Endothelial cells form the largest endocrine organ in the body, that cover huge, inner surfaces of the entire cardiovascular system. In turn, endothelial dysfunction (ED) is a hallmark of various diseases and may be regarded as a barometer of cardiovascular risk. Moreover, studies on ED development in mice *in vivo*, constituting a basic model in preclinical studies, are essential for the better understanding of the role of endothelial cells in the disease progression, for indication the drugs with detrimental or beneficial effects on the endothelium or for identification of sensitive parameters of ED. Currently, various tests are used for the endothelial phenotype assessment, but most of them are not sensitive to detect the early phase of ED. However, based on our preliminary results, we hypothesize that among many methods of ED measurements, assessment of changes in permeability *in vivo* may provide one of the most sensitive approaches to detect changes in endothelial phenotype, but this avenue was not explored further and presents the subject of this grant proposal. The number of publications describing preclinical studies on endothelial permeability *in vivo* in mice is limited, due to requirement of using specialized tools and methodology. One of the methods allowing detection of endothelial permeability changes *in vivo* is magnetic resonance imaging (MRI)-based assessment of the gadolinium contrast agent (CA) accumulation in the vessel wall. However, there is still lack of sensitive CAs, which are uptaken by endothelium in a similar way as low-density lipoproteins (LDLs) to mimetic pathophysiological events, occurring in the early stage of increased endothelial permeability, in atherosclerosis.

Therefore, the overall aim of this project is: firstly, to develop optimized MRI-based methodology for the endothelial permeability assessment in murine models *in vivo* and secondly to use this optimized method to characterize endothelial permeability changes induced by disease development and in response to pharmacotherapy.

The accumulation of LDL in the intimal layer of arteries represents a pivotal step in atherosclerosis development, therefore in the present project, the optimization of CA will rely on the development of LDL-mimetic liposomal CA, in order to increase the local concentration of CA in the vessel wall, uptaken in a similar way as LDL during increased endothelial permeability, in the very early phase of ED. In particular, liposome physicochemical parameters including size, composition, surface charge or surface accessibility, will be modified. Moreover, in the frame of optimization, liposomes will be decorated with apolipoprotein B100-mimetic peptide (the primary protein in LDL). During this studies the mechanism of the CA uptake in the vessel wall will be also identified. Alternatively to the MR imaging of increased endothelial permeability detected as an increased signal from accumulated in the vessel wall gadolinium-loaded liposomes, the imaging of 19-fluorine (^{19}F)-loaded liposomes will also be used, what will might provide a more sensitive way for detection of changes in endothelial permeability, because of the lack of any ^{19}F background in the body. In the second part of the project, optimized methodology will be used to assess the progression of increased endothelial permeability in E3L.CETP mice, the unique mice model for mild hyperlipidemia that displays human-like lipoprotein metabolism and representing a clinically-relevant model of ED. In E3L.CETP mice long term development of ED recapitulates slow progression of ED in humans with age-dependent changes in endothelium accelerated by mild hyperlipidemia. Our approach will allow for determining whether increased endothelial permeability assessment is suitable for the earliest ED detection, preceding other features of ED. Optimized MRI-based methodology for endothelial permeability assessment *in vivo*, will be also used to perform proof-of-concept study on effects of nuclear factor 2 (Nrf2) activators on endothelial permeability, which despite beneficial effects on the endothelium, evidenced by a decrease in reactive oxygen species production, displayed a differential effect on endothelial barrier function in human microvascular endothelium. Moreover, the role of endothelin-1 and Nrf2 pathways involvement in the regulation of endothelial permeability by Nrf2 activators will be studied, what has not been performed to this date.

Apart from knowledge-enhancing value, the presented project has also methodological significance. Studies performed in this project will provide a unique, pathophysiology-relevant method for early ED detection and comprehensive results increasing our knowledge on the significance of the endothelial permeability as a marker of early ED development *in vivo*, will explain mechanisms of LDL-mimetic liposomal CA uptake and will validate the developed method in murine studies. To the best of our knowledge, this type of study will be performed for the first time taking advantage of state-of-the-art methodologies, unique murine models and altogether it will open up new translational perspectives.