TITLE: Repurposing mitochondrial metabolism with SGLT2 inhibitors in obesity-induced chronic kidney disease

Authors and affiliations : Francesca Luna Vargas [1], Hélène Marlier [1], Jean-Marie Colet [2], Frédéric Debelle [1,3], Emmanuel Estève [1,4], Anne-Emilie-Declèves [1].

[1] Department of Metabolic and Molecular Biochemistry, Research Institute for Health Sciences and Technology, University of Mons, Mons, Belgium
[2] Laboratory of Human Biology & Toxicology, Research Institute for Health Sciences and Technology, University of Mons, Mons, Belgium
[3] Department of Nephrology and Dialysis, CH EpiCURA, Baudour, Belgium
[4] Inserm UMR S1155, Nephrology and dialysis, Tenon Hospital, AP-HP, UPMC

Chronic kidney disease (CKD) is a major healthcare burden. It already concerns 850 million patients worldwide and its prevalence is steadily increasing. This rise can be at least partially explained by the current epidemiology of obesity with or without diabetes, two independent well recognized CKD risk factors. Besides glomerular damage, proximal tubular epithelial cells (PTEC) are one of the main sites of injury in obesity related CKD. Obesity can lead to lipid and glucose overload that causes deleterious cellular effects in PTEC. Lipid overload can induce lipotoxicity and alter tubular structures and functions, notably because of mitochondrial and lysosomal dysfunctions.

The sodium-glucose co-transporter 2 inhibitors (SGLT2i) have emerged through the last decade as exerting a pleiotropy of beneficial effects beyond glycemic control in all stages of type 2 diabetes mellitus (T2DM). Several large clinical trials on gliflozins reported additional **nephroprotection**, even in non-diabetic patients. Experimental *in vitro* and *in vivo* studies revealed that the protective impact of SGLT2i in proximal tubular cells is associated with decreased oxidative stress, improved mitochondrial function, and reduced epithelial-mesenchymal transition notably preventing kidney fibrosis. Since obesity induced lipotoxicity disrupts mitochondrial function, a key target of SGLT2i action, we want to decipher how SGLT2i affect lipid loaded PTEC.

Therefore, HK-2 cells will be treated with either palmitic acid (PA, 300 µM or 500 µM) alone to stimulate excessive fatty acid influx and induce lipotoxicity, high glucose (20 mM) to induce glucotoxicity, or a combination of PA and glucose to further mimic T2DM. Two optimal SGLT2i concentrations will be selected for further analyses. Treatments are applied during 6, 24 or 48 hours. Cell metabolism and viability are assessed using conventional biochemical methods namely MTT and LDH release assays. In further experiments, intracellular lipid content, mitochondrial superoxide production and mitochondrial membrane potential will be assessed. **Mitochondrial bioenergetics** will be studied using the Seahorse XFe 24 Analyzer respirometry device. These analyses will be complemented with metabolomics approaches using **proton-nuclear magnetic resonance (NMR) spectroscopy** and mass spectroscopy to identify metabolites related to mitochondrial function beyond oxidative phosphorylation.
Eventually, elucidating the cellular and molecular mechanisms underlying SGLT2i tubular preservation could pave the way to a better stratification of CKD patients. Accordingly, new strategies could be established for a more personalized management of these patients.

Keywords: CKD, obesity, PTEC, mitochondrial metabolism, SGLT2 inhibitors