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High-Density Lipoprotein Function, Dysfunction, and Reverse Cholesterol Transport

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Abstract—Although high high-density lipoprotein (HDL)-cholesterol levels are associated with decreased cardiovascular risk in epidemiological studies, recent genetic and pharmacological findings have raised doubts about the beneficial effects of HDL. Raising HDL levels in animal models by infusion or overexpression of apolipoprotein A-I has shown clear vascular improvements, such as delayed atherosclerotic lesion progression and accelerated lesion regression, along with increased reverse cholesterol transport. Inflammation and other factors, such as myeloperoxidase-mediated oxidation, can impair HDL production and HDL function, with regard to its reverse cholesterol transport, antioxidant, and anti-inflammatory activities. Thus, tests of HDL function, which have not yet been developed as routine diagnostic assays, may prove useful and be a better predictor of cardiovascular risk than HDL-cholesterol levels. **(*Arterioscler Thromb Vasc Biol*. 2012;32:2813-2820.)**

Key Words: apolipoprotein A-I ■ atherosclerosis ■ plaque regression ■ macrophage foam cell

Human high-density lipoprotein (HDL) is a heterogeneous collection of lipoprotein particles with a density between 1.063 and 1.21 g/mL. When human HDL is run on a size exclusion column or nondenaturing gradient gels, it is evident that HDL is polydisperse with several discrete particle sizes evident. Ultracentrifugation can separate 2 major density subfractions, HDL_2 (density between 1.063 and 1.125 g/mL) and HDL_3 (density between 1.125 and 121 g/mL). The proteomics of HDL is very complex,[1] but the overwhelming majority of HDL particles contain apolipoprotein A-I (apoAI), which is the most abundant apolipoprotein in normal human plasma. Many HDL particles also contain apoAII, the second most abundant protein in HDL, and those that do carry apoAII can be separated by immunoisolation. Many of the less abundant proteins associated with HDL are found on only a small fraction of HDL particles, increasing the diversity of HDL particles. A useful way to get a snapshot of the diversity of apoAI-containing particles is through 2-dimensional nondenaturing gel electrophoresis, followed by blotting and staining with an antibody against human apoAI, which yields a complex pattern of pre-β, α, and pre-α particles of various sizes. Generally speaking, the pre-β migrating particles represent small lipid-free and lipid-poor apoAI, whereas the α1, 2, and 3 particles represent spherical HDL of decreasing sizes.[2]

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The metabolism of HDL initiates with apoAI synthesis in the liver and intestine, but to form HDL, apoAI must interact with cells expressing ABCA1, the gene defective in Tangier disease. Mouse models of tissue-specific ABCA1 deficiency demonstrate that hepatic ABCA1 plays the largest role in generating HDL, but that nonhepatic tissues also play significant roles in HDL formation.[3] Nascent HDL released by ABCA1-expressing cells contains cellular phospholipids and free cholesterol, and this particle is the substrate for lethicin:cholesterol acyltransferase which esterifies free cholesterol into cholesteryl ester, building up the hydrophobic core necessary to generate spherical α HDL particles. Furthermore, HDL remodeling by plasma and cell surface enzymes is complex and includes processes mediated by ABCG1, hepatic lipase, endothelial lipase, cholesterol ester transfer protein, and phospholipid transfer protein. In humans, HDL-cholesterol (HDL-C) can be returned to the liver via 2 pathways: direct hepatic uptake by scavenger receptor B1 (SR-B1); or through cholesterol ester transfer protein exchange of HDL-cholesteryl ester for triglycerides in apoB-containing lipoproteins, followed by hepatic uptake of these apoB-containing particles by the low-density lipoprotein (LDL) receptor.

