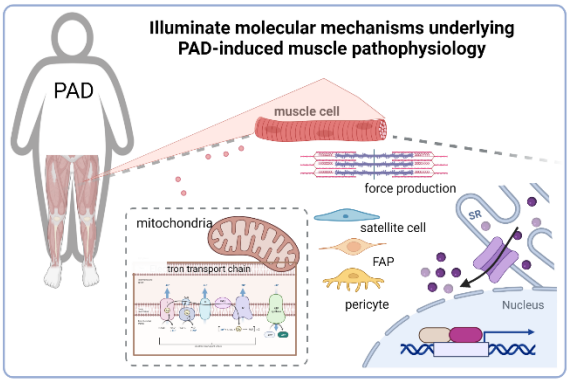
Illuminating mechanisms linked to muscle dysfunction in peripheral artery disease

Background: Comorbidities of type II diabetes (T2D) are major health concerns worldwide, including peripheral artery disease (PAD). The prevalence of PAD is three to four times higher and severe in diabetic individuals compared with nondiabetic individuals. The global prevalence of PAD is estimated to be ~200 million. PAD lowers blood flow to the lower limb, causing muscle ischemia and dysfunction, reduced exercise tolerance, which ultimately may lead to tissue necrosis[1]. Muscles of PAD patients exhibit skeletal muscle myopathy characterized by fibrosis, adipocyte accumulation, immune cell infiltrates, microvascular abnormalities, myofiber degeneration and myofiber dysfunction. Functionality of limb skeletal muscle is a key component for determining morbidity and mortality risk in patients with PAD. Interventions that improve distal arterial pressures (ie. bypass surgery or endovascular procedure) generally fail to normalize the structural and functional abnormalities of muscles, which point towards pathophysiological mechanisms inside the skeletal myofibers that reduce overall muscle function. However, despite serious muscle abnormalities, the field is lacking molecular understanding of PAD-induced muscle dysfunction. **Here we aim to use a translational approach involving PAD patients and mouse models and state-of-the-art methods going from functional analyses to single cell profiling to illuminate molecular mechanisms underlying PAD-induced muscle dysfunction.**



Objectives. This postdoctoral project has been divided into two objectives:

1. Determine the contractile properties and mitochondrial quality of myofibers from patients with PAD and healthy controls

2. Elucidate intramuscular communication in PAD using single cell sequencing of human biopsies followed by validation in vitro and in vivo

Methodology.

1. ***Determine the contractile properties and mitochondrial quality of myofibers from patients with PAD and healthy controls.*** Impaired mitochondrial function is observed in skeletal muscle from PAD patients[2]. In agreement, our preclinical mouse model of PAD (femoral artery ligation, FAL[3]) show that muscle weakness is accompanied by altered mitochondrial respiration and gene set enrichment analyses from the RNAseq of these muscles show alterations in signaling pathways related to mitochondrial assembly and cellular respiration (unpublished). Thus, altered mitochondrial function appears as one of the pathophysiological processes that contribute to PAD-induced muscle weakness. *Experimental layout:* Human skeletal muscle biopsies will be obtained from patients diagnosed with PAD and with and without T2D. The muscle biopsies will be used for multiple assessments, including **i)** RNA and metabolite extraction to obtain a global transcriptomic and metabolomic signature, respectively[4], from two unique muscle, **ii)** histopathological analysis, including electron microscopy **iii)** primary culture of human myocytes for mitochondrial respiration[3], and **iv)** *in vitro* motility assays for contractility (force) measurements[5]. Primary muscle cultures, adult muscle fibers and preclinical models will be used for gain- or loss-of-function experiments for selected candidate genes from the RNA-seq and metabolomics results. Determination of clinical parameters, incl. exercise/walking capacity and differential blood counts, electrolytes, serum lipids and HbA1c will also be assessed.

