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High-density lipoprotein carbamylation and dysfunction in vascular disease

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1. ABSTRACT

High-density lipoprotein (HDL) is cardioprotective because of its anti-atherogenic properties. Nevertheless, our goal to optimize HDL cholesterol (HDL-C) levels have had little effects on the atherothrombotic burden and suggests a closer look be taken at HDL function and dysfunction. HDL is a group of complex macromolecules composed of a lipid- and proteome that work in synergy to execute its anti-inflammatory, anti-atherogenic, and anti-thrombotic effects. However, throughout its life-span in circulation, HDL undergoes significant modification. Carbamylation, a non-enzymatic and irreversible post-translational modification of protein, is one effector of HDL which has growing evidence that it plays a crucial role in the development and progression of atherosclerotic cardiovascular disease (ASCVD), particularly in chronic kidney disease (CKD). We summarize HDL's function, susceptibility to modification, and discuss HDL carbamylation and its effect in cardiovascular disease.

2. INTRODUCTION

For decades we have known that HDL and HDL-C are cardioprotective, primarily for its atheroprotective properties. Nevertheless, studies aiming to reduce the atherothrombotic burden via increase of HDL-C, with rare exception (Helsinki trial), have been disappointing. These data suggest that targeting the serum concentration of HDL-C is not the answer. Still, there is a strong and increasing evidence that edifies HDL's ability to protect arteries from the development and progression of atherosclerosis, and facilitate vascular repair (1-2).

HDL is a dynamic, extremely complex and heterogeneous conglomerate of lipoproteins with multidimensional functionality (3) (Figure 1*)*. In circulation, it undergoes continual remodeling which may affect its functionality. As a result, more and more research is focused on HDL's components and the perturbations that cause its dysfunction.

Carbamylation is a non-enzymatic and irreversible post-translational modifier of proteins and free amino acids. Carbamylation additionally targets HDL proteins with growing evidence that it plays a crucial role in the development and progression of ASCVD (Figure 1). Herein, we summarize HDL's function, susceptibility to modification, and discuss HDL carbamylation and its effect in ASCVD.

3. HDL STRUCTURE AND FUNCTION

HDL is composed of a mixture of lipoproteins and phosphatidylcholine molecules that form an amphipathic shell where unesterified cholesterol is imbedded surrounding a core of water-insoluble cholesterol esters (4) (Figure 1). The amphipathic shell is, in turn, associated with many proteins and lipids (1, 5-6). In fact, HDL can transport at least 96 different proteins (7-8) and over 200 different lipid species (5), in addition to its major structural components, apolipoprotein A-I (ApoA-I) and apolipoprotein A-II (ApoA-II) (9).

ApoA-I is the principal protein fraction of HDL and confers most of the atheroprotective

