SRP Diabetes Postdoctoral Fellowship Program 2024

Project title: Differential local and systemic impact of adipose tissue on vascular function in patients with coronary artery disease and type two diabetes

Background. Even with optimal disease management, patients with Type 2 Diabetes (T2D) have a significantly heightened risk for developing atherosclerosis and subsequent cardiovascular disease[1]. T2D prevalence is increasing, underlining the need for novel measures that could protect people living with T2D from developing atherosclerosis. A pivotal, early step in atherosclerosis development is endothelial cell (EC) dysfunction[2]. EC dysfunction is marked by reduced endothelial nitric oxide bioavailability, which is exacerbated in T2D due to increased activity by the enzyme Arginase. While the role of Arginase in promoting atherosclerosis is established[3], **the influence of obesity and adipose tissue accumulation on EC dysfunction and Arginase activity is not known**. Moreover, how the arterial vasculature in T2D patients differentially is influenced by adjacently-located (perivascular, PVAT) and peripherally located (subcutaneous, SAT) adipose tissue remains unclear.

PVAT encapsulates larger vessels like arteries, and in the healthy state releases vaso-protective factors, including nitric oxide, directly affecting EC function[4]. Obesity triggers dysfunction of both PVAT and SAT, causing their secretory patterns to instead become pro-inflammatory and disease-promoting[4]. PVAT adjacent to atherosclerotic plaques is also altered in its tissue composition[5]. Despite progress, **knowledge gaps persist regarding the specific PVAT alterations** in patients with atherosclerotic coronary artery disease (CAD) that drive disease, and the contribution of altered Arginase activity within PVAT itself. How T2D affects Arginase activity in PVAT versus other fat depots, such as SAT that represents 80% of our fat mass[5], also remains unknown. By identifying how local changes in PVAT and Arginase activity promote EC dysfunction and atherosclerosis in patients with T2D, new disease pathways affecting plaque development can be identified, and potential therapeutic targets illuminated.

Objectives & Methodology. The postdoc will investigate **how T2D promotes endothelial dysfunction by altering the PVAT secretome and Arginase activity**. This is done combining clinical and *in vitro* assessments of vascular function with mechanistic and histological analyses of paired PVAT and SAT biopsies from CAD patients +/- T2D undergoing coronary bypass surgery (**Fig.1**). It will allow the postdoctoral fellow to both apply their clinical skills, and learn basic research methods, to identify disease pathways in PAT that are affected by T2D, thus potentially contributing to future development of treatments for high-risk patient groups such as those with T2D and cardiovascular disease.

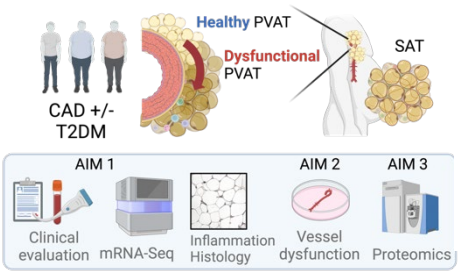

Fig. 1. Schematic summary. This proposal combines **Hagberg's** expertise of adipose tissue with **Kövamees'** expertise in vascular dysfunction and Arginase signaling to study how perivascular (PVAT) and subcutaneous (SAT) adipose tissue differentially affects vessel function in patients with CAD+/-T2D, with the aim to identify disease driving pathways and secreted proteins that in the future can be targeted as novel vasoprotective interventions.

Work plan. Our preliminary data shows conditional media from PVAT of T2D patients (T2D-PVAT) exerts decreased vessel vasorelaxation as compared to media from PVAT of patients without T2D (H-PVAT), suggesting the vasoprotective effects of the PVAT secretome is impaired by T2D (**Fig.2**).

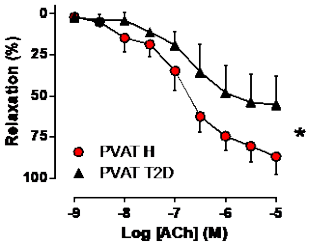

Fig.2. **Effect of PVAT secretome on vascular function.** Endothelial-dependent relaxation in aortic vessel segments incubated with healthy PVAT-medium (PVAT-H) or diabetic PVAT medium (PVAT-T2D). Tension is measured by myography during increasing concentration of the known endothelial dependent stimulator acetylcholine, reduced relaxation (as in the PVAT-T2D samples) notes EC dysfunction. *p<0.05. ACh; acetylkolin