HDL-C is commonly known as the good cholesterol because high levels of HDL-C are associated with reduced levels of cardiovascular disease (CVD), and low levels of HDL-C are associated with increased CVD, in multiple epidemiological studies. The concept that HDL-C is protective against incident coronary heart disease for subjects in different strata of LDL-cholesterol levels was first demonstrated in the Framingham

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study in the 1970s and 1980s.[4,5] In addition, the incidence of low HDL-C (<35 mg/dL) was ≈3-fold higher among men with premature (<60 years of age) coronary heart disease than in age-matched controls.[6] However, recent human studies have cast some doubt on the good cholesterol HDL hypothesis. A genetic method called mendelian randomization found that a score derived from 14 common genetic variants that is associated with HDL-C levels, and not other lipoprotein traits, is not associated with myocardial infarction.[7] Furthermore, 2 recent drug trials, one of the cholesterol ester transfer protein inhibitor torcetrapib, and the other of extended-release niacin, did not show beneficial cardiovascular outcomes despite increased HDL-C levels.[8,9] However, torcetrapib had off-target effects raising blood pressure, and the niacin trial had many design flaws. Because there is abundant evidence in mouse models (described below) that increased HDL is associated with decreased atherosclerosis progression and accelerated atherosclerosis regression, a new theory has surfaced, that the levels of HDL-C may not be an ideal indicator of coronary heart disease risk, rather HDL function may be a better indicator. This was also made clear in the mouse model of SR-B1 deficiency, in which high levels of plasma HDL-C accumulate, but these mice developed more severe atherosclerosis when bred onto the hyperlipidemic apoE-deficient background.[10] In this review, we will discuss one of the major functions of HDL, its role in promoting reverse cholesterol transport (RCT), along with several mechanisms by which HDL can become dysfunctional, and new clinical evidence linking HDL functionality to cardiovascular outcomes.

RCT and Assays for HDL/ApoAI Function

HDL has been ascribed with many atheroprotective activities, such as its antioxidant, anti-inflammatory, endothelial cell maintenance functions, and its activity in mediating RCT. Although the relative role of the above activities in mediating HDL's protective effect has not been directly compared, it is our opinion that RCT likely plays an important role in its atheroprotective effect, making this topic the focus of our review. The RCT hypothesis, first put forth by Glomset, proposes that HDL acts to accept cholesterol from the periphery, such as arterial wall cells, and deliver it to the liver, where it can be directly excreted into the bile or be metabolized into bile salts before excretion.[11]

HDL function can be measured in several in vitro assays. Fogelman and colleagues pioneered cell-based and cell-free assays to measure the anti-inflammatory and antioxidant activities of HDL.[12,13] LDL added to endothelial cells cocultured with smooth muscle cells undergoes oxidation and induces expression of monocyte chemotactic factors and increases monocyte binding and transmigration. The addition of HDL can impair this response, demonstrating HDL's antioxidant and anti-inflammatory activities. However, HDL from patients undergoing an acute phase reaction did not possess antioxidant activity and did not inhibit monocyte chemotaxis but actually increased it, demonstrating proinflammatory activity.[14] Through these and similar studies, Fogelman's group concluded that HDL can become dysfunctional. Another example of the anti-inflammatory activity of apoAI is its ability to reduce the macrophage response to endotoxin, suppressing the type I interferon response.[15] Several of the HDL accessory proteins, such as paraoxonase and apoL-I, are associated with the antioxidant activity of apoAI.[16]

The activities of HDL relevant to endothelial cell maintenance and inflammation can be assayed by measuring: (1) the ability of HDL to promote NO production by cultured endothelial cells; (2) the protection of cultured endothelial cells from apoptotic stimuli, such as exposure to ultraviolet light; and (3) the ability of HDL to reduce endothelial cell expression of the proinflammatory adhesion protein vascular cell adhesion molecule-1 after treatment with an inflammatory cytokine.[17,18] HDL isolated from type 2 diabetic patients has reduced levels of these endothelial protective activities, indicative of diabetes mellitus inducing HDL dysfunction.[17]

The first step in RCT is the efflux of cellular cholesterol. ApoAI, other exchangeable apolipoproteins, and mimetic peptides that share the amphipathic helical structure of these apolipoproteins can all accept cellular free cholesterol and phospholipids in an ABCA1-dependent fashion.[19,20] Plasma-derived HDL can also accept cholesterol via ABCG1 or SR-B1. However, depending on its method of preparation, plasma HDL may also contain some ABCA1-dependent lipid acceptor activity. There is some evidence to suggest that this may be attributable to lipoprotein remodeling releasing lipid-free apoAI, because HDL_2 or reconstituted HDL made in vitro from apoAI and phosphatidylcholine is virtually devoid of this activity.[21,22] It is relatively simple to measure the activity of HDL, apoAI, or plasma/serum fractions for their function in RCT via their ability to act as acceptors of cholesterol from cholesterol-loaded macrophages or cells with regulated expression of ABCA1, ABCG1, or SR-B1. One prepares [^{3}H or ^{14}C]cholesterol-labeled cells, chases these cells with the specific acceptor, and calculates cholesterol efflux as the percentage of the cellular [^{3}H or ^{14}C]cholesterol that appears in the media. HDL recovered from sepsis patients is reported to have decreased cholesterol-accepting activity, indicating that sepsis is associated with dysfunctional HDL in regard to its role in RCT.[23]