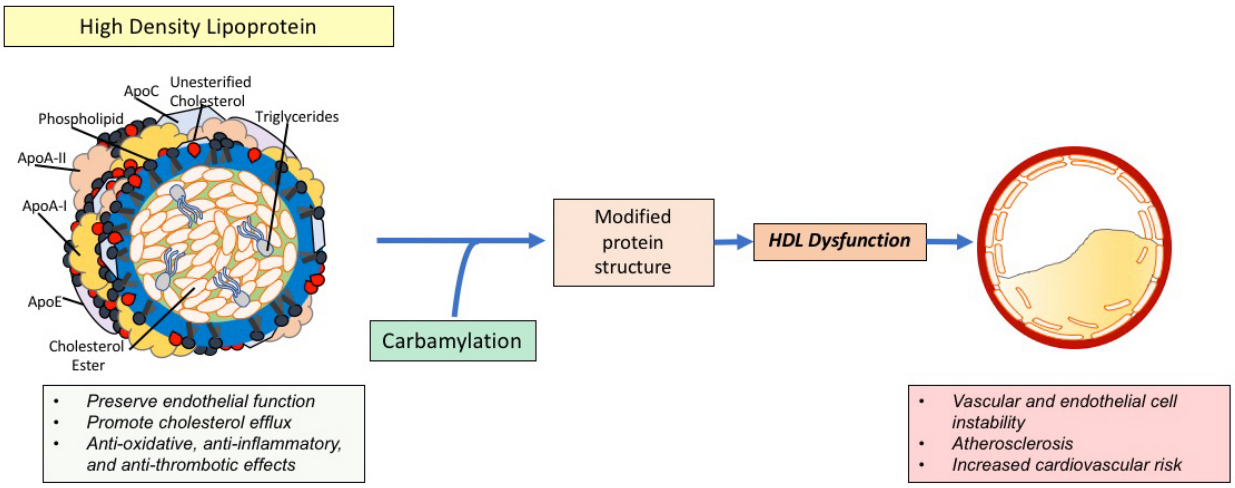

Figure 1. Carbamylation alters HDL protein structure which leads to HDL dysfunction and transformation of HDL into a pro-atherogenic lipoprotein.

and anti-inflammatory functions of HDL (10). It is a 28kDa protein with 243 amino acid residues (11). Its secondary structure consists of class A amphipathic alpha-helices separated by proline hinges. In these helices, apolar residues occupy one face and polar residues occupy the other. Positively charged lysine and arginine residues separate the two faces of the helix. This amphipathic structure allows the interaction of ApoA-I with lipids. The proline hinges confer a high steric flexibility on the ApoA-I molecule, allowing important conformational changes needed to occupy HDL particles of different sizes which allows for reverse cholesterol transport (RCT) (12).

One of HDL's most studied and well recognized function, is its role in the RCT pathway. RCT describes HDL's ability to remove excess cholesterol from lipid-laden macrophages within arterial walls and transporting it to the liver for excretion into the bile or to adrenals, testes, and ovaries for steroid hormone production (1, 13). The critical step being the efflux of cholesterol from the lipid-laden cells within atherosclerotic lesions, which is mediated by HDL cell membrane transporters like ABCA1, ABCG1, and SR-B1 (13-14).

Additionally, HDL can attenuate inflammation and play a vital role in vasodilatation and anti-coagulation (15). At the vascular endothelial level, for example, HDL increases endothelium nitric oxide synthase (eNOS) protein expression and activation, and even, delays eNOS mRNA degradation (16-20). In turn, eNOS stimulates the release of nitrous oxide (NO) that promotes vasodilation and attenuates smooth muscle cell migration (16, 21-22) and platelet aggregation (23). Further, HDL inhibits adhesion molecule expression and white blood cell adhesion (24-26). Also, HDL can enhance endothelial repair and bare anti-apoptotic properties (2, 20, 27).

Paraoxanase-1 (PON-1) is a hydrolytic enzyme that associates with HDL and lends the capability of protecting against lipid oxidation (28) and inflammation. It consists of 354 amino acids with a molecular mass of 43kDa (29). It is expressed primarily in the liver (30) and mainly transported by HDL and to a much lesser extent with VLDL and chylomicrosns (31). PON-1 specifically binds to HDL through interaction of hydrophobic N-terminus to phospholipids, and through PON-1-ApoA-I interaction (32). HDL associated PON-1, protects LDL and HDL form oxidative modification from reactive oxygen species (33).

4. HDL SUSCEPTIBILITY TO MODIFICATION

In circulation, however, HDL and its components are susceptible to structural modifications. Modifications are mediated by various mechanisms such as carbamylation, oxidation, glycation (34-35), nitration (36) and homocysteinylation (37). These mechanisms are examples of posttranslational modifications of proteins and play a major role in the biological activities of HDL. As a result, vital components like ApoA-I, PON-1, and lecithin-cholesterol acyltransferase (LCAT) are altered, ultimately attenuating HDL's cyto- and vascular-protective properties (2, 35, 38). In inflammatory conditions like type 2 diabetes (39-40), CAD (24, 28, 31), and CKD (2, 41) HDL modification is amplified and, as a result, its function is severely impaired.

