Diabetic nephropathy (DN) is one of the chronic complications of diabetes which remains the leading cause of renal failure requiring renal replacement therapy. Structural and functional impairment of podocytes, which are localized on the external side of glomerular basement membrane, plays an important role in the development of the DN. Podocytes are characterized by high metabolic activity that enables these non-dividing cells to constitute one of the layers of the glomerular filtration barrier, which is constantly subjected to the proteomic renewal considering its functional burden connected with the filtration of large volumes of serum and high protein load. Podocytes are characterized by dense and extensive network of mitochondrial cisterns of various size and morphology, suggesting significant energy needs of these cells. Therefore, podocytes are required to maintain an adequate mitochondrial number, as well as to control their proper function, which in turn is dependent on mitochondrial fusion/fission processes and their intracellular transport. These processes are tightly controlled by regulator mechanisms, among which a particular type of autophagy, mitophagy, plays an prominent role. Mitophagy is a process of selective degradation of non-functional or damaged mitochondria, moreover, it serves for programmed removal of mitochondrial, e.g. in developing erythrocytes and in fiber cells of the embryonic eye lens. The proper mitophagy process determines efficient elimination of impaired mitochondria, accumulation of which triggers cellular stress (e.g. oxidative stress) and leads to apoptotic cell death. On the other hand, mitochondrial fusion is important especially under conditions of an increased cellular energy demands, since it promotes oxidative phosphorylation, allows biodistribution of fatty acids, and maintains effective ATP production.

It has been reported, that an impairment of mitochondrial dynamics is connected with aging processes, and also underlies the pathogenesis of various neurodegenerative diseases and type 2 diabetes. Accumulation of damaged mitochondria in muscle cells results in the disturbance of insulin signaling pathways and suppression of cellular glucose uptake. Additionally, the downregulation of one of the key markers of mitochondrial biogenesis in pancreatic beta cells in rodents leads to significant decrease of mitochondrial DNA content, reduced efficiency of oxidative phosphorylation and results in the development of diabetes. In podocytes mitochondrial biogenesis and mitophagy processes are poorly understood so far, although it seems plausible that they may be particularly important in the development of podocyte insulin resistance in the course of diabetes. Therefore, the main goal of the project is to analyse the potential changes in podocyte bioenergetics in diabetes, and in addition, to determine the role of mitochondrial dynamics and mitophagy in the pathogenesis of the DN.

The project involves a number of detailed tasks, which will be accomplished in order to reply the following questions: 1) What are the effects of high insulin and glucose concentration on the podocyte bioenergetic profile? 2) What is the role of mitophagy and mitochondrial biogenesis in the proper podocyte function and cell viability? and 3) Whether an impairment of mitochondrial dynamics and mitophagy in podocytes leads to the development of cell insulin resistance and dysfunction of the renal filtration barrier? The research will be conducted on human immortalized podocyte cell line, as well as on the primary rat podocytes. Additionally, a part of the study will employ animal models of diabetes: rats with chemically induced diabetes (through streptozotocin injection), and rodent model of genetically determined hyperinsulinemia and insulin resistance (Zucker rats). The evaluation of the bioenergetic profile will be based on the analyses of oxygen consumption rates and extracellular acidification rates in podocytes cultured in high insulin and glucose environment, which is typical for the early stages of the DN. In these cells multiparametric analysis of mitochondria will be carried out, concerning their number, size and shape (using electron microscopy), mitochondrial DNA content, intracellular localization of the organelles, and their co-localization with autophagy markers and with autophagic vacuoles. The project also includes studies on the role of mitophagy and mitochondrial biogenesis in the development of podocyte insulin resistance and in their proper function, which will be assessed after inhibition of the expression of chosen gene responsible for mitochondrial fusion/fission, or involving in the mitophagy processes (e.g. Drp1, Mfn1/2, parkin, PINK1). Functional analyses will be conducted through measurements of podocyte permeability to albumin *in vitro*, and also by assessment of albuminuria in diabetic animals, along with the evaluation of the levels of mitophagy markers in their kidneys.

On the assumption that diabetic milieu affects podocyte function, as well as modulates mitophagy and mitochondrial dynamics in other cells, attempts to determine the role of mitophagy in podocyte bioenergetics in high glucose and insulin environment seem to be of great importance to obtain a new and valuable knowledge on DN pathogenesis. It seems plausible that better understanding of the pivotal mechanisms contributing to bioenergetics regulation in podocyte in diabetes may provide not only promising therapeutic targets to establish more effective treatment of the DN, but also may indicate the potential molecular pathways which can be pharmacologically or genetically modulated in order to prevent the development of the DN in the course of diabetes.