Cholesterol efflux assays are quite useful for examining the first steps in the RCT pathway; however, it had been a long-standing challenge to prove the RCT hypothesis in vivo. Rader and colleagues developed a simple in vivo RCT assay in mice in which cholesterol-labeled macrophages are injected intraperitoneally, and the cholesterol radioactivity can be followed into the plasma, liver, and feces; and this study showed that RCT is modulated by apoAI expression levels.[24] RCT is calculated as the percentage of the injected radioactivity found in the plasma, liver, and feces. This assay can be modified using different types of donor cells and different sites of donor cell injection;[25] and a recent review has cataloged all studies through 2011 using this assay.[26] Although this assay has been widely adapted, what is really measured is not net efflux of cholesterol mass from the donor cells, but unidirectional movement of the radiolabeled cholesterol tracer from the donor cells to the plasma and onward, and this movement may occur by exchange with endogenous cholesterol pools rather than by net efflux. To get around this caveat, Weibel et al[27] developed a method in which macrophages were encapsulated in hollow fibers and then implanted into recipient mice. After 1-day incubation in vivo, the cells are recovered and the cholesterol mass of the cells is compared before and after in vivo incubation.[27] Whereas there is a reduction in foam cholesterol mass after implantation into wild-type mice (indicative of foam cell regression), there is an increase after implantation into hyperlipidemic LDL receptor–deficient mice (indicative of foam cell formation). Although numerous HDL turnover studies have been performed in humans, it would be attractive to have a good method to assess the entire RCT pathway from macrophages to feces in humans. Although this methodology has not been developed yet, recently a [13C]cholesterol infusion study in humans demonstrated the ability to monitor cholesterol in different pools and use kinetic modeling to estimate the RCT pathway.[28] However, this method does not specifically assess the contribution of foam cells, and thus, whether this design is germane to coronary atherosclerosis is not known.

Several studies demonstrate changes in HDL structure and function associated with inflammation. For example, de Beer et al[29] have shown marked HDL remodeling after treatment of mice with endotoxin, or in humans after surgery, producing acute phase HDL, in which serum amyloid A and group IIa secretory phospholipase A2 are induced, whereas apoAI levels are repressed. Because serum amyloid A has cholesterol acceptor activity, the consequences of inflammation on RCT must be determined empirically. Two studies used lipopolysaccharide injections in mice to induce inflammation and the acute phase response, and both reported impaired RCT, with the block occurring primarily in cholesterol mobilization from the liver to the intestine, consistent with large decreases in hepatic Abcg5, Abcg8, and Cyp7a1 gene expression.[23,30] A third study used zymosan to induce inflammation and the acute phase response, but zymosan is also effective in leading to the release of myeloperoxidase (MPO), an enzyme known to impair apoAI and HDL function (discussed below). This study also found impaired RCT, but at the first step where cholesterol is mobilized from the injected foam cells to the plasma compartment. Furthermore, diluted plasma from the zymosan-treated mice had decreased ABCA1-dependent cholesterol acceptor activity.[31] In addition, direct injection of MPO and H_2O_2 into mice also leads to impaired RCT to the plasma, liver, and fecal compartments.[23]

HDL Effects on Atherosclerosis Progression and Regression

Given the many studies establishing HDL particles as cholesterol acceptors in vitro, the extrapolation of its effects to the in vivo situation predicted protection from atherosclerosis. The simple reasoning was that by its promotion of cholesterol efflux from macrophages in plaques, increasing HDL particles would either retard the formation of foam cells from macrophages (in the setting of progression) or unload excess cholesterol from these cells after they are formed (in the setting of regression). As additional pleiotropic effects of HDL have been uncovered (discussed above), the expectation that HDL would be beneficial in reducing coronary artery disease (CAD) risk has further increased. This optimism is sustained by many investigators in the face of recent reports that higher levels of plasma HDL-C in genetic or pharmacological studies were not associated with decreased CAD risk,[7,32] most likely because there is scant evidence that HDL-C is a reliable biomarker of HDL function; in fact, there is accumulating evidence to the contrary, reviewed below. HDL proponents point to the direct evidence that raising the level of functional HDL particles by either increasing their hepatic production or by HDL infusion results in atheroprotective effects (decreased progression or increased regression of plaques). Although these data have largely come from preclinical studies, there are also reports from the clinical literature. Both types of studies will now be briefly summarized, starting with preclinical investigations.

Progression

In the standard mouse models of atherosclerosis ($LDLr^{-/-}$, $apoE^{-/-}$), increased production of HDL particles has been achieved by either transgenic or adenoviral means. Only a few examples can be given in this brief review, but in all cases, the content of macrophages and macrophage-derived foam cells, the central cellular components of the atherosclerotic plaque, was decreased as a result. Two groups made human apoAI transgenic mice on the apoE knockout background (hAI/EKO mice) and showed that atherosclerosis progression was suppressed by >80% even after 8 months on chow diets, on which the mice sustained non–HDL-C levels of >400 mg/dL.[33,34] Both apoAI levels (a rough indicator of HDL particle number) and HDL-C were increased. These results were extended to show atheroprotection when the mice were fed a Western-type diet, which further elevated non–HDL-C to >1000 mg/dL.[35] Examples of the viral approach are the reports in which either $apoE^{-/-}$[36] or $LDLr^{-/-}$ mice[37–39] fed chow ($apoE^{-/-}$) or Western-type diet ($LDLr^{-/-}$) were infected with an adenovirus or an adeno-associated virus-containing DNA encoding human apoAI. Again, plasma levels of apoAI were increased (although not always HDL-C),[38] and the early progression of atherosclerosis was suppressed. The infusion approach was also successful in suppressing atherosclerosis progression in rabbits.[40]

There are many clinical studies in which plasma levels of HDL-C have been raised, with inconsistent outcomes on CAD risk observed (some showed benefit, others did not). It is not possible to summarize this complex area here, but it has been discussed in many recent articles, including one by some of us.[41] Simply stated, a major difficulty in interpreting the available clinical studies is that in none of them has it been established whether an increase in functional HDL particles was achieved.

Regression

Although retarding the progression of atherosclerosis is highly desirable, perhaps regression is more relevant to the typical clinical scenario, in that by the time risk factor reduction is medically undertaken, many people will already have significant plaque burden, and in secondary prevention situations will already have documented CAD. As in the progression studies above, there are strong supporting data from preclinical studies that by increasing the number of functional HDL particles, preexisting plaques can undergo remarkable remodeling, particularly in the content and inflammatory phenotype of plaque macrophages and macrophage-foam cells. There are also clinical studies, albeit limited, consistent with these findings.

Considering first the preclinical models, the raising of plasma levels of apoAI by viral or infusion means after plaques formed resulted in significant reductions of the macrophage and macrophage-foam cell content in LDLr$^{-/-}$[42] or apoE$^{-/-}$[43] mice. These data agreed with earlier work in rabbits, in which human HDL infusions also regressed atherosclerosis.[44] One of the limitations of both the viral and infusion approaches to study regression of atherosclerosis has been the relative short-term nature of the treatments, either because of the cumbersome logistics of repeated injections/infusions or the transient expression of viral vectors. To overcome these limitations, we adopted an aortic transplant approach by using as recipients hAI/EKO mice.[45,46] By transferring an atherosclerotic aortic arch from a donor mouse into a recipient with a different plasma lipoprotein profile, the environment that the plaque cells are exposed to is changed quickly and is spontaneously sustained for long periods of time. To study the effects of increasing HDL-C levels on plaques, aortic arches from apoE$^{-/-}$ mice (low HDL-C, high non–HDL-C) were transplanted into recipient hAI/EKO mice (normal HDL-C, high non–HDL-C). With regard to the abovementioned distinction between HDL-C and HDL particles, it is important to note that there is good correlation between plasma levels of apoAI and HDL-C in hAI/EKO mice.

