Biomedical implications of high-density lipoprotein: its composition, structure, functions, and clinical applications

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High-density lipoprotein (HDL) is a proven biomarker for the monitoring of changes in antioxidant and anti-inflammation capability of body fluids. The beneficial virtues of HDL are highly dependent on its lipids and protein compositions, and their ratios. In normal state, the HDL particle is enriched with lipids and several HDL-associated enzymes, which are responsible for its antioxidant activity. Lower HDL-cholesterol levels (<40 mg/dL) have been recognized as an independent risk factor for coronary artery disease, as well as being a known component of metabolic syndrome. Functional and structural changes of HDL have been recognized as factors pivotal to the evaluation of HDL-quality. In this review, I have elected to focus on the functional and structural correlations of HDL and the roles of HDL-associated apolipoproteins and enzymes. Recent clinical applications of HDL have also been reviewed, particularly the therapeutic targeting of HDL metabolism and reconstituted HDL; these techniques represent promising emerging strategies for the treatment of cardiovascular disease, for drug or gene therapy. [BMB reports 2009; 42(7): 393-400]

Biochemical properties of HDL in reverse cholesterol transport

It is well-known that plasma high-density lipoprotein-cholesterol (HDL-C) levels are inversely correlated with the risk of cardiovascular disease, as was first demonstrated during the Framingham study (1). HDL plays a critical role in reverse cholesterol transport (RCT), which is involved in the removal of excess cholesterol from peripheral cells, and its delivery to the liver and steroidogenic cells for catabolism (2). The RCT pathway works against pathological accumulations of lipid deposit, especially in oxidized LDL (low-density lipoprotein), in the peripheral cells—a process that eventually results in atherosclerosis and coronary artery disease (3). The RCT concept was first introduced by Glomset (4), and is described as follows: HDL facilitates the uptake of peripheral cholesterol and its return to the liver for excretion in bile acid and feces. As shown in Fig. 1, excess cholesterol in the peripheral cells can be taken up by nascent HDL (discoidal type), and the nascent particles are forced to mature into more spherical HDL (HDL₃ and HDL₄, by lecithin: cholesterol acyltransferase (LCAT)) during their translocation to the liver for excretion via the bloodstream. Until now, this RCT pathway has been thought to be a uniquely natural cholesterol excretion pathway (5). Numerous reports have corroborated the assertion that RCT functions as a protective mechanism against atherosclerosis, and Miller and Miller have suggested that HDL may protect against atherosclerosis via the promotion of RCT (6).

Among several protein constituents, apolipoprotein (apo)A-I is the principal protein component of HDL. ApoA-I is synthesized within the liver (70%) and intestine (30%), and is secreted into the serum in a lipid-free state (7). The lipid-poor or pre-beta forms (discoidal shape) of apoA-I conformations, different from the bulk of plasma apoA-I, may prove to be particularly efficient at the promotion of free cholesterol (FC) efflux during the first step of RCT (Fig. 1). Although little remains known regarding the details of this process, lipid-poor beta HDL (nascent HDL) is capable of taking up cholesterol from macrophages in the artery wall via the ATP-binding cassette A-1 receptor (ABCA-1) (8, 9). Once associated with phospholipid (PL), FC and apoA-I form disc-shaped HDL-particles, ranging in diameter from 7 to 12 nm. While in circulation, these nascent particles are remodeled by lecithin: cholesterol acyltransferase (LCAT), which can esterify FC into cholesteryl ester (CE); apoA-I functions as a crucial modulator of this reaction (10). The resulting CE is packaged into the core of the particle, resulting in the formation of the spherical HDL₂ and HDL₃ particles that constitute the bulk of plasma HDL. On the delivery end of the pathway, apoA-I appears to be important in that it provides HDL-cholesterol for steroid hormone and bile acid synthesis in the adrenals and liver, via interactions with the scavenger receptor type B class I (SR-BI) (11). Alternatively, HDL-cholesterol can be transferred to apo-B-containing proteins (LDL, IDL, VLDL), via the action of cholesteryl ester transfer protein (CETP), while in the bloodstream. This results in a recycling of cholesterol and an increasing attenuation in blood circulation, with the potential to enter back into the artery wall (12).
Biomedical importance of HDL
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As illustrated in Fig. 2, HDL exerts many beneficial effects for the maintenance of a healthy physiological system, including antioxidant, anti-inflammatory, and anti-thrombotic effects (5, 13). These activities are exerted in accordance with the composition of essential apolipoproteins and associated enzymes. However, the favorable characteristics and functions of HDL can be hampered via interaction with reactive oxygen species (ROS) and oxidized LDL (oxLDL)-induced inflammation (Fig. 2).

