Published in final edited form as:

J Am Coll Cardiol. 2016 October 11; 68(15): 1677–1679. doi:10.1016/j.jacc.2016.08.006.

Carbamylated Low Density Lipoprotein and Thrombotic Risk in Chronic Kidney Disease

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Carbamylation is a form of post-translational modification in which cyanate/isocyanate covalently modifies nucleophilic groups on proteins (e.g., N-terminus and Ne-amine group of lysine residues, or thiol group of cysteine residues) forming a carbamyl group (1). Historically, carbamylation was believed to predominantly be fostered by uremia. This is because urea is in equilibrium with cyanate/isocyanate, and carbamylation of proteins dramatically increases with severe impairment in renal function (1). Recent studies also discovered a second pathway for generation of the reactive cyanate/isocyanate species and protein carbamylation-leukocyte heme peroxidases like myeloperoxidase (MPO) (2). MPO can use plasma levels of thiocyante as a co-substrate to generate cyanate and catalyze protein carbamylation. Since plasma levels of thiocyanate are heightened in smokers and second-hand or work-place smoke exposures, this pathway has been linked to heightened cardiovascular disease risks in subjects who smoke (2). MPO has been shown to catalyze protein carbamylation at sites of inflammation, such as within the atherosclerotic artery wall where MPO-catalyzed oxidative processes are enhanced (2). Elevated levels of carbamylated proteins in plasma or serum are associated with adverse prognosis including enhanced mortality rate in stable cardiac patients (2), subjects with end stage renal disease on hemodialysis (3), and in patients with chronic heart failure (4).

The mechanisms through which carbamylation of proteins and LDL are linked to atherosclerosis development have been studied for decades. Early *in vitro* studies showed that both “uremic” LDL and carbamylation of LDL, often monitored by either loss of protein lysine residues or generation of carbamyllysine (also known as homocitrulline), alters the ability of LDL to interact with scavenger receptors and an almost total loss in the ability to interact with apolipoprotein B-E receptors, suggesting its atherogenic propensity (5,6). Subsequent animal studies have demonstrated heightened levels of carbamylated LDL (cLDL) and atherosclerotic burden in atherosclerosis prone apoliprotein $E^{-/-}$ mice in which uremia was induced by unilateral nephrectomy (7). These early studies similarly showed

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CONFLICTS OF INTEREST

Dr. Tang has reported that he has no relationships relevant to the contents of this paper to disclose.

cLDL possessed atherogenic properties distinct from oxidized LDL, and was co-localized with ICAM-1 and macrophage infiltration within atherosclerotic laden aorta (7). Promising data have showed the ability for cLDL to directly induce endothelial dysfunction (8,9), potentially via the endothelial scavenger receptor LOX-1 activation, eNOS uncoupling and increased reactive oxygen species production (10). Carbamylation of LDL and other proteins appears to be a downstream consequence of processes fundamentally linked to enhanced cardiovascular risks—namely, uremia, inflammation and smoking.

In this issue of the *Journal*, Holy and colleagues extended previous work by demonstrating in an elegant set of in vitro experiments that cLDL induced expression of tissue factor and PAI-1 in cultured endothelial and vascular smooth muscle cells, and heightened platelet responsiveness. They also showed cLDL fostered increased levels of LOX-1 receptor on the surface of platelets, suggesting a feed-forward cycle whereby cLDL-induced platelet hyper-responsiveness and thrombosis risk may be amplified(11). In addition to in vitro studies, the authors also isolated LDL from subjects with CKD, or chemically carbamylated LDL, and compare the effects of infusion of these modified LDL versus LDL isolated from healthy subjects. When infused into mice, heightened pro-thrombotic effects were evaluated an in vivo thrombosis model. One strength of their study is the reductionist approach taken. Another is the comprehensive examination of the effects of native LDL versus cLDL, both endogenously carbamylated and experimentally by reaction with potassium cyanate, on different mouse cell types relevant to the vessel wall and platelets in vitro, and following in vivo infusions. The results provide a compelling story to suggest cLDL can amplify thrombotic potential in vivo. However, one feature not focused on is whether the carbamylation of alternative proteins can elicit similar effects to those observed with cLDL since, particularly with uremia, all circulating and membrane-bound proteins are exposed to the heightened carbamylation process. For example, carbamylation of abundant circulating proteins such as albumin and hemoglobin have been identified in patients with CKD, suggesting a possible pathogenic role (1). Also, it is important to point out that beyond carbamyllysine as a measure of extent of carbamylation, other residues (namely cysteine) or nucleophilic groups (e.g., anionic lipids that contain reactive amine moieties) can also be targets of carbamylation.

Since carbamylation is a conserved chemical pathway that links inflammation and uremia, the findings reported by Holy and colleagues implied that carbamylation can contribute not only to an accelerated atherogenic process but can amplify a prothrombotic process that results from multiple pathways and impacts multiple cell types in the artery wall, as well as within platelets. Another point worth noting is the interesting relationship between cLDL and LOX-1 identified. cLDL is proposed to be recognized by the LOX-1 receptor and to trigger down-stream pathways that heighten thrombosis potential, including increasing platelet surface expression of LOX-1. The authors observed that unstimulated platelet LOX-1 expression is relatively low, but increased following exposure of animals to cLDL infusion. These findings imply that the presence of cLDL itself is not the direct trigger of prothrombotic process, but appears to amply its pathogenicity once a thrombotic event is initiated. Clearly, much effort is needed to understand if such mechanistic pathways are physiologically relevant in vivo to translate these findings to the clinical settings. For example, rather than inhibitor based studies, more definitive experiments inducing uremia in

LOX-1 receptor null animal models will permit direct experimental testing of an obligatory role of LOX-1 in platelet hyper-responsiveness mediated by cLDL. It would also be intriguing to see if LDL isolated from stable patients with marked reduction in uremia (e.g., ESRD patients undergoing stable hemodialysis without uremia) will attenuate cLDL generation and its thrombogenic potentials. How these processes may be affected by standard CKD and ESRD medical regimens such as aspirin, statins, and even dialysis will also be of broad clinical interest beyond exploring LOX-1 targeting strategies. The biggest clinical question, however, is to what extend does this cLDL-mediated process play in the grand schema of the uremic CKD patient where multiple other pro-thrombogenic mediators (e.g., uremic toxins, metabolic factors, and inflammatory mediators) are concurrently operational. Nevertheless, the present studies add to the growing body of data suggesting that reduction in protein carbamylation (especially cLDL) and the disruption of the receptor recognition of cLDL may be of therapeutic benefit in vulnerable patients. The present studies should help to further stimulate investigations into this important area.

Acknowledgments

Funding: This work was supported by grants from the National Institutes of Health (NIH) P01 HL076491, R01 DK106000, R01 HL126827.

Dr. Hazen is named as co-inventor on pending and issued patents held by the Cleveland Clinic relating to cardiovascular diagnostics and therapeutics. Dr. Hazen has received research funds from P&G, Pfizer Inc., Roche Diagnostics and Takeda. Dr. Hazen has the right to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics or therapeutics from Cleveland HeartLab, Siemens, Esperion, and Frantz Biomarkers, LLC.

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