The vascular endothelium, considered the largest endocrine organ, produces numerous mediators that maintain vascular homeostasis. Among different features of the endothelial dysfunction, impaired endothelium-dependent vasodilatation and increased permeability are of special importance. Endothelial dysfunction represents a key step in the development of atherothrombosis, and is also a hallmark of various other diseases, including hypertension, diabetes, heart failure, as well as non-cardiovascular diseases such as cancer. Thus, pharmacotherapy targeting the endothelium provides a novel approach to the treatment of many such diseases. However, preclinical studies, evaluating the efficacy of endothelial-targeted pharmacotherapies , require continuous development due to the lack of real and robust methods to assess the phenotype of endothelial in animal models. Used in clinical studies, non-invasive methods of endothelial function assessment are not adequate for measurements performed in experimental condition due to the size of the animals (especially mice, which are currently the basic model in preclinical studies in biomedicine) and their frequency of the heart beat. Therefore, creation of new methods that will be able to overcome of the requirement of high spatio-temporal resolution, is necessary.

In this project, non-invasive method of imaging which is based on the phenomenon of nuclear magnetic resonance (MRI), characterized by high sensitivity and repeatability, will be used. Despite being less available, MRI technology provides an excellent tool with which to gain better insight into endothelium-dependent mechanisms of health and disease, both in clinical research as well as experimental studies. Simultaneously, this method provides a number of techniques and possibilities of the use of contrast agents, which allow for the study of endothelial phenotype, even in such small animals as mice are. The aim of this study is to develop and validate sensitive method for endothelial imaging *in vivo*, allowing for an effective assessment of impaired, endothelial-dependent vasodilatation response and permeability of endothelial cells, which are the main features of the phenotype of endothelial dysfunction; and secondly, use the developed methodology to assess the effects of various endothelial pharmacotherapies in murine model of atherosclerosis, in which endothelial dysfunction develops spontaneously.

In particular, this method will allow for assessment of:

- endothelial-dependent response to acetylcholine, causing vasodilatation in normal conditions and paradoxical, vessel contraction in the case of endothelial dysfunction;
- vasodilatation in response to increase in flow (FMD), which is a standard method for measuring endothelial function in humans and therefore it will open the way for translational research;
- changes in endothelial permeability by using new and standard contrast agents accumulating in the wall of the damaged vessel and shortening the relaxation time T_1 in vessel wall, which is identified with increased permeability of the vessel.

These protocols will be used in development of unique methodology of simultaneous assessment of endothelium-dependent vasodilatation response and changes in endothelial permeability *in vivo*. In turn developed methodology will be used in progression of endothelial dysfunction assessment in murine model of atherosclerosis, allowing to identify of early stage of changes in endothelial phenotype in atherosclerosis, indicating the point at which endothelial pharmacotherapy, should be initiated. Additionally, validation of the developed methodology, in terms of the detection of possibility of improvement and deterioration of endothelial function caused by drugs with proven beneficial (angiotensin converting enzyme inhibitors, perindopril) and detrimental (proton pump inhibitors, omeprazole) effects on endothelial function. Changes in endothelial function will be also confirmed by biochemical measurements of endothelial NO production in isolated blood vessels *ex vivo* with the use of electron paramagnetic resonance (EPR). Finally, developed methodology will be used in assessment of the impact of vitamin K_2 treatment on endothelial dysfunction development in atherosclerosis. Recent studies indicate that vitamin K_2 is absolutely necessary for the proper proliferation of endothelial cells, and this effect may also be responsible for vascular protection effect of vitamin K_2, reported in clinical trials. To date, impact of vitamin K_2 on endothelium, has not been characterized. Accordingly, this study will expand the knowledge in the field of impact of vitamin K_2 on the vascular system.

To sum up, as a result of the proposed project, comprehensive and multiparameter method to assess phenotype of endothelial cells, will be developed, which will allow to characterize the main features of endothelial dysfunction, even on its early stages of development. Therefore, the results obtained during the project will allow for a better insight into endothelium-dependent mechanisms of disease. In addition, the project will determine the effect of vitamin K_2 on the condition of the vascular endothelium, which to date has not been studied and characterized. These studies can open up new perspectives for research on pharmacotherapy of endothelium and on profiling of drugs effects on endothelium in murine models *in vivo*.