SRP Diabetes Postdoctoral Fellowship Program 2025

Project title: Molecular mechanisms causing skeletal muscle dysfunction in polycystic ovary syndrome

Background

Polycystic ovary syndrome (PCOS) affects ~15% of women globally, posing a significant yet underexplored health challenge[1]. Characterized by hyperandrogenism and infertility, PCOS is associated with comorbidities such as insulin resistance (~75%), type 2 diabetes (>40% by age 40), and cardiovascular disease[1], contributing to a multibillion-euro annual healthcare burden. [1]Lifestyle modification is the first-line treatment for PCOS and insulin resistance, often followed by antidiabetic drugs like metformin[1]. In hyperandrogenic PCOS, skeletal muscle exhibits impaired energy and glucose metabolism, resulting in insulin resistance. Studies from our lab have identified altered protein and gene expression, DNA methylation, and lipotoxicity in PCOS skeletal muscle[2-4]. **However, the specific cell populations, molecular mechanisms, and intercellular crosstalk driving PCOS-related metabolic dysfunction remain unexplored.**

Objectives of this postdoctoral project is to uncover the molecular and cellular pathways driving PCOS-specific skeletal muscle dysfunction, a key contributor to insulin resistance and type 2 diabetes risk. Leveraging state-of-the-art single-cell technologies, the study will characterize cellular and transcriptional profiles in skeletal muscle biopsies from women with and without insulin resistance and hyperandrogenemia. This approach aims to identify candidate therapeutic targets for drug development. Integrating single-cell transcriptomics with unique *in vitro* models, the project will delineate PCOS-specific phenotypic abnormalities and unravel the intercellular interactions driving skeletal muscle dysfunction in PCOS.

Specific aims are to:

1) Identify cell type-specific disease signatures, variations in cellular composition, and gene regulatory networks in skeletal muscle from women with and without PCOS.
2) Determine the efficacy of lifestyle modification, muscle contractions, and metformin in reversing PCOS-specific cellular and transcriptional signatures.
3) Investigate molecular mechanisms and disease signatures in fibro-adipogenic progenitors, focusing on their mitochondrial function and glucose metabolism *in vitro*.

Methodology and workplan

Single-nuclei RNA sequencing (snRNA-seq) has been conducted on frozen skeletal muscle biopsies from 8 controls and 20 women with PCOS, collected at baseline and after 16 weeks of treatment: lifestyle management, with or without metformin or electrical stimulation-induced muscle contractions. This transcriptional dataset enables detailed mapping of PCOS-specific skeletal muscle cellular composition, identification of cell-type-specific features, and exploration of subtype-specific molecular mechanisms and treatment effects.

Given the emerging significance of intercellular communication in skeletal muscle responses to hyperandrogenism and obesity, CellChat analysis will be utilized to assess interactions among identified cell types based on ligand-receptor expression. To determine genetic influences on specific skeletal muscle cell types, CELLECT will integrate snRNA-seq data with genome-wide association studies (GWAS), leveraging established links between obesity, type 2 diabetes, glycemic traits, and PCOS[5,6].

Preliminary snRNA-seq analyses highlight dysregulation in fibro-adipogenic progenitors (FAPs), characterized by aberrant extracellular matrix remodelling, mitochondrial function, BMP and TGFß-signalling, which are reversed by metformin. Preliminary CellChat analyses depicts a clear shift in FAP and skeletal muscle cells in BMP, collagen, laminin and ANGPTL signalling network. FAPs, crucial for glucose tolerance, muscle repair, and regeneration, will be isolated from a subset of participants and androgen-induced PCOS-like mouse models. This will enable mechanistic comparisons between women

with PCOS and controls with similar age, weight and BMI, refining insights into cell-type-specific disease signatures and molecular pathways.

Materials and Methods

The ongoing clinical trial (Ethical approvals: 2015/1656-31/2, 2024-0063-02; ClinicalTrials.gov: NCT026478274) recruit women after written and oral consent at the Women's Health Unit, Karolinska University Hospital, Stockholm, in collaboration with Prof. Angelica Linden Hirschberg. PCOS diagnosis follows the 2003 Rotterdam criteria, with mandatory hyperandrogenism. Matched controls (age, weight, BMI) lack hyperandrogenism and exhibit regular menstrual cycles (28 ± 2 days). At baseline, women with PCOS are randomized into three groups for a 16-week intervention: (1) Lifestyle management, (2) Metformin + lifestyle management, or (3) Electrical stimulation (muscle contraction) + lifestyle management. Comprehensive phenotyping is conducted at baseline and post-intervention, assessing reproductive and endocrine parameters, glucose metabolism, and body composition.

Serum and Tissue Collection. Serum and *vastus lateralis* muscle biopsies are collected during the proliferative phase (days 6–8) between 8–10 a.m., minimizing variations due to the menstrual cycle or circadian rhythms. Biopsy samples are processed as follows: one portion is snap-frozen in liquid nitrogen and stored at -80°C; a small portion is fixed for detailed histology; the remainder is used for muscle stem cell isolation.

Nuclei Extraction and snRNA Sequencing. Nuclei has been extracted from frozen skeletal muscle tissue, and single-nuclei RNA sequencing (snRNA-seq) libraries prepared using the 10X Genomics platform. Sequencing (~10,000 nuclei/sample and ~30,000 raw reads/cell) was performed at Novogene-Europe, UK. The postdoc will work with data processing using Cell Ranger (10X Genomics) and analysis with Seurat 4.0. Following our established pipeline[7]—including filtering, integration, clustering, dimensionality reduction, and differential gene expression analysis—CellChat will be used to infer and analyze intercellular communication, while CELLECT will identify genetic contributions to PCOS-specific dysfunction in candidate cell types.

Cellular and Molecular Function of FAPs. To investigate dysregulation in the FAP subpopulation in skeletal muscle of women with and without PCOS, as well as in an androgen-induced PCOS mouse model, the postdoc will employ a multifaceted approach. Skeletal muscle tissue will undergo FACS sorting to isolate FAPs, followed by differentiation using fibrogenic and adipogenic cocktails. Bulk RNA sequencing (Prime-seq) will profile their transcriptional landscape, while bioenergetic function will be evaluated using the Seahorse XFe96 platform. Glucose uptake will be assessed via a 2-Deoxy-D-glucose assay. Fibrotic capacity will be assessed with Sirius Red staining. The response of FAPs to treatments, including metformin and semaglutide, and their potential as targets for novel therapeutic strategies will also be explored.

Diabetes Relevance

With 75% of women with PCOS exhibiting insulin resistance and 40% developing type 2 diabetes before age 40, identifying key contributors to skeletal muscle metabolic dysfunction is crucial. This project addresses the urgent need for better prevention and treatment strategies by**:**

1. **Identifying Key Drivers**: Pinpoint cell types and molecular contributors to skeletal muscle dysfunction in PCOS, including their biomarker potential and response to treatment.
2. **Unravelling Mechanisms**: Investigate the molecular basis of PCOS-specific muscle dysfunction and its systemic metabolic effects.
3. **Advancing Therapies**: Discover novel therapeutic targets to address metabolic complications in PCOS.

References – all publications are from postdoc lab

1. Stener-Victorin, E.*, et al.* Polycystic ovary syndrome. *Nat Rev Dis Primers* **10**, 27 (2024).
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3. Benrick, A.*, et al.* Electroacupuncture mimics exercise-induced changes in skeletal muscle gene expression in polycystic ovary syndrome women. *J Clin Endocrinol Metab* **105**, 2027-2041 (2020).

4. Nilsson, E.*, et al.* Transcriptional and Epigenetic Changes Influencing Skeletal Muscle Metabolism in Women With Polycystic Ovary Syndrome. *The Journal of Clinical Endocrinology & Metabolism* **103**, 4465-4477 (2018).
5. Liu, Q.*, et al.* A genome-wide cross-trait analysis identifies shared loci and causal relationships of type 2 diabetes and glycaemic traits with polycystic ovary syndrome. *Diabetologia* **65**, 1483-1494 (2022).
6. Liu, Q.*, et al.* Genomic correlation, shared loci, and causal relationship between obesity and polycystic ovary syndrome: a large-scale genome-wide cross-trait analysis. *BMC Med* **20**, 66 (2022).
7. Eriksson, G.*, et al.* Single-Cell Profiling of the Human Endometrium in Polycystic Ovary Syndrome. *Nature Medicine* (2025) Mar 20. doi: 10.1038/s41591-025-03592-z. Online ahead of print.

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