**Anti-atherosclerotic function of HDL**
Beginning several decades ago, the results of epidemiological data and cohort studies began to prove that HDL concentration is an inverse predictor of progressive atherosclerosis and of future coronary artery disease (1, 14, 15). Although HDL’s physiological effects were determined a fairly long time ago, the first in vivo experiment designed to characterize the
anti-atherosclerotic functions of HDL was conducted in 1990, using New Zealand White rabbits in which atherosclerosis was induced. Weekly blood infusions of HDL-VHDL (50 mg of protein) for 30 days into a high-cholesterol diet induced a reduction in the development of aortic fatty streaks in the experimental rabbits (16).

**Apolipoprotein A-I**

The principal functions of HDL, namely lipid binding, cholesterol removal from peripheral cells, the activation of LCAT, and the recognition of receptors in the liver and steroidogenic tissues-are due, in large part, to apoA-I, the primary protein constituent of HDL. ApoA-I is synthesized as a 267-amino acid precursor, preproapoA-I, which harbors an 18-amino acid prepeptide and a 6-amino acid Acid (Arg-His-Phe-Trp-Gln-Gln) prepeptide (29). Following cleavage and posttranslational modification, mature human apoA-I is comprised of a single polypeptide chain of 243 amino acids without containing Ile or Cys, which is characterized by the 11 and 22 amino acid homologous repeats, and referred to as the helix domain (30). This starts at residue 44 of the sequence and continues for the entire length of the protein. Many of these 22 amino acid repeat, beginning with Pro residues, and all are predicted to form amphipathic alpha-helices.

**Apolipoprotein A-II**

ApoA-II is the second most important constituent of the HDL apolipoproteins, and exists as a homodimer with two polypep-
tide chains, each 77 amino acids in length, which are linked by a disulfide bond at Cys-6 (31). Induced increases in the transcription of the apoA-II gene might represent a strategy for the augmentation of HDL-C levels in a murine model; however, the implications of this strategy for atherosclerosis control in humans remain uncertain (32). Although the physiological role of apoA-II in atherosclerosis remains controversial, 20% of patients with coronary artery disease (CAD) have evidence of increased serum apoA-II levels (40-60 mg/dL) compared to control 30-35 mg/dL (32); this suggests that increased apoA-II levels might be associated with the development of CAD. Furthermore, human apoA-II enrichment in HDL displaces paraoxonase from HDL and disrupts or inhibits its antioxidant properties (33). Recently, the Cho group reported the case of a Caucasian male with very low blood cholesterol and low apoA-II, who evidenced no signs of atherosclerosis (34). This case demonstrates that reduced apoA-II protein levels in the serum and increased HDL-cholesterol and particle size may protect against hyperlipidemia and the atherosclerotic process, even in patients suffering from severe obesity. Collectively, apoA-II is considered to be an atherogenic factor that hinders the beneficial functions of HDL, including antioxidant ability.

Apolipoprotein C-II and C-III
ApoC-II and apoC-III have been determined to be involved in the regulation of lipoprotein lipase (LPL) activity and serum TG levels (35). Acute increases in VLDL-TG levels and acute reductions in HDL levels have been associated with the over-expression of apoC-III and the down-regulation of HDL, thereby indicating that the specific composition of apolipoproteins might exert some influence on abnormal lipid composition and lipoprotein quality. ApoC-III is a primary component of the triglyceride-rich lipoproteins (VLDL and chylomicron), and its concentration is both high and positively correlated with the concentration of TG (36). It has been reported that the apoC-III levels are elevated and that the apoC-II/apoC-III levels were reduced in patients undergoing chronic hemodialysis (37), as apoC-III is an inhibitor and apoC-II is an activator of LPL. Thus, these alterations in the levels of apoC-III and apoC-II may result in a marked reduction in the catabolism of VLDL. Therefore, VLDL must remain its size as a larger particle with enriched TG content in chronic or acute renal failure, such as in the oliguric phase of hemorrhagic fever with renal syndrome. Similarly, lipoprotein profiles of sera in the oliguric phase have evidenced alterations of lipid metabolism, including familial disease (FED), and are rarely associated with premature CHD (48).