Specifically, inflammation impairs the RCT pathway (42) and HDL's ability to induce NO production independent of HDL-C levels (40, 43). In patients with myocardial infarction for example, cholesterol efflux and anti-inflammatory effects of HDL is impaired independent of the plasma HDL-C level (44-45). However, new cardiac events were independently associated with HDL anti-inflammatory capacity

Table 1. HDL carbamylation and vascular effect

Significance	Reference
Increased foam cell formation → HDL transformation to a pro-atherogenic lipoprotein	34
Decreased lecithin-cholesterol acyltransferase activity → Impaired endothelial cell maturation and reverse cholesterol transport pathway	35
Decreased paraoxanase-1 Activity →Decreased capability of protecting against lipid oxidation and inflammation	2, 35
Decreased endothelial cell repair → vascular wall injury	2
Decreased reverse cholesterol transport activity and cholesterol efflux capacity	41

(44). Mechanistically, HDL from patients with CAD is reported to gain access to the endothelial cell by the lectin-like oxidized LDL receptor-1 (1). Thus, eNOS activation is inhibited, and HDL's anti-inflammatory and endothelial repair capabilities are compromised.

In high-risk post-infarction patients, for example, elevated levels of HDL-C were predictive of risk of recurrent coronary events (46). During ST wave elevated myocardial infarction (STEMI), high HDL-C was associated with a greater decline in endothelial function due to the structural and functional changes of HDL (47). In a large cohort of CAD patients undergoing isolated first-time elective coronary artery bypass grafting, pre-operative HDL-C levels were not associated with reduced but rather increased of major adverse cardiovascular events (48). In maintenance hemodialysis patients, higher mortality rates were correlated with an increased HDL inflammatory index via measure of HDL's inflammatory and anti-inflammatory properties (49). Chang and company found that increasing serum HDL-C over time is paradoxically associated with significantly higher all-cause and cardiovascular mortality (50). Furthermore, HDL from diabetics and patients with abdominal obesity had a reduced capacity to reverse the inhibition of aortic ring endothelium-dependent relaxation compared to HDL from healthy patients (39). Altogether, these studies show that the function of HDL can be significantly altered particularly in certain abnormal metabolic conditions and therefore underscore the need to analyze mechanisms that alter HDL function.

5. HDL CARBAMYLATION

Carbamylation is a form of permanent post-translational protein modification that results from the interaction between isocyanate and proteins, Figure 2. Isocyanate itself can be generated from either the dissociation of urea or from the activity of myeloperoxidase (MPO) (35, 51). Plasma urea normally and spontaneously dissociates into isocyanate (52). MPO is stored in azurophilic granules of neutrophils, monocytes, and macrophages and catalyzes the oxidation of thiocyanate, a plasma anion mainly derived from the diet or smoking, to form cyanate which plays an important role increasing the risk of atherosclerosis in smokers (51).

Isocyanate non-enzymatically reacts with proteins forming an irreversible covalent bond. Carbamylation occurs when isocyanate reacts with the N-terminal alpha-NH_2 groups or the epsilon-NH_2 groups of lysine side-chains of target proteins, or with free amino acids (52-53), and further leads to altered structure and properties of affected proteins. A prominent effect is positive-charge elimination, which changes protein–water interactions and disrupts ionic interactions on the protein surface (54). Because these interactions stabilize secondary and tertiary structures of proteins, the loss of charge in carbamylated proteins leads to dramatic conformational changes. As a result, protein function like enzymatic activity, degradation capacity and receptor-ligand recognition is compromised (54-56).

Mass spectrometry analysis revealed that protein carbamylation is a major posttranslational modifier of HDL and is directly proportional to the plasma urea concentration (34). Holzer and company found that the carbamyl-lysine content of HDL derived from atheromatous plaques is more than 20-fold higher than the amount mediated by MPO; and demonstrated that only one carbamyl-lysine residue per HDL-associated ApoA-I was necessary to induce net cholesterol accumulation and lipid-droplet formation in macrophages (34) (Table 1). This remarkable finding suggests that carbamylated HDL participates in the creation of foam cells and highlights a remarkable ironic finding of carbamylated HDL, that is, its transformation to a pro-atherogenic lipoprotein.