Remarkably, despite persistent elevated non–HDL-C in hAI/EKO recipients, the plaque content of CD68+ cells (macrophages and macrophage-derived foam cells) decreased by >50% 1 week after transplantation. Interestingly, the decreased content of plaque CD68+ cells was associated with their emigration from the plaques and induction of their chemokine receptor 7, a factor we have previously shown to be required for regression in the transplant model.[47] Based on a recent study of another mouse model of atherosclerosis regression,[48] it is also possible that some reduction of plaque macrophage content was because of decreased monocyte recruitment, but this remains to be determined. The induction of chemokine receptor 7 is likely related to changes in the sterol content of foam cells when they are placed in a regression environment, given that its promoter has a putative sterol regulatory element that seems to be active in vivo.[49] In support of this mechanism are the data from our study showing that there were reductions in cholesteryl ester content of the plaques in the hAI/EKO recipients, compared with the donor mice, accompanied by the induction of the classic sterol regulatory element–regulated gene, 3-hydroxy-3-methylglutaryl-coenzyme A-reductase in CD68+ cells captured from plaques.[46]

Another important result was the change in the inflammatory state of plaque CD68+ cells. There was decreased expression of inflammatory factors and enrichment of markers of the M2 (anti-inflammatory) macrophage state in the hAI/EKO recipients, compared with the donor mice.[46] Macrophage heterogeneity in human atherosclerotic plaques is widely recognized, with both M1 (activated) and M2 markers being detectable in lesions,[50,51] but little is known about the factors that regulate M2 marker expression in plaques in vivo. How raising HDL-C accomplished this in our model is under investigation, but in this regard, it is interesting to note that an apoAI-mimetic peptide inhibited M1 and promoted M2 changes in macrophages in vitro.[52,53]

Another approach to raise the level of functioning HDL particles has come from microRNA research. MiR-33, an intronic microRNA located within the gene encoding sterol-regulatory element binding protein-2, inhibits hepatic expression of both ABCA1 and ABCG1, reducing HDL-C concentrations, as well as ABCA1 expression in macrophages, thus resulting in decreased cholesterol efflux.[54] In LDLr$^{-/-}$ mice treated with an inhibitor of miR-33, HDL-C levels rose concomitant with enrichment of M2 markers in plaque CD68+ cells.[55] The treated mice also exhibited plaque regression with fewer macrophages and macrophage-derived foam cells. The therapeutic potential of miR-33 inhibitors to cause similar benefits in people was suggested by plasma levels of HDL-C and apoAI being raised in treated nonhuman primates.[56] Thus, antagonism of miR-33 may represent a novel approach to enhancing macrophage cholesterol efflux and raising HDL-C levels in the future.

Clinical Studies of Regression by ApoAI/HDL Therapies

Turning to the clinical investigations, there are a limited number of human studies in which HDL levels have been manipulated by infusion and the effects on plaques assessed. In the first,[57] patients at high risk for CVD were infused with either an artificial form of HDL (apoAI milano/phospholipid complexes) or saline (placebo) once a week for 5 weeks. By intravascular ultrasound, there was a significant reduction in atheroma volume (−4.2%) in the combined (high and low dose) treatment group, although no dose response was observed in a higher versus lower dose of the artificial HDL. There was no significant difference in atheroma volume compared with the placebo group, but the study was not powered for a direct comparison. In the second infusion study, high-risk patients received 4 weekly infusions with reconstituted HDL (containing wild-type apoAI) or saline (placebo).[58] Similar to the previous study, there was a significant decrease in atheroma volume (−3.4%) (as assessed by intravascular ultrasound) after treatment with reconstituted HDL compared with baseline, but not compared with placebo (which the study was not powered for). However, the reconstituted HDL group had statistically significant improvements in a plaque characterization index and in a coronary stenosis score on quantitative coronary angiography compared with the placebo group. In the third infusion trial,[59] a single dose of reconstituted human HDL was infused into patients undergoing femoral atherectomies, with the procedure performed 5 to 7 days later. Compared with the control group (receiving saline solution), in the excised plaque samples in the HDL infusion group, macrophage activation state (eg, vascular cell adhesion molecule-1 expression) as well as cell size (attributable to diminished lipid content) was reduced.

Based on the evidence reviewed above, as well as on other reports in the literature, HDL has the potential to retard the progression of atherosclerosis or promote its regression by modulating several steps, including the oxidation of LDL, the activation of endothelium, the recruitment of circulating monocytes and their conversion to foam cells, the activation and inflammatory state of macrophages, and their retention or emigration. From the small amount of clinical work consistent with plaque-protective benefits of functional HDL, it is tempting to speculate that at least some of these effects will be operative in people; however, 1 major limitation that necessarily limits enthusiasm is that there are no outcome studies to show that any of the clinical end points measured to date (eg, plaque volume, inflammatory state of macrophages) are correlated with decreased events.

MPO Modification of ApoAI/HDL

Posttranslational modification of apoAI can directly lead to HDL dysfunction. For example, copper oxidation, malondialdehyde, or lipid peroxide treatment of HDL alters apoAI structure and function.[60] HDL from diabetic subjects can have glycated apoAI with altered lipid-binding activity, and incubation of HDL with glucose impaired its anti-inflammatory and antioxidant activities.[61,62] One of the best studied modifications of apoAI is mediated by MPO, a leukocyte-derived heme protein abundant in neutrophils, monocytes, and a subset of tissue macrophages. Part of the innate immune host defense system, MPO uses H_2O_2 to generate an array of reactive oxidant and free radical species that are antimicrobial, such as hypochlorous acid (HOCl). These same species can also foster spurious oxidative injury to normal tissues as well, such as within atherosclerotic plaque, where MPO has been shown to promote both protein modifications and initiate lipid peroxidation. Once released from activated leukocytes, in the circulation and within lesions, MPO has been shown to bind to HDL. This tight binding, which has been mapped to helix 8 region of apoAI,[63] likely accounts for the selective oxidative targeting of apoAI within HDL for modification by MPO-generated oxidants.[63] These and other recent observations support a role for MPO serving as an enzymatic catalyst for site-specific modification of apoAI and HDL, leading to functional impairment within the artery wall. Indeed, HDL isolated from human atherosclerotic plaque has been shown to coimmunoprecipitate with MPO. Moreover, mass spectrometry analysis of apoAI within HDL recovered from human atheroma is markedly enriched in protein-bound 3-chlorotyrosine and 3-nitrotyrosine, posttranslational modifications of protein tyrosine residues indicative of protein exposure to MPO-generated reactive chlorinating and nitrating oxidants.[63,64] In several clinical studies, circulating apoAI recovered from CAD subjects demonstrates dramatic increases in chlorotyrosine, a specific molecular fingerprint of MPO-catalyzed oxidation, than apoAI isolated from plasma from healthy controls.[63–66] The 100- to 500-fold enrichment in levels of MPO-specific oxidation products within apoAI recovered from either plasma or human atherosclerotic plaque serves as strong evidence for MPO selectively targeting apoAI for oxidative modification in vivo.[63] In 1 clinical study, subjects with an elevated apoAI chlorotyrosine or nitrotyrosine content were shown to have a 16-fold or 6-fold, respectively, greater likelihood of having CVD.[63] Consistent with the notion that MPO selectively modifies apoAI in the artery wall, histological studies demonstrate colocalization of apoAI along with both MPO and other MPO-generated oxidation products within human plaque.[67,68] Additional studies have shown that the extent to which apoAI harbors MPO-generated oxidative modifications, such as chlorotyrosine, is strongly associated with further impairment in the cholesterol efflux function of apoAI, particularly via the ABCA1-dependent cholesterol efflux pathway.[63–66]

MPO-generated reactive chlorinating oxidants are known to favor modification of Cys, Met, Lys, His, Tyr, and Trp residues on proteins. The sites of oxidative modification to apoAI recovered from human plasma and atherosclerotic plaque have been mapped by mass spectrometry.[63,64] Initial in vitro studies demonstrated MPO-catalyzed oxidative modification occurs preferentially at residues on helix 8 (eg, Tyr 192), in close spatial proximity with the site on apoAI where MPO has been shown to bind.[63] Additional residues in close spatial proximity to this region have been shown to serve as preferred sites of oxidative modification, such as Trp 72, and Tyr 166.[63–66] Critical to these studies is the recognition that oxidized apoAI binds to lipid less effectively and is often not HDL associated,[69] so traditional buoyant density lipoprotein isolation methods before mass spectrometry analyses can substantially underestimate the degree of oxidative modification, and lead to spurious conclusions.[70] Although there is general consensus that MPO, or its reactive product HOCl, inactivates apoAI's cholesterol acceptor activity, the actual site of apoAI modification responsible for this dysfunction is controversial. The creation of Trp-free apoAI isoforms has been informative in this regard. Replacing all 4 Trp residues with Leu (the 4WL isoform) led to a dysfunctional apoAI; however, replacing the Trp residues with Phe (the 4WF isoform) led to a fully functional apoAI, but one that is resistant to becoming dysfunctional after MPO or HOCl treatment.[71] Thus, the Trp residues of apoAI appear to be the Achilles heel in leading to its loss of cholesterol acceptor activity from MPO-generated HOCl. It is possible to speculate that the oxidant resistant 4WF apoAI isoform may be a better therapeutic reagent to promote the regression of atherosclerotic plaques, making good cholesterol even better, because it would be anticipated to have a prolonged biological half-life within the prooxidant MPO-rich vulnerable atherosclerotic plaque.

Subsequent studies have revealed that MPO-induced modification of apoAI/HDL inhibits additional HDL functions. For example, oxidative modification of apoAI Tyr166 through MPO-catalyzed nitrating and chlorinating pathways is linked to functional impairment of HDL binding to lethicin:cholesterol acyltransferase, and lethicin:cholesterol acyltransferase activation and activity.[72] Similarly, oxidation of apoAI Met148 also impairs lethicin:cholesterol acyltransferase activation.[73] HDL exposure to MPO or HOCl results in the loss of antiapototic and anti-inflammatory activities of HDL, and specifically the loss of SR-B1–binding activity, while increasing its proinflammatory activities, such as endothelial cell nuclear factor-kB activation and vascular cell adhesion molecule-1 expression.[18] Some of the effects of MPO on generating dysfunctional apoAI and HDL are summarized in Figure. HDL exposure to MPO-generated oxidants can serve as a potent inhibitor of platelet activation and aggregation induced by physiological agonists, suggesting that not all oxidative modifications of HDL result in adverse cardiovascular phenotypes.[74] F

