# Heterogeneity of perivascular adipose tissue in the browning process: at the crossroads of single-cell Raman spectroscopy and pathophysiology of adipocytes

Worldwide obesity has almost tripled since 1975. In 2019, more than half (53%) of adults living in the European Union were overweight or obese, and the situation worsened after the Covid-19 pandemic. Although obesity is preventable, due to its growing evidence, there is an urgent need for effective pharmacological therapy, the development of new systemic therapeutic strategies and understanding the physiology and pathological alterations of adipose tissue. Until now, it has been known that the status of the circulatory system is inseparable related to the condition of the vascular endothelium and research has focused on endothelial dysfunction. Recent research revealed that perivascular adipose tissue (PVAT) surrounding the aorta may contribute to cardiovascular and lifestyle diseases i.e. atherosclerosis and obesity. Thus, **PVAT is identified as a novel factor involved in the progression of vascular diseases**, and thus, becomes a new target of non-evaluated therapeutic potential.

The heterogeneity of the periaortic PVAT adipocytes results in regional differences in the phenotype of cells enabling to distinguish brown adipose tissue (BAT)-like region in the thoracic aorta and white adipose tissue (WAT)-like spots in the abdominal part. Such a variety of cell phenotypes carries consequences in terms of functionality and is also exposed in pathological processes. In high-fat diet (HFD)-induced obesity, changes related to lipid metabolism lead to increased fat accumulation in WAT and disturbance of the thermogenic function of BAT. **Recent studies have shown that HFD process is reversible** and proposed that the treatment of WAT adipocytes by specific stimuli leads to the formation of recently identified – **beige adipocytes.** The mentioned process is called **adipocyte browning** and due to their increased energy expenditure present a promising strategy to treat obesity. Consequently, inducing PVAT browning in the WAT like abdominal PVAT, and preserving the brown phenotype of thoracic PVAT is a crucial strategy to maintain a healthy vasculature.

Due to the plasticity of PVAT, **we hypothesize that the PVAT heterogeneous response to HFD can be restored by diet reversal, and this process can be further enhanced by induction of the brown-like phenotype of PVAT, which in turn will have a vasoprotective effect on the aorta (Fig. 1).** Among the relatively limited number of conventional techniques to investigate adipose tissue, the advantages of Raman-based methodologies and their versatility make them promising tools in such research. As a label-free and unbiased technique, Raman microscopy enables to study of chemical composition, especially lipid profile, and combines it with the high-resolution imaging and visualization of sample components. **Raman imaging is a rapid technique to study the effect of the active substance on the chemical changes in single adipocytes and the heterogeneity of the PVAT response.** Some relevant Raman markers have been established including the degree of lipid unsaturation to characterize qualitatively and quantitatively lipids in adipose tissue. The degree of lipid unsaturation degree is one of the most frequently used parameters and is evidenced to function as a chemical indicator of PVAT/vascular inflammation. Here, lipid unsaturation will be subjected as spectroscopic markers of adipocytes browning.

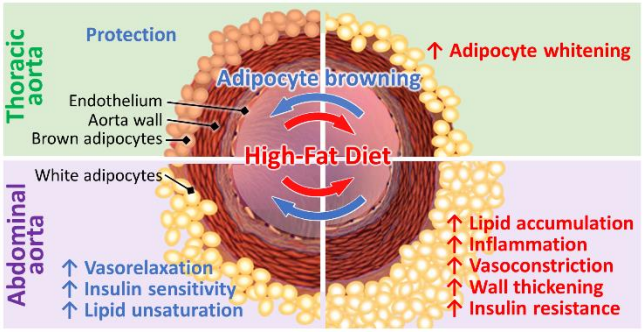


**Fig. 1. Working hypothesis of adipocytes browning effects on heterogeneous PVAT.** Increased fat accumulation and vasoconstriction in abdominal aorta by two-week HFD (marked in red) can be restored by diet reversal enhanced by adipocyte browning (blue), and reflected in Raman marker – lipid unsaturation degree.

The aim of the project is to **develop and validate a unique single-cell Raman imaging methodology for rapid analysis of PVAT adipocytes** and to **elucidate the molecular mechanisms behind PVAT heterogeneity and changes in PVAT lipid composition upon high-fat diet and its reversal through RNA sequencing**. The developed methodology will be applied for the examination of PVAT heterogeneity, the influence of high-fat diet on PVAT lipid profiles, and in pharmacological studies of PVAT adipocyte browning. The project employs development of *in vitro* model conjoined with molecular biology techniques, followed by *in vivo* verification of vasoprotective influence PVAT browning in physiological and HFD-induced obesity contexts. The far-reaching goal of this project is the implementation of Raman-based techniques as a tool for rapid screening for new or structurally modified molecules of browning potential. Given the cardiovascular relevance of PVAT, reversing HFD effects via lipid composition changes in PVAT with positive influence on endothelial function poses a significant challenge and opportunity for vascular biomedicine. Overall, **the project addresses novel methodological, mechanistic and functional aspects to explore PVAT pathophysiology.**