Cholesteryl ester transfer protein (CETP)
As serum TG and CE components in lipoproteins are not spontaneously transferred between isolated lipoprotein particles at a biologically significant rate, all TG and CE transfer activities in human plasma are facilitated by CETP. CETP promotes the redistribution and equilibration of hydrophobic lipids packaged within the lipoprotein core (CE and TG) between HDL and apo-B-harboring proteins (LDL, IDL, VLDL, chylomicrons) (44). CETP is a hydrophobic, glycosylated, and single-polypeptide protein consisting of 476 amino acids, which circulates in plasma bound to lipoproteins (45). The CETP functions can be summarized as lipid transfer activity, size-redistribution of lipoprotein particles, and cellular cholesterol movement. The net effect of the action of CETP on HDL is the depletion of CE, enrichment with TG, with an overall net reduction in the size of the HDL particle. It has been fairly well established that CETP is an atherogenic factor. Studies conducted with CETP-deficient patients have revealed that these patients evidence remarkably high plasma levels of HDL-C and apoA-I (46). Thus, it has been proposed that the inhibition of CETP would result in an augmentation of the levels of HDL-C (47).

Paraoxonase
Human serum paraoxonase, PON, (E.C. 3.1.1.2) is an HDL-associated calcium-dependent enzyme, with demonstrated evidence of strong antioxidant activity. It catalyzes the hydrolysis of oxidized fatty acids from phospholipids and prevents the accumulation of oxidized lipids in lipoproteins, particularly LDL (48). Oxidized LDL tends to be small and dense, readily affixing to macrophages in atherosclerotic lesions of rabbit and human (49). This process is part of the initiation of the pathological progress of atherosclerosis, which is stimulated by the generation of the cytokines, IL-1beta, IL-6, and TNF-alpha, coupled with the induction of adhesion to endothelial cells. Low PON activity has been closely correlated with an increased risk of CAD and it also has been observed in patients evidencing alterations of lipid metabolism, including familial
Platelet activating factor-acetyl hydrolase (PAF-AH)

PAF-AH (E.C. 3.1.1.47) consists of 441 amino acids and is involved in the antioxidant and anti-inflammatory functions associated with the surfaces of HDL (54), and is a Ca²⁺-independent enzyme belonging to group 7 of the PLA2 family (55). PAF-AH performs 2 pivotal functions against the oxidation process. First, PAF-AH hydrolyzes PAF (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine), which is a potent phospholipid mediator with pro-inflammatory properties. Second, the enzyme participates in the degradation of oxidized phospholipids (56). PAF activates leukocytes and platelets, and enhances the adhesion of leukocytes to vessel walls. PAF accumulates within the atherosclerotic plaques of individuals suffering from CAD, thereby suggesting that this phospholipid mediator may be actively involved in the pathophysiology of atherosclerosis (57). Consequently, via the degradation of such phospholipids, PAF-AH may function as a profoundly anti-atherogenic enzyme.

Clinical application of HDL

For more than 3 decades, lipid-lowering therapeutic modalities have focused on LDL-cholesterol levels, as is the case in statin therapy. However, the focus of research in this area is currently shifting to the regulation of HDL levels. Two approaches are currently being exploited in HDL-oriented therapy. One of these involves the use of synthetic HDL with recombinant apoA-I and its mutants, and the other employs apolipoprotein-mimetic peptides, such as D4F. In a study performed in mice, oral administration of D4F reduced atherosclerosis, without changing plasma cholesterol levels (58).

