Exploring a novel signalling in "dysfunctional" adipose tissue, mediating the cardiovascular complications of diabetes

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Background

Adipose tissue releases numerous mediators that exert endocrine and paracrine effects on the vascular wall. We recently found that in patients with obesity or type 2 diabetes, perivascular adipose tissue is "dysfunctional" and secretes highly pro-atherogenic mediators.[1] We have established, in recent years, the Oxford Heart Vessels Fat cohort (ox-HVF),[1-5] which builds a unique, very well characterised cohort of patients undergoing cardiac surgery and stores a wide range of tissue samples, including various types of adipose tissue/adipocytes, human vessels/vascular cells, myocardial samples and others. By screening the tissue samples of the ox-HