shown significant reductions in major CHD events, particularly in subgroups with elevated triglycerides and/or insulin resistance. However, other trials in high-risk patients have shown no reduction in CHD events. Whether the HDL-raising effect of fibrates contributes to their cardiovascular benefits remains unknown.

Nicotinic acid (niacin) is the most effective HDL-raising drug currently available, with increases of 15% to 35%. Although limited clinical trial data are available, the Coronary Drug Project suggested that niacin resulted in reduced coronary events. The update to the ATP III guidelines and a position paper from a European consensus panel note that fibrates and niacin may have a role in the treatment of high-risk patients with low HDL-C once the LDL-C and non–HDL-C goals are met. Thiazolidinediones, agonists of PPAR-γ, are used for the treatment of type 2 diabetes; they also have a modest effect in increasing HDL-C by 5% to 15% in diabetics as well as in nondiabetics. The recently presented Prospective Pioglitazone Clinical Trial in Macro-
An important concept is that simply raising HDL-C levels may not necessarily be the optimal target for the development of new therapies targeted toward HDL. Plasma steady state HDL-C levels are not an assay of the rate of RCT, which is a dynamic process that can only be assessed through kinetic measures of cholesterol flux.30 Thus some new therapeutic approaches are targeted toward promoting RCT, even if they do not raise HDL-C levels per se. It could be that the function of HDL is more important than its concentration and that therapies that improve HDL “function,” even if they do not increase HDL-C levels, may have important antiatherogenic and vascular protective effects.34 Advances in the area of HDL-related biomarkers that will allow the assessment of the rate of RCT and other measures of HDL function are extraordinarily important to the development of new HDL-targeted therapies. There is a critical need to determine whether a new therapy targeted toward HDL improves RCT and/or HDL function in preclinical models and early in clinical development before the performance of large atherosclerosis imaging and cardiovascular outcome trials.

Thus, HDL metabolism and RCT are complex and pose a much more complicated target for new therapeutic development than, for example, reduction in LDL-C levels. Below, we outline several categories in which new therapies targeted toward HDL and RCT are being developed. Some of these therapies require parenteral administration and thus are being developed primarily for acute and short-term use, whereas others are orally administered and are being developed for chronic use.

**Apolipoprotein A-I–Directed Therapies**

Apolipoprotein A-I (apoA-I) is the main protein in HDL, and is present on virtually all HDL particles.30 Lipid-poor apoA-I is an important acceptor of macrophage cholesterol efflux mediated by the cellular transporter adenosine triphosphate–binding cassette protein A1 (ABCA1) (Figure 2).35 ApoA-I may also be critical to other aspects of HDL function such as its antioxidant and antiinflammatory effects.36 ApoA-I levels are an independent risk factor for CHD, and some studies suggest that there is an even stronger inverse relationship between apoA-I and CHD than between HDL-C and CHD.37 Deficiency of apoA-I in atherosclerosis-prone mice results in significantly more atherosclerosis despite preserved levels of HDL-C.38 Transgenic overexpression, somatic gene transfer, and intravenous infusions of apoA-I substantially reduce...
progression and even induce regression in both rabbits and mice. Thus, upregulation of endogenous apoA-I expression is widely considered the most desirable approach to target HDL therapeutically. Despite substantial effort, however, it has been difficult to identify small-molecule upregulators of apoA-I transcription.

Given the abundance of animal data supporting apoA-I overexpression and the lack of small molecules that upregulate apoA-I expression, alternative approaches to targeting apoA-I have been developed. Some studies have assessed the acute effects of bolus intravenous infusion of apoA-I in humans. These studies demonstrate relatively little increase in HDL-C levels but measurable increases in apoA-I and phospholipids. Intriguingly, a study in 4 subjects with heterozygous familial hypercholesterolemia infused with a single bolus of proapoA-I demonstrated a 39% increase in fecal sterol excretion, suggesting promotion of RCT. This evidence remains the best to date that apoA-I infusion may promote RCT in humans. In theory, repeated infusions of apoA-I might be expected to be beneficial with regard to atherosclerosis in humans, but in part because there is no intellectual property position around “normal” apoA-I, this approach has never been developed.

