Reprograming insulin signalling in the human cardiovascular system

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Please note that a Novo Nordisk mentor will also be assigned to this project.

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Project outline

Background: Insulin therapy, the mainstay of treatment in advanced type 2 diabetes mellitus (T2DM), has recently been shown to exert detrimental cardiovascular effects in patients with advanced atherosclerosis, due to the presence of insulin resistance (IR) in the cardiovascular cells and the surrounding perivascular adipose tissue (PVAT). Activation of dysregulated insulin signalling in the human vascular wall and PVAT, results in increased vascular oxidative stress and activates pro-atherogenic processes. There is, therefore, an unmet need to better understand the pathogenesis of organ-specific, molecular IR in the context of CVD, and to develop novel strategies of insulin sensitisation, to harness the full therapeutic potential of insulin.

Hypothesis: We hypothesise that: 1) cardiovascular cells are characterised by molecular IR in patients with atherosclerosis, independently of systemic IR status; 2) adipose IR may be influenced by systemic parameters such as obesity, diabetes and log-grade inflammation; 3) cardiovascular insulin sensitisation may reverse the direct detrimental effects of insulin, inducing cardioprotective phenotypes.

Aims: 1) to characterise insulin signalling in cardiovascular cells [endothelial cells (ECs), vascular smooth muscle cells (VSMCs) and PVAT adipocytes], in the context of human atherosclerosis; 2) to investigate the effects of low-grade systemic and local inflammation, obesity and diabetes on cardiovascular adipose insulin signalling, in patients with atherosclerosis; 3) to propose novel regulators of molecular insulin signalling in human vessels and PVAT that could act as targets for organ-specific cardiovascular insulin sensitization.

Experimental work plan: This programme of work will be built on the Oxford cohort for Heart, Vessels and Fat (OxHVF), one of the most well-phenotyped cohorts of cardiac surgery patients: a large biobank of vascular (internal mammary arteries-IMA, saphenous vein-SV), myocardial and adipose tissue (AT) biopsies (from 5 different deports, including epicardial, pericardial, perivascular, subcutaneous and gluteal) along with circulating biomarker measurements, whole genome data and already extracted RNA sequencing tissue data for the participants (n>1600), as well as cardiac CT images. There is ongoing tissue collection (~3-5 patients /week), and samples are used for *ex vivo* tissue culture as well as cardiovascular cell isolation for human cell culture experiments.

Aim 1: We will interrogate the details of insulin signalling in ECs, VSMCs isolated from patients with advanced coronary atherosclerosis (both from diabetic and non-diabetic patients), as well as primary cells cultured with established atherosclerosis-mimicking protocols. Our characterisation will use

standard molecular biology techniques such as western blotting and quantitative real-time polymerase chain reaction, and will span the whole range of insulin signalling, from receptor expression to downstream second messenger expression and/or selective phosphorylation [e.g., insulin receptor substrates (IRSs), Ak, MAPK). The dominant pathway directions will then be further investigated, in each cell type, with targeted known-down cell culture models (e.g., siRNA for IRSs). We will also test the direct effects of insulin signalling on redox state (previously shown by us to be affected by dysregulated insulin signalling in intact arteries), in individual cell types. Finally, we will investigate the whole spectrum of insulin effects on human arteries, ECs and VSMCs with transcriptomics and gene ontology enrichment analysis (RNA sequencing data already extracted for arterial samples from the OxHVF cohort).

Aim 2: We will isolate pre-adipocytes from human AT of obese vs non-obese, and diabetic vs non-diabetic OxHVF patients, and we will investigate the elements of insulin signalling in each sub-group, as in aim 1. The proof-of-concept for the effect of inflammation on adipose IR will be assessed by adipocyte culture in the context of *in vitro* inflammation (e.g., by inflammatory cytokine cocktail pre-treatment). We will also assess the effect of insulin on pre-adipocyte differentiation in each sub-group. Finally, we will investigate the integrated effects of insulin on adipocyte function and secretome via multi-omics analyses (e.g., tissue transcriptomics and secretome proteomics and/or metabolomics). This will also reveal novel secreted AT modulators with potential endocrine and paracrine cardiovascular effects. This aim will also utilise RNA sequencing AT data that are available in the OxHVF cohort, to explore novel links between AT biology and IR.

Aim 3: Previous data from our group have shown that endogenous molecules such as dipeptidyl peptidase 4, may regulate molecular insulin signalling in humans. We aim to expand on existing pilot data available, to test the efficiency of novel cardiovascular insulin sensitizers, in the context of atherosclerosis. These proposed endogenous regulators of cardiovascular insulin signalling will first be validated in intact tissues *ex vivo* (i.e., IMA and SV for vascular redox state, SV rings for endothelial function by vasomotor studies), to confirm their ability to modulate clinically relevant phenotypes in response to insulin. Underlying mechanisms will then be investigated by targeted knock-down experiments in primary cardiovascular cells (e.g., knock-down of relevant membrane receptors or downstream second messenger inhibition in ECs and/or VSMCs). Transcriptomic analyses in primary cells and intact arteries will also be undertaken, to elucidate the full effect spectrum of the proposed insulin signalling modulators. Finally, we will test the ability of these regulators to influence insulin signalling in primary pre-adipocytes.

Expected value of the results: We will reveal novel, previously undescribed effects of insulin in the vasculature and AT, in the context of atherosclerosis in real-life patients. We will further propose novel ways of pharmacologically "reprogramming" cardiovascular insulin signalling in humans with atherosclerosis. Our results will be derived from already available human tissue RNA sequencing data as well as targeted *in vitro* and *ex vivo* mechanistic models with intact tissues and primary cells, which will hugely increase their impact and clinical translation potential. These may revolutionize the treatment of diabetic patients with atherosclerosis (particularly in insulin-dependent T2DM), with the potential to significantly improve cardiovascular outcomes in such patients.

Supervisor's recent relevant publications

1. Carena MC….**Antoniades C**; "Role of Human Epicardial Adipose Tissue-Derived miR-92a-3p in Myocardial Redox State". J Am Coll Cardiol. 2023;82(4):317-332
2. Kondo H, **Akoumianakis I**…**Antoniades C**; "Effects of canagliflozin on human myocardial redox signalling: clinical implications". *Eur Heart J* 2021;42(48):4947-4960
3. Akawi N….**Antoniades C**; "Fat-Secreted Ceramides Regulate Vascular Redox State and Influence Outcomes in Patients with Cardiovascular Disease". *J Am Coll Cardiol* 2021;77(20):2494-2513

4. **Akoumianakis I**…**Antoniades C**; "Insulin-induced vascular redox dysregulation in human atherosclerosis is ameliorated by dipeptidyl peptidase 4 inhibition". *Sci Transl Med* 2020;12(541):eaav8824
5. **Akoumianakis I**…**Antoniades C**; "Adipose tissue-derived WNT5A regulates vascular redox signaling in obesity via USP17/RAC1-mediated activation of NADPH oxidases". *Sci Transl Med* 2019;11(510):eaav5055