The two specific aims of the project are to:

1. *Elucidate the morphological, transcriptional, and secreted differences between PVAT and SAT that associate with vascular dysfunction in patients with CAD +/- T2D.*

CAD-patients with and without T2D undergoing coronary artery bypass surgery will be included from the Karolinska thoracic department through Kövamees. The internal mammary artery is isolated together with PVAT, and SAT biopsies and blood work are taken at a later visit. All ethics and practicalities are in place, enrollment has started 02/2024. The postdoc will clinically evaluate the subject's **vessel function** from the brachial artery using flow-mediated vasodilatation (FMD) together with Kövamees (N=20 subjects/group based on power calculations). **Paired patient PVAT, vessel and SAT samples** will be collected during the first year (with Kövamees) and phenotyped by the postdoc (with Hagberg) for overall adipose histology and expression (bulk mRNA-Seq). A third of the biopsy will be cultured for 18 hrs and the media collected for vessel function assays (**Fig.2**) and proteomics analyses of secreted factors (see below). The postdoc will learn from Hagberg to analyze the data comparing paired PVAT and SAT samples, and biopsies from patients with CAD versus CAD+T2DM, mapping A) differentially expressed pathways, B) gene signatures for adipose tissue dysfunction[6] C) changes in Arginase activity and D) expression of cytokines and other secreted factors, and relate it to the patients' vascular function and adipose tissue morphology (cell size, inflammation, fibrosis). **This will allow mapping of the pathological changes specifically in PVAT that associate with vessel dysfunction and T2D.**

2. *Identify secreted mediators from PVAT that induce vessel dysfunction upon T2D, and the influence of Arginase activity on those mediators.*

To pinpoint paracrine mediators from PVAT affecting vascular function, the postdoc will learn to use the myograph model, of which Kövamees is expert, to record **tension measurements of isolated mouse vessel segments**. Vessel segments from healthy mice will be incubated 30 min with half of the conditioned media from human T2D-PVAT, or H-PVAT (collected under *Aim1*) before measuring their vasoreactivity (**Fig.2**). The other half of the **media will be analysed by proteomics** at the SciLife proteomics core facility together with collaborator Allan Zhou, and the postdoc will identify relevant secreted factors by correlating the proteomics results to the functional myograph tests. As mitigation Multiplex immunoassays will be used to detect specific secreted factors based on our RNA-Seq data from *Aim1*. Identified factors will be functionally validated using recombinant proteins or neutralizing antibodies against them in the above myograph model, experiments of which Hagberg is expert. To understand the unique role of the PVAT, identified factors can be measured in plasma from the same patients, or in conditioned media from the paired SAT biopsies. Lastly, the role of PVAT Arginase activity will be assessed by A) querying the RNA-Seq data from *Aim1*, B) evaluating Arginase activity through the measurement of urea production to the media, and C) resonance-measurements of reactive oxygen radicals with Kövamees. **Taken together, the postdoc will identify factors by which PVAT affects vessel function, and map the involvement of Arginase and nitric oxide in those effects**.

References: 1) Haffner SM. *et al*., N Engl J Med, 339(4):229-34 (1998) **2)** van Sloten TT. *et al.,* Hypertension. 64(6):1299-305 (2014) **3)** Ryoo S. *et al.*, Circ Res. 102(8):923-32 (2008) **4)** Cheng CK. *et al.*, Cardiov. Drugs Therapy 32:481–502 (2018) **5)** Hagberg & Spalding. Nat Rev Mol Cell Biol. 25(4):333 (2024) **6)** Li, Hagberg *et al*. Nat Med 27(11):1941-1953 (2021).

SRP Diabetes Postdoctoral Fellowship Program 2024

Contact details:

Main supervisor
Name and title: Carolina Hagberg, PhD
Associate Professor, Group Leader
Affiliation: Dept. of Medicine Solna, Karolinska Institutet
Email: carolina.hagberg@ki.se
Phone: +46 (0)707572204
Webpage: www.Hagberglab.com
https://ki.se/en/people/carolina-hagberg

Co-supervisor
Name and title: Oskar Kövamees, PhD
MD residency in Cardiology, Karolinska University Hospital
Affiliation: Dept. of Medicine Solna, Karolinska Institutet
Email: oskar.kovamees@ki.se
Phone: +46 (0)704070784
Webpage: https://ki.se/en/people/oskar-kovamees