In contrast, there is substantial interest in genetic variants of apoA-I as potential therapeutic approaches. ApoA-I Milano is one of several rare, naturally occurring point mutations in apoA-I that cause low HDL-C levels but do not necessarily increase cardiovascular risk. The apoA-I Milano mutation was discovered >3 decades ago in a small number of individuals in a town in northern Italy. It is believed by some to confer a gain of function resulting in increased antiatherogenic effects, although few comparisons to wild-type apoA-I have been performed. Studies in animals have shown that apoA-I Milano expression is associated with reduced atherosclerosis. Importantly, in contrast to normal apoA-I, there is an intellectual property position around “normal” apoA-I, this approach has never been developed.

Parenterally administered apoA-I–related therapies are in early clinical development, and ultimately studies of atherosclerosis and hard clinical end points will be required. Given that parenteral administration presents a logistical barrier to long-term administration, the focus of this approach has been on potential clinical use as “acute induction therapy” for rapid plaque regression or stabilization in patients with acute coronary syndromes (ACS). This is conceptually attractive because ACS patients have a high rate of recurrent events. However, it presents considerable challenges in the clinical development of such therapies because not only dose but also frequency and duration of administration are variables that must be addressed in the design of expensive atherosclerosis imaging and cardiovascular outcome studies. Nevertheless, should any such therapies ultimately be shown to be effective and thus be approved, apoA-I “induction therapy” would likely be given to ACS patients initially in the hospital, with subsequent infusions as outpatients in the cardiologist’s office.

Clearly the “holy grail” of HDL-based therapies is the discovery of small molecules that turn on expression of the apoA-I gene, resulting in increased apoA-I production. Everything we know suggests that this antiatherogenic strategy should be effective. Interestingly, fibrates (PPAR-agonists) have been shown to upregulate apo-A-I expression in a transgenic mice and increase apoA-I production rates in humans. Thus, fibrates may be weak inducers of apoA-I transcription in humans. New more potent PPAR-agonists are reportedly in clinical development and could provide more powerful approaches to apoA-I upregulation. There have been major attempts to discover novel small molecules that specifically upregulate apoA-I, but to our knowledge
there are no such molecules in clinical development at this time; the entry of such molecules into clinical development in the future would create major excitement in the field. However, one oral apoA-I–based therapy in clinical development is an oral apoA-I mimetic peptide called D-4F. D-4F is an 18–amino acid peptide almost identical in sequence to the aforementioned L-5F but differs in that it is has 1 less phenylalanine on the hydrophobic face and is composed of all D-amino acids. As a result, it is not recognized by gut peptidases, as are naturally occurring proteins containing L-amino acids, and is therefore not degraded in the gut and is orally bioavailable. Oral administration of D-4F has been shown to dramatically reduce atherosclerosis in mice without raising levels of HDL-C.52 Although its mechanism remains uncertain, some data suggest that its major effect may be to enhance the antiinflammatory function of HDL.53 D-4F has also been shown to promote macrophage RCT in vivo in mice.53 Results from early clinical trials of D-4F are eagerly anticipated given that D-4F may provide a fascinating test of the hypothesis that enhancing HDL function without increasing plasma HDL-C levels can reduce atherosclerosis or cardiovascular risk.

Therapies Directed to Promotion of Macrophage Cholesterol Efflux and RCT

The theory that RCT protects against atherogenesis is conceptually appealing.30 Although RCT is difficult to measure in vivo, studies in mice have confirmed that apoA-I overexpression promotes macrophage RCT.54 There have been major advances in our understanding of the molecular regulation of RCT over the last decade.50 A major area in HDL-based therapeutics is the exploitation of this information to develop pharmacological approaches to enhance components of the macrophage RCT pathway. The area of the most intense focus has been the first step of RCT, namely, efflux of cholesterol from the macrophage. Indeed, the macrophage “foam cell” has been widely considered to be a target for therapeutic intervention.55

As mentioned above, the best-established pathway for macrophage cholesterol efflux involves the ABCA1 transporter promoting efflux to lipid-poor apoA-I (Figure 2).56 Interest in this pathway as a therapeutic target was spurred by the seminal discovery that mutations in ABCA1 are the cause of Tangier disease, which is characterized by extremely low plasma HDL-C and apoA-I levels and massive accumulation of cholesterol in macrophages.57 Overexpression of ABCA1 in mice leads to significant increases in HDL-C and apoA-I levels58 and is associated with a significant reduction in aortic lesion area compared with control mice.59 Subsequent studies established that the major regulator of ABCA1 gene expression is the nuclear receptor liver X receptor (LXR) with its heterodimer partner retinoid X receptor (RXR).60 More recently, ABCG1 was identified as a transporter that promotes macrophage cholesterol efflux to mature HDL (Figure 2);ABCG1 is also regulated by LXR.62,63

