Carbamylation-Induced Vascular Calcification: Implications for Hypertension Pathogenesis

An estimated 850 million people worldwide is affected by chronic kidney disease (CKD). Despite continuous improvements, dialysis can only replace approximately 20% of kidney function, leaving patients with a chronic overload of toxic metabolites. Hypertension is an established risk factor for cardiovascular disease (CVD), and patients with CKD are at high risk of CVD, including hypertension, heart failure, coronary heart disease, stroke, and sudden cardiac death. In fact, CVD is the leading cause of morbidity and mortality in dialysis patients.

Carbamylation is a recognized hallmark of molecular aging and has been linked to various CKD and age-associated pathologies, including vascular health. It affects the structural and functional integrity of proteins through a non-enzymatic reaction of isocyanic acid with the N-terminus or ε-amino groups of lysine residues. This irreversible modification can occur at multiple sites within a single protein, altering protein charge, structure, and function. There are two sources of isocyanic acid *in vivo*: inflammation and kidney insufficiency. In **the first one**, carbamylating ions are generated as a byproduct of the myeloperoxidase (MPO) system activity, utilized by neutrophils for bacterial defense (MPO/H2O2/SCN-). These highly reactive ions can cause carbamylation locally or systemically if neutrophil activation is widespread, potentially contributing to inflammageing, the accelerated aging due to chronic mild inflammation. The **second source of isocyanic acid** is the natural decay of urea, a nitrogen metabolite circulating in the blood. Blood cyanate concentrations, in equilibrium with urea at approximately 1:100, can reach levels as high as 1 mM in CKD patients, leading to excessive protein carbamylation. Kidneys, humans fastest-aging organs, experience a yearly decline in the glomerular filtration rate by about 1%, causing a steady accumulation of carbamylated proteins that adversely affect protein functionality and contribute to age-related diseases. Thus, carbamylation is a critical area of research in both CKD and aging, promising novel strategies for managing and potentially reversing the impact of aging at the molecular level. Although the carbamylation of plasma proteins has been extensively studied and linked to mortality in hemodialysis patients, little is known about its impact on the arterial wall and the development of CVDs. Most studies focus on carbamylated lipoproteins, which exhibit proatherogenic activities like inducing cell death, promoting macrophage foam-cell formation, and vascular smooth muscle proliferation. While carbamylation affects critical extracellular matrix proteins such as elastin and collagen and might promote calcification, the molecular mechanisms responsible for arterial stiffening and calcification remain largely unexplored. Understanding the mechanistic link between carbamylation and pathological changes in arterial wall is crucial for devising new protective methods against age-related molecular aging and CKD arterial pathologies.

Based on preliminary results, we hypothesize that carbamylation-triggered alterations are overlooked elements in hypertension pathogenesis. Our project will investigate the impact of carbamylation on aberrant extracellular matrix (ECM) formation, arterial wall remodeling, and extraosseous calcification, all pivotal in hypertension and atherosclerotic plaque development. We will explore the biochemical and molecular pathways through which carbamylation contributes to atherosclerotic plaques and hypertension. Given the evidence that carbamylation may serve as a potential preventative and therapeutic target to reduce morbidity and mortality from cardio-renal-metabolic diseases, we will also investigate how elasmobranchs (sharks, rays, skates) attenuate carbamylation reactions, potentially leading to the development of novel protective agents.

Therefore, the overall objectives of the project are as follows:

- Evaluate effect of carbamylation on biological activity of inhibitors of active mineralization.
- Characterise the impact of carbamylation on ECM formation, endothelial calcification *in vitro* and in clinical samples from CKD patients.
- Evaluate the impact of carbamylation on the artery stiffness in the different compartments of vascular wall *in vitro* and in animal model using the atomic force microscope (AFM).
- Correlate the carbamylation on ECM within the arterial wall with age-related pathologies.
- Explore novel paths to attenuate carbamylation reactions in the context of elasmobranchii physiology.