Healthy individuals have an isocyanic acid level of about 50 nanomols/L, while patients with ESRD have levels as high as 150 nanomols/L and more than a 2-fold elevation in carbamylated HDL which may cause detrimental cardiovascular effects (2, 57). To that end, it has been shown in *in-vitro* studies that carbamylation of ApoA-I, a cofactor for LCAT, results in significant suppression in LCAT activity, and thereby diminishing the crucial role it plays in RCT (35). Additionally, carbamylated HDL *in-vitro* can impair HDL-associated PON-1 activity (20). Recently, Sun *et al.* reported that HDL-associated PON-1 activity was significantly decreased (~50%) and its activity was found to be negatively correlated with the level

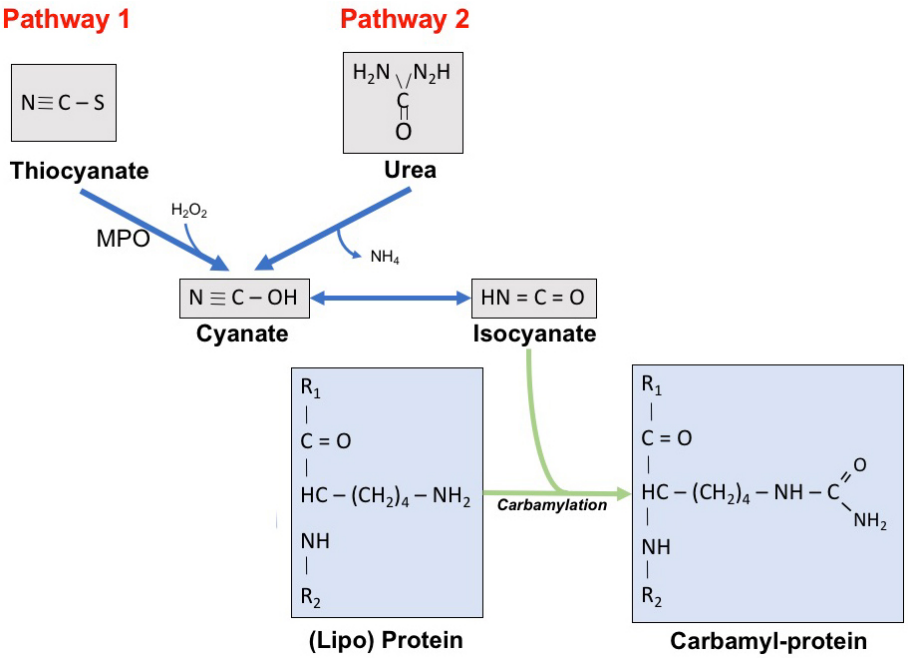

Figure 2. The possible pathways to HDL protein carbamylation is illustrated. Carbamylation results from the interaction between isocyanate and proteins. Isocyanate itself can be generated from either the dissociation of urea or from the activity of myeloperoxidase (MPO). Isocyanate specifically reacts with the N-terminal alpha-NH_2 groups or the epsilon-NH_2 groups of lysine side-chains of target proteins, or with free amino acids.

of carbamylated HDL in patients with ESRD (2) (Table 1). Holzer and colleagues show that carbamylation of HDL, resulted in a loss of anti-inflammatory and anti-oxidative properties (35) (Table 1).

Further, in CKD, carbamylated HDL suppressed the expression of VEGFR-2 and SR-B1 signaling pathways in endothelial cells and significantly inhibited endothelial cell migration and repair; nevertheless no significant elevation in either MPO concentration or activity was observed in patients with ESRD suggesting that the urea driven system for protein carbamylation supersedes that of the MPO driven system (2, 34). However, in a separate study that looked at MPO levels in maintenance hemodialysis patients versus controls, higher MPO levels in ESRD independently predicted three-year mortality risk (58). As previously noted HDL is heterogenous, comprised of several subclasses, namely HLD2 and HDL3. However we are unaware of the extent of carbamylation among the subclasses of HDL.

6. SUMMARY AND PERSPECTIVE

There is now substantial evidence that carbamylation of (lipo)proteins is a key contributory factor of atherosclerosis be it driven by the action of MPO or by elevated plasma urea. These data suggest that the static measure of HLD-C is absolete and indicate the need for clinicians to measure HDL function, particularly in patients with inflammatory conditions, such as CAD and CKD.

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