2. Elucidate intramuscular communication in PAD using single cell sequencing of human biopsies followed by validation in vitro and in vivo. In addition to contractile multinucleated muscle fibres, skeletal muscle contains many mononucleated cells that contribute to maintain healthy muscle

regeneration and function and hence when altered may interfere with muscle health. This intramuscular niche includes different populations of muscle resident cells, e.g., satellite cells, perivascular stem cells, and mesenchymal progenitor cells[6]. Satellite cells have traditionally been implicated as the primary cell population to be responsible for the normal regeneration of skeletal muscles. However, non-satellite interstitial cells have gained attention for their involvement in muscle regeneration and health, including pericytes and fibro/adipogenic progenitors (FAPs)[7,8]. Also, FAPs and pericytes can contribute to fibrosis and ectopic fat accumulation[9,10], common characteristics in PAD-induced muscle dysfunction. In the muscle field, intermuscular communication between cell types is predominantly based on histological analyses and, to our knowledge, currently no human data available of single cell RNA sequencing (sc-seq) of muscles of PAD individuals. *Experimental layout:* We will use single cell profiling linked to tissue histology. Muscles will be processed and single cell RNA-sequencing using the 10x-Chromium system will be used. Based on prior experience of collaborators from analysis of healthy human muscle biopsies, ~5000 cells per muscle will be sequenced, generating an overall dataset with transcript information for ~30 000 cells from both weakened and healthy muscles, respectively. These data will be processed using a pipeline developed for analysis of cell types from human skeletal muscle to cluster cells of similar expression patterns, and to categorize specific cell-types. Top selected candidate genes will be used for linked with tissue histology and validation *in vitro* (in primary human myocytes or adult mouse muscle) and *in vivo* (in mouse models; genetic and/or inducible models). This will be a first-of-its-kind study, leading to detailed molecular understanding of intramuscular signaling and contractile function, which will yield substantial novel insights into PAD muscle pathology.

Work plan: The postdoc will play a key role in the project and bring new ideas into the research group which complements the labs' work on muscle pathophysiology. Lanner's research team currently consists of two postdocs, two PhD students, and a Master student. Working in a smaller sized group, will allow the postdoc to become an integral part of the research team and be engaged in all aspects of this collaborative project to achieve the scientific output. With guidance from the PI, the postdoc will lead the overall scientific work including collecting data, running analyses (including global and single cells analyses) and draft manuscripts to achieve the scientific output. The postdoc will also supervise doctoral and undergraduate students, allowing for progress to a more senior role than previously. Moreover, **the SRP Diabetes network** offers the postdoc a strong environment for muscle and metabolic research, hence the postdoc will gain invaluable experience and gain access to networks. Altogether, a postdoc in Lanner lab will promote the recruit's expertise in curiosity-driven research and leading a team, a skill set that will be crucial in me becoming a successful independent group leader in the future.

Relevance of project for diabetes: T2D is reaching epidemic proportions worldwide and comorbidities of diabetes are major health problems, including PAD. Muscles of PAD patients exhibit debilitating skeletal muscle myopathy, which can cause significant long-term disability and sick-leave, and thus a socioeconomic burden, as well as reduced quality of life for afflicted patients. Patients with PAD require surgical intervention to restore blood flow to the affected limb, but the restoration of arterial flow does not largely improve skeletal myopathy and highlights the need for new efficient treatment options for these patients. ***A SRP Diabetes postdoc will be a highly appreciated recruit and will act as a catalyst, allowing us to untangle molecular aspects of PAD-induced muscle dysfunction which will lay the groundwork for future pharmacological discoveries to improve muscle function for patients afflicted by muscle dysfunction.***

References. 1: Heffron, S. P. et al. Atherosclerosis 292 (2020). **2:** Thompson, J. R. et al. Ann. Surg. 261, (2015). **3:** Liu, Z. et al. FASEB J 35, (2021). **4:** Agudelo, L. Z. et al. Nature comm 10, (2019). **5:** Ušaj, M. et al. Commun biol 4, (2021). **6:** Dellavalle, A. et al. Nature comm 2, (2011). **7:** Joe, A. W. et al. Nat Cell Biol 12, (2010). **8:** Giordani, L. et al. Mol. Cell 74, (2019). **9:** Birbrair, A. et al. Stem cells and development 22, (2013). **10:** Uezumi, A., et al. Nat Cell Biol 12, (2010).

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