Synthetic LXR agonists upregulate macrophage ABCA1 and ABCG1 and increase cholesterol efflux to both lipid-poor apoA-I and mature HDL in vitro.64 An LXR agonist was recently shown to promote macrophage RCT in vivo in mice.65 LXR agonists have also been shown to reduce atherosclerosis in mice.64,66 Thus, LXR agonists are conceptually attractive as an approach to promoting RCT and reducing atherosclerosis. However, some nonselective LXR agonists induce hepatic expression of SREBP1c, which in turn induces expression of fatty acid synthetic genes, resulting in increased hepatic triglyceride synthesis, hepatic steatosis, and hypertriglyceridemia.67 In addition, LXR agonists have been shown to increase LDL-C levels in animal models, such as hamsters and nonhuman primates, that express cholesteryl ester transfer protein (CETP).68 These adverse effects have slowed the progression of LXR agonists into clinical development. It would be desirable to develop LXR modulators that are relatively selective for specific cells or tissues (ie, macrophages over liver) or for specific genes (ie, ABCA1/G1 over SREBP1c). One LXR agonist has been reported to be partially selective and to induce less hepatic steatosis;69 however, this same compound elevated LDL-C as above.68 Alternatively, selective modulation of LXR-β but not LXR-α (which is more abundant in liver) would be a theoretically desirable approach, but the discovery of β-selective LXR modulators has been challenging. There remains hope that LXR agonists or modulators will be developed that have the appropriate pharmacological and toxicological profile to allow testing in humans.

As noted above, fibrates are agonists of the nuclear receptor PPAR-α and have been shown to increase HDL-C levels and reduce cardiovascular events.51 Importantly, macrophages express PPAR-α, and treatment of macrophages in vitro with fibrates has been shown to upregulate ABCA1 and increase cholesterol efflux,51 possibly via upregulation of LXR itself. Coadministration of an LXR agonist and a PPAR-α agonist in mice resulted in a synergistic elevation in HDL-C levels.70 Additionally, administration of a potent synthetic PPAR-α agonist to mice reduced atherosclerosis and inhibited foam cell formation in an ABCA1-independent but LXR-dependent manner.71 However, no direct data exist yet that PPAR-α agonists directly promote RCT in vivo. Nevertheless, the concept that fibrates may reduce atherosclerosis and cardiovascular risk by promoting macrophage cholesterol efflux and RCT is provocative and clinically important. Fibrates are relatively weak PPAR-α agonists, and new more potent PPAR-α agonists are reportedly in clinical development. The effects of these compounds on HDL metabolism and RCT need to be investigated in animals and humans. If more potent PPAR-α agonists are ultimately approved and advance to market, they could potentially provide an important new approach to promoting RCT and reducing atherosclerosis.

Macrophages also express PPAR-γ, and thiazolidinediones, agonists of PPAR-γ used for the treatment of type 2 diabetes, have also been shown to promote macrophage cholesterol efflux in vitro, potentially also through upregulation of LXR, ABCA1, and ABCG1 expression.71–74 Given that thiazolidinediones are known to increase HDL-C levels and reduce atherosclerosis progression and also have recently been shown, at least for pioglitazone, to potentially reduce cardiovascular events in type 2 diabetics,29 it is tempting to speculate that thiazolidinediones may promote RCT as well.
Administration of a PPAR-γ agonist to mice reduced atherosclerosis and inhibited foam cell formation in an LXR- and ABCA1-independent manner but upregulated ABCG1.71 PPAR-γ agonists have not yet been shown to directly promote RCT in vivo. The discovery that both PPAR-α and PPAR-γ agonists promote macrophage cholesterol efflux makes their combined use, as well as the potential introduction of “dual PPAR agonists” that have activity at both PPAR-α and PPAR-γ, particularly interesting from the point of view of RCT and atherosclerosis. Muraglitazar modestly increases HDL-C levels and was the first of such compounds to be presented to a Food and Drug Administration Advisory Panel for approval. However, a subsequent analysis of the data suggested an increased incidence of adverse cardiovascular events.73 Thus, the future of muraglitazar is uncertain at present. Other dual PPAR agonists, such as tesaglitazar, also raise HDL-C levels76 and are still in clinical development.

Finally, macrophages express a third PPAR, namely, PPAR-β/δ. Relatively little is known about the function of this nuclear receptor in macrophages. One study demonstrated that a synthetic PPAR-β/δ agonist raised HDL-C levels by 80% in a rhesus monkey model of the metabolic syndrome.77 In vitro studies suggested that the PPAR-β/δ agonist promoted macrophage cholesterol efflux, but this has not been corroborated and remains uncertain. Studies are needed to formally test whether PPAR-β/δ agonists are capable of promoting RCT in vivo. There has also been the suggestion that macrophage PPAR-β/δ modulates the inflammatory response.78,79 PPAR-β/δ agonists are in clinical development, and early human studies suggest relatively modest effects on HDL-C levels.80 It will be of substantial interest to follow the clinical development of PPAR-β/δ agonists and to determine whether they have any role in modulation of plasma lipids or prevention of atherosclerotic disease.

The PPAR agonist field as a whole will likely continue to evolve, with compounds that have varying degrees of specificity for all 3 of the PPARs. It will be important to establish whether some combination of PPAR agonism or selective modulation will be an effective approach to promoting macrophage cholesterol efflux and RCT and thus ultimately reducing atherogenesis. It is important to remember that PPAR agonists have antiinflammatory effects as well that could contribute to antiatherogenic effects.79 Additional approaches to pharmacological promotion of macrophage RCT, for example, by directly targeting the transporters themselves, are the subject of discovery efforts and may eventually add to the armamentarium of potential ways to promote RCT.

A final strategy to improve RCT is through intravenous infusion of large unilamellar liposomes (LUVs) consisting solely of phospholipids. In animals, infusions of LUVs appear to promote “cholesterol mobilization” based on increases in plasma levels of free cholesterol,81 although the mechanisms of the cholesterol mobilization remain unknown. Studies of LUVs in rabbits have demonstrated regression in atherosclerosis.82 There are no published data to date with regard to human studies of LUV infusions. LUVs represent yet another parenteral approach that would presumably be focused on ACS patients for short-term therapy; it will be interesting to see how this approach evolves compared with infusion of apoA-I Milano/phospholipid complexes and apoA-I mimetic peptides.

Therapies Intended to Alter HDL and ApoA-I Metabolism to Raise Their Levels in Plasma
From a kinetic standpoint, plasma levels of HDL-C and apoA-I are determined by the combination of the rates of their production and the rates of their catabolism or removal from the blood.89 In the first section we addressed the desirability of promoting apoA-I production and the status of apoA-I–based therapeutic approaches. In the second section we addressed the desirability of promoting cholesterol efflux, essentially the source of HDL cholesterol, and the status of approaches geared toward promotion of efflux and RCT. In this section we address the other side of the metabolic picture, namely, the concept that slowing the removal of HDL cholesterol and apoA-I from the blood would be expected to increase their plasma levels. In humans, the rate of HDL and apoA-I catabolism is the most important factor in determining the variation in HDL-C and apoA-I levels.80 Thus, attempting to slow the catabolic rate to increase plasma concentrations seems in many ways like a logical approach. As we have mentioned, however, the flux of cholesterol through the RCT pathway is believed to be an important aspect of the protective function of HDL. Thus, this approach, though in some ways the most amenable to pharmacological manipulation, is also the most uncertain with regard to benefit in terms of atherosclerosis. One approach in this category, niacin therapy, is already an established clinical therapy, and the discovery of the niacin receptor has spurred tremendous new interest in this area. Another approach, CETP inhibition, is already in advanced phase III trials. There are several other possible approaches to this area that could be taken as well.

As noted above, niacin has been used for decades as a cholesterol-lowering drug and is also the best HDL-raising drug currently available. The mechanism by which niacin raises HDL-C levels is the topic of continued investigation. In vivo studies in humans suggest that the catabolic rate of apoA-I is slowed by niacin therapy.83 Studies in hepatocytes in vitro have shown reduced uptake of HDL apoA-I after niacin treatment.84 The paradigm has been that in addition to indirect effects on HDL metabolism through reduction in triglycerides, niacin acts on an unknown pathway in the liver to slow HDL and apoA-I catabolism. The recent discovery of a niacin receptor, a G protein–coupled receptor known as HM74A (also known as GPR109A),85 has fueled tremendous new interest in the mechanisms of niacin action and the development of new compounds that act on this receptor. The niacin receptor is primarily expressed in adipocytes, and its activation by niacin results in inhibition of hormone-sensitive lipase, reduced triglyceride hydrolysis, and reduced flux of free fatty acids from adipose to liver,86 which is thought to account for the triglyceride-lowering effects of niacin. Although the niacin receptor is also expressed in other cell types, hepatocytes do not appear to be a major site of expression. Thus, linking this receptor to the mechanisms by which niacin raises HDL-C levels has been difficult, and considerably more work is required on this important topic. Intriguingly, activated macrophages express the niacin recep-
tor, and niacin treatment of macrophages upregulates ABCA1 expression. It seems likely that new compounds targeting the niacin receptor will be discovered, and if they enter into clinical development it will be fascinating to see whether they raise HDL-C and reduce atherosclerotic cardiovascular disease to the extent that niacin itself does.

CETP is a plasma glycoprotein that mediates transfer of cholesteryl esters from HDL to apoB-containing lipoproteins in exchange for triglycerides (Figure 1). The importance of CETP in HDL metabolism in humans was established by the discovery in 1989 of Japanese individuals with extremely high HDL-C levels who were genetically deficient in CETP. This discovery led naturally to the concept that pharmacological inhibition of CETP might be a novel approach to raise HDL-C levels. More than a decade later, CETP inhibitors are in advanced stages of clinical development. One CETP inhibitor, torcetrapib, was shown in a phase I study to increase HDL-C levels in a dose-dependent fashion, with a 16% increase in HDL-C at the 10-mg dose and up to 91% increase at the 120-mg twice daily dose. In a fixed sequence crossover trial in subjects with low baseline HDL-C levels designed to study the metabolic effects of CETP inhibition, torcetrapib was studied either as monotherapy or when added to stable atorvastatin therapy. Torcetrapib 120 mg daily, HDL-C concentrations increased by 46% in the monotherapy group and by 61% in the combination treatment group; torcetrapib 120 mg twice daily as monotherapy increased HDL-C by 106%. ApoA-I levels also increased, although to a lesser extent than HDL-C levels, and this increase was demonstrated to be due to slower catabolism of apoA-I. LDL-C and apoB levels were also reduced by torcetrapib treatment. In larger studies, also in patients with low HDL-C, 8 weeks of treatment with torcetrapib again resulted in substantial, dose-dependent increases in HDL-C in patients randomized to torcetrapib monotherapy as well as in those randomized to the combination of torcetrapib and a stable dose of atorvastatin. Similar results were also seen in another, larger, phase II dose-ranging trial in patients meeting NCEP ATP III criteria for the treatment of elevated LDL-C. This trial evaluated the lipoprotein effects of the combination of 60 mg of torcetrapib in combination with doses of atorvastatin ranging from 10 to 80 mg and demonstrated significant increases in HDL-C levels of 52% to 65% from baseline levels along with dose-dependent significant reductions in LDL-C. Torcetrapib is now being studied in phase III at the 60-mg daily dose in combination with atorvastatin (compared with atorvastatin alone) in atherosclerosis imaging trials and a large cardiovascular outcomes trial. The question of whether CETP inhibition with torcetrapib when added to atorvastatin will slow progression (or induce regression) of atherosclerosis and reduce cardiovascular events compared with atorvastatin alone is one of the most important questions in the entire lipid/atherosclerosis field to be answered over the next several years.

A second CETP inhibitor, JTT-705, is also in clinical development. In healthy subjects with mild hyperlipidemia, a randomized, double-blind, placebo-controlled trial demonstrated that treatment with JTT-705 alone for 4 weeks at the highest dose, 900 mg, led to a 37% decrease in CETP activity and a 34% increase in HDL-C. Another trial showed that 600 mg of JTT-705 in combination with pravastatin 40 mg for 4 weeks in a dyslipidemic population yielded a 28% increase in HDL-C levels. JTT-705 continues in clinical development but is not as advanced as torcetrapib. Given the attractiveness of CETP inhibition as an HDL-raising strategy, it would not be a surprise if other small-molecule CETP inhibitors enter into clinical development. Finally, a novel strategy of using a CETP-based peptide for immunization to raise inhibiting autoantibodies against CETP is in clinical development. In a phase I trial of 36 subjects, 1 subject who received a single injection of the CETP peptide at the highest dose developed anti-CETP antibodies, and 8 subjects who received a second injection of the active vaccine developed anti-CETP antibodies, although there were no increases in HDL-C levels. This is an interesting approach that has the uncertain advantage of avoiding the need to take a daily pill.

Despite the fact that CETP inhibition clearly increases HDL-C levels in humans, there remains controversy about whether it is likely to be effective in reducing atherosclerosis progression and cardiovascular risk. The arguments that have been made against CETP inhibition include issues related to vascular disease in CETP-deficient patients, inconsistent animal (particularly mouse) studies, and especially the concept that CETP inhibition might impairment RCT. Space does not permit a full discussion of these issues here. With regard to the CETP-deficient patients, the relatively small number of homozygotes has not been studied with sufficient rigor and compared with appropriate controls to make adequate conclusions about their cardiovascular risk. Heterozygotes, who only have a modest increase in HDL-C levels, do not appear to be at increased risk and may have a modest reduction in risk. Furthermore, large prospective observational studies of CETP mass indicate a positive relationship to cardiovascular risk, suggesting that CETP is a risk factor. With regard to mice, they naturally lack CETP, and therefore studies in mice require the introduction of the CETP gene, making it difficult to extrapolate any results to human physiology. In contrast, rabbits have abundant CETP, and studies of CETP inhibition in rabbits have been consistently positive demonstrating reduction in atherosclerosis.

The issue that is most frequently raised is that of the potential effect of CETP inhibition on RCT. There is a school of thought that CETP-mediated transfer of cholesteryl ester from HDL to apoB-containing lipoproteins with subsequent clearance by the liver is a major pathway of RCT. This belief was enhanced by the report from Schwartz and colleagues that in healthy volunteers the majority of radiolabeled HDL cholesteryl ester that appeared in bile had been transferred first to apoB-containing lipoproteins. Because macrophage-to-bile (or -feces) RCT cannot be measured effectively in humans at present, there is no way to directly test whether CETP inhibition slows, has no effect, or even possibly promotes RCT. A small study found that torcetrapib had no significant effect on fecal neutral sterol excretion but was not powered to see a difference, and fecal sterol excretion is a crude measure of RCT at best and less useful in the steady
state setting. However, the hope is that by increasing the pool of mature HDL through CETP inhibition, the efflux of macrophage cholesterol via the ABCG1 and potentially scavenger receptor class B1 (SR-B1) pathways (Figure 2) will be increased. Finally, if the non-RCT properties of HDL such as inhibition of oxidative stress and inflammation contribute to its benefit, then raising HDL-C levels might be expected to be beneficial through these mechanisms. Ultimately, the studies of the effects of CETP inhibitors on atherosclerosis progression and clinical events should tell the real story underlying this fascinating debate.

As mentioned above, low levels of HDL-C and apoA-I are usually accompanied by increased catabolism of apoA-I. The kidney appears to be an important site of apoA-I catabolism (Figure 1), probably after glomerular filtration of lipid-poor apoA-I and subsequent receptor-mediated catabolism by the renal tubular cells. Thus, the rate at which HDL is “remodeled,” resulting in generation of lipid-poor apoA-I, is probably an important determinant of apoA-I catabolism and thus apoA-I and HDL-C levels. Therefore, HDL remodelling may be an important target of new HDL-based therapies (Figure 1). CETP and its cousin, the phospholipid transfer protein (PLTP), play a role in HDL remodelling. Lipases also play an important role in this process. Hepatic lipase (HL) has been the most studied, and indeed genetic deficiency of HL is associated with modestly elevated HDL-C levels in mice and humans. However, because HL also acts to hydrolyze lipids in apoB-containing lipoproteins, it has not been considered a major target for the development of inhibitors. However, a relative of HL called endothelial lipase (EL) has been more recently described. EL appears to have more specificity than HL for phospholipids and for HDL in vivo; overexpression of EL in mice results in reduction in HDL-C and apoA-I due to increased catabolism, and loss of function of EL in mice results in significant increases in HDL-C levels. In addition, the EL knockout mouse has reduced atherosclerosis on an apoE knockout background. Genetic studies in humans have suggested a possible association of EL gene variants with high HDL-C levels, and recent studies in humans indicate that plasma levels of EL mass are inversely associated with HDL-C levels and positively associated with coronary calcification. Thus, EL is an intriguing and potentially attractive new target for the development of inhibitors as a novel way to inhibit catabolism of apoA-I, raise apoA-I and HDL-C levels, and potentially reduce atherosclerosis risk.

