DESCRIPTION FOR THE GENERAL PUBLIC

A glomerulus is a tuft of small blood vessels (capillaries) located at the beginning of a nephron in the kidney. The unique, highly specialized and terminally differentiated glomerular visceral epithelial cells (podocytes) are an essential and integral part of the glomerular filter. These cells are the final barrier that restricts entry of plasma proteins from the circulation into the urine. Blood enters the glomerular capillaries and is filtered across the endothelium and the glomerular basement membrane and through the slit diaphragms between podocyte foot processes to produce the primary urinary filtrate. In healthy glomeruli, this barrier restricts the passage of macromolecules but is permeable to water and small solutes. Injury to podocytes leads to proteinuria, a hallmark of most glomerular diseases. Because podocytes, similarly as neuronal cells, are not able to proliferate, they are the most vulnerable component of glomerular filter. The loss of podocytes from the kidney glomerulus is an early and key event in the development of diabetic nephropathy, a chronic progressive disease that affects up to 40% of patients with diabetes mellitus. In advanced stage of the disease dialysis and even transplantation are necessary, which is related to growing costs of the treatment.

Anaerobic glycolysis converting glucose to pyruvate and then to lactate was recently demonstrated as the predominant metabolic pathway in podocytes. Correct podocyte energy metabolism and regulation are key elements in maintaining cellular structure and related kidney function. However, accumulating evidence has indicated that during diabetes, high glucose levels affect podocyte metabolism and function, and attenuate its insulin responsiveness, leading to the abolition of insulin-stimulated glucose uptake, and the increase of albumin transfer across the podocyte monolayer.

Glucose homeostasis is controlled by endogenous glucose production and glucose utilization rates. The two major sources of endogenous glucose production comprise the degradation of glycogen stores via glycogenolysis and the *de novo* synthesis of glucose via gluconeogenesis. In hyperglycemia, glycogen content accumulates in the kidneys as a result of different pathogenic mechanisms, including the defects in the activity of enzymes involved in glycogen synthesis and degradation. To the best of our knowledge, no studies have reported the ability of podocytes to produce glucose. Also in podocytes, the mechanisms regulating glycogen metabolism and the role of glycogen itself remain unknown. Our preliminary studies demonstrated considerably increased glycogen particles and enhanced activity of a key enzyme of gluconeogenesis in podocytes exposed to hyperglycemic conditions. We hypothesize that suppressed insulin action may underpin increased glucose production in podocytes. Additionally, the energy sensing pathways crucial for metabolic control, such as the protein deacetylase, SIRT1 and AMP-dependent protein kinase (AMPK), whose activities are impaired in insulin resistant podocytes, may be involved in modifications of endogenous glucose production in these cells. We hypothesize that the impairment of SIRT1-AMPK signaling caused by hyperglycemia may be related to defects within insulin-dependent regulation of glycogen metabolism and glucose production, thereby disturbing cellular glucose homeostasis in podocytes. Thus, the principal objective of the project is to examine effects of hyperglycemia on glycogen metabolism and endogenous glucose production in podocytes. We will also examine SIRT1-AMPK-dependent mechanisms of regulation of glycogen metabolism and gluconeogenesis in podocytes, and analyze its impairment caused by hyperglycemia.

The research project is divided into two parts: *in vivo* and *in vitro*. In *in vivo* study, we will use Zucker diabetic fatty (ZDF) rats, widely used as a model of type 2 diabetes and diabetic nephropathy. In experiments *in vitro*, we will use primary podocytes isolated from Wistar rats and immortalized line of human podocytes.
The molecular, biochemical, and functional studies will aim to examine effects of hyperglycemia on glycogen metabolism and gluconeogenesis regulation in podocytes and SIRT1-AMPK-dependent mechanism of regulation of these processes. Understanding the mechanisms of the regulation of intracellular glucose homeostasis in podocytes could provide further insights into glomerular disease pathogenesis and generate novel therapeutic targets for glomerulopathy treatment in diabetes. The effects of our study should bring us closer to understanding the alterations that take place in the course of diabetic nephropathy, leading in the final stage to impaired renal function.