Blood infusion of rHDL for regression

In synthetic HDL therapy, wildtype plasma apoA-I, recombinant human apoA-I, and its mutants were employed to constitute rHDL with various phospholipids. Mass-purification of human plasma apoA-I and synthesis of reconstituted HDL in industrial amounts for therapeutic applications were reported first in 1996 (59). In 2007, a phase II clinical trial was performed with 145 acute coronary syndrome (ACS) to test regression effect of plasma apoA-I-rHDL, ERASE (Effect of rHDL on atherosclerosis-safety and efficacy) trial (60). Four weekly infusions of CSL-111, a synthetic rHDL manufactured from human plasma apoA-I and soybean phosphatidylcholine, given in doses of 40 mg/kg demonstrated reduction in plaque characteristics compared to placebo. Although there were no statistical differences between CSL-111 and placebo, there was a reduction in coronary plaque volume after infusions of CSL-111, from a level of 3.4% compared to placebo levels of 1.6%.

In contrary to plasma apoA-I, 2 recombinant apoA-I mutants were reported to be potent candidates: one is R173C apoA-I, (apoA-I(Kao)), the other is V156K of apoA-I. Since R173C-apoA-I was initially discovered in northern Italy in the 1980 (61), a series of reports has shown that blood infusions of R173C-rHDL are effective in the removal of pre-existing atherosclerotic plaques in animal studies and in human clinical trials (62). However, the regression effect of R173C-rHDL had, at that time, yet to be compared with wildtype-rHDL.

Contrary to what has been observed with R173C, V156K-apoA-I was recently developed (63). It demonstrated potent antioxidant activity and anti-inflammatory activity both in vivo and in vitro (20), as well as an in vivo anti-atherosclerotic effect (64, 65). The pharmaceutical effects of V156K-rHDL were compared directly to WT-rHDL and R173C-rHDL. Although their anti-atherosclerotic effects were far stronger than those of WT-rHDL, the effects were very similar between V156K-rHDL and R173C-rHDL.

HDL as a drug delivery vehicle

Classically, rHDL has been studied from the viewpoint of anti-atherosclerotic therapies. In addition, to its demonstrated beneficial effects on HDL as described above, hydrophobic biomolecules, including anti-cancer (66), antifungal (67), and anti-viral drugs (68) have already been incorporated into rHDL. The greatest difficulty with such potent agents is achieving specific delivery methods with high degrees of efficiency. The various sizes of rHDL have been theorized to represent an attractive vehicle for delivery in such cases. Lacko et al. demonstrated that 3 molecules of taxol per rHDL particle could be incorporated into the rHDL complexes (66). Oda et al. reported that amphotericin B-rHDL represented a novel formulation that effectively solubilized the antibiotic, and elicited significant in vitro and in vivo antifungal effects without any observed toxicity at therapeutic doses (67). These reports have demonstrated that rHDL might be utilized as an efficient drug and gene delivery vehicle for therapeutic agents, owing to the ability of peripheral cells to absorb HDL’s core components.

Conclusion

In this review, various aspects of HDL, including its structure and function correlations, its protein components and roles, and the clinical applications of HDL and strategies for raising HDL-cholesterol levels, have been discussed. It has been established clearly that HDL is a valuable target in treatments for atherosclerotic cardiovascular disease, including CETP inhibitors, PPAR-alpha agonist, PPAR-gamma agonist, and HDL-blood infusion therapies. In addition to its cardioprotective effects, the anti-oxidant ability of HDL and apoA-I is an important property to suppress senescence and degenerative metabolic disorders. HDL-C prevents aggregation and polymerization of beta-amy-
loid (69). HDL-C is involved in diminishing the risk of dementia (70), by providing a prophylactic-neuroprotection effect.

Determining the quality and function of HDL is an emerging area of research in which the physiological defense mechanisms of HDL against oxidizing and inflammatory attacks might be determined. It has been postulated that the composition of apolipoproteins and lipids may influence the quality and function of HDL. It seems clear that improved HDL function and quality will allow for the generation of more favorable outcomes with regard to the augmentation of HDL-cholesterol levels, thereby allowing for enhancements of the innate therapeutic functions associated with HDL.

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REFERENCES


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