EL modulates HDL metabolism through its ability to hydrolyze HDL phospholipids. Other phospholipases in plasma may also influence HDL metabolism. For example, the secretory phospholipase A2 group IIA (sPLA2-IIA) is upregulated by inflammation (as is EL) and has been shown to have the ability to hydrolyze HDL phospholipids and increase its catabolism. sPLA2-IIA and potentially other members of the large sPLA2 family are additional interesting targets for the development of inhibitors that might raise HDL-C levels and reduce atherosclerosis. Finally, HDL metabolism might be able to be modulated by administration of phospholipids themselves. Phosphatidyllynositol is a minor phospholipid species in HDL that modifies HDL charge and potentially HDL metabolism. In a small clinical trial, 2 weeks of treatment with oral phosphatidylinositol resulted in a significant increase of 13% to 18% in HDL-C levels. Another group reported that oral administration of the phospholipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine to apoE-null mice led to an increase in HDL-C levels and HDL function and a reduction in atherosclerosis. One appeal of phospholipids as therapeutic agents is that they are natural substances and would be expected to have a good safety profile. It will be interesting to see whether oral administration of specific types of phospholipids advances in clinical development over the next several years.

Summary

Low levels of HDL-C are a major independent risk factor for atherosclerotic cardiovascular disease and events, even in patients who are aggressively treated for LDL-C reduction. Although existing drugs have modest effects on HDL-C levels, this area remains a major unmet medical need in cardiovascular medicine. HDL metabolism is exceedingly complex, and because the protective ability of HDL may relate to the flux of cholesterol through the RCT pathway and to other aspects of HDL functionality, the plasma level of HDL-C alone is almost certainly not an adequate predictor of the potential clinical benefit of an HDL-targeted therapy. Some interventions that raise HDL-C may not reduce atherosclerosis or cardiovascular events; conversely, other interventions may not raise HDL-C but through effects on RCT or HDL function may have major effects on atherosclerosis or cardiovascular events. There is a great need for the development of novel biomarkers and kinetic methods to assess the effects of novel interventions on RCT and HDL function. Despite the challenges, a large number of HDL-targeted therapies are in various stages of preclinical and clinical development. Because CETP inhibition is the furthest along, it will provide the first information about whether a therapy specifically targeted to raising HDL-C levels will reduce atherosclerosis and cardiovascular events. This issue is one of the most critical questions for the field of HDL and atherosclerosis in the next couple of years.

Many additional important questions in the area of HDL therapeutics will be addressed in the next several years. Will parenteral administration of apoA-I Milano, apoA-I mimetic peptides, or large unilamellar vesicles in ACS patients regress atherosclerosis and/or reduce cardiovascular events, thus supporting the concept of “acute induction therapy” for atherosclerosis? Do PPAR agonists actually promote macrophage cholesterol efflux and RCT in vivo, and what is the optimal “mix” of PPAR-modulating activities for effects on RCT and atherosclerosis? Will LXR agonists or modulators enter into clinical development, and, if so, will they have the same effects on hepatic lipogenesis and RCT as in preclinical models? Will we see new compounds specifically targeted to the niacin receptor, and will they increase HDL-C levels in humans? Will EL-specific inhibitors be discovered and enter into clinical development? Will phospholipid-based therapies advance in clinical development? There will undoubtedly be further advances in drug development as more is discovered through basic and translational research with regard to the
multiple mechanisms of HDL action and the effects of altering HDL metabolism. Much as the last 2 decades have seen a revolution in our ability to reduce LDL-C and thus cardiovascular events, the next 2 decades are likely to witness major advances in the development of novel therapeutics targeted to HDL metabolism and RCT with the goal of inhibition and even regression of atherosclerosis and a further substantial reduction in cardiovascular events.

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References


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