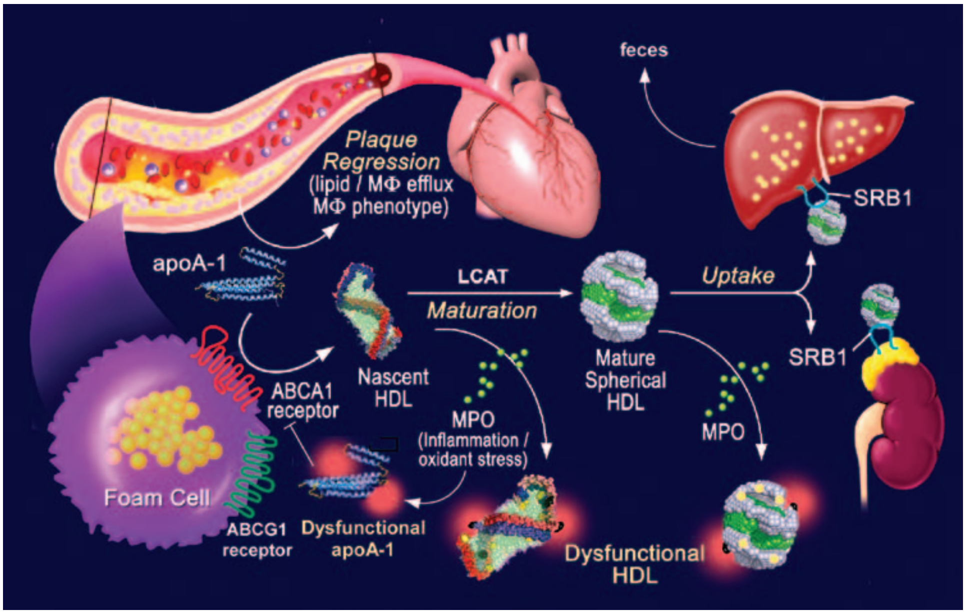

Figure. High-density lipoprotein (HDL) dysfunction impairs reverse cholesterol transport. Reverse cholesterol transport is initiated from arterial macrophages by the interaction of lipid-free apolipoprotein (apo)AI with cellular ABCA1 to generate nascent HDL. ABCG1 adds additional cholesterol to HDL, and lethicin:cholesterol acyltransferase (LCAT) esterifies HDL cholesterol (HDL-C) to generate mature spherical HDL particles. HDL-C uptake by steroidogenic tissues and the liver is mediated by scavenger receptor B1 (SR-B1). The liver excretes sterols and bile acids into the intestine for output in the feces. Myeloperoxidase (MPO)-generated oxidants modify apoAI, blocking its lipidation by cellular ABCA1 and leading to decreased nascent HDL biogenesis. MPO also impairs LCAT-mediated particle maturation and generates dysfunctional and proinflammatory HDL.

HDL Function as a Diagnostic Indicator

Tests for HDL function or biomarkers associated with dysfunctional HDL may be useful for identifying subjects at risk for CAD. For example, plasma MPO levels are positively associated with CAD and the risk of a subsequent major adverse cardiac event.[75,76] Similarly, the level of apoAI chlorotyrosine, detected by mass spectrometry, is also a predictor of CVD.[63] Although this assay may be for a potential means of quantifying dysfunctional HDL levels, it is not suitable for routine clinical use. The identification of specific modified amino acid residues linked to loss of function in apoAI may facilitate the development of clinical immunoassays specific for oxidized apoAI/dysfunctional HDL. Several tests of HDL function were described above, and one high impact study used the cholesterol acceptor activity of human apoB depleted serum in cultured macrophages as a surrogate indicator of HDL function. Khera et al[32] measured this activity in 3 large patient cohorts and found lower acceptor activity in CAD versus control subjects. Although cholesterol acceptor activity was correlated with HDL-C levels, variation in HDL-C was associated with only 26% of the variation in cholesterol acceptor activity. After partitioning of the subjects into quartiles based on their cholesterol acceptor activity, those in the highest quartile were associated with an odds ratio for CAD of 0.28 versus those in the lowest quartile; and this effect remained significant after adjustment for traditional risk factors including apoAI and HDL-C. In a logistic regression model containing traditional risk factors, efflux capacity was significantly associated with decreased CAD risk, although this measure did not appreciably add to the sensitivity versus specificity compared with the model using just HDL-C. As an add-on to their study, Khera et al[32] measured the change in cholesterol acceptor activity in a small set of subjects after treatment with the peroxisome proliferator-activated receptor γ agonist Pioglitazone or with a statin. Although both treatments led to small increases in HDL-C, only the Pioglitazone-treated group had a small (11%) but significant increase in cholesterol acceptor activity.[32]

In the future, we anticipate additional large clinical studies using cholesterol acceptor activity and other measures of HDL function and apoAI modification. Because the Khera et al[32] study was a retrospective study, it will be of interest whether these measures can also predict prospectively who will go on to have subsequent events. If these studies are successful, measures of HDL function/dysfunction may be useful as a criterion to select patients for treatment to prevent CAD, as well as to select the specific drug therapy used.

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Disclosures

Dr Hazen reports being listed as co-inventor on pending and issued patents held by the Cleveland Clinic relating to cardiovascular diagnostics and therapeutics. Dr Hazen reports having been paid as a consultant or speaker for the following companies: Abbott Diagnostics, Cleveland Heart Laboratory, Esperion, Lilly, Liposcience Inc, Merck & Co, Inc, and Pfizer Inc. Dr Hazen reports receiving research funds from Abbott, Cleveland Heart Laboratory, Liposcience Inc, and Pfizer Inc. Dr Hazen reports having the right to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics or therapeutics from the companies shown below: Abbott Laboratories, Inc, Cleveland Heart Laboratory, Esperion, Frantz Biomarkers, LLC, Liposcience Inc, and Siemens. Dr Smith reports being listed as co-inventor on pending and issued patents held by the Cleveland Clinic relating to cardiovascular diagnostics and therapeutics. Dr Smith reports having been paid as a consultant for Esperion, and receiving research funds from Esperion. Dr Smith reports having the right to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics or therapeutics from Cleveland Heart Laboratory and Esperion. The other authors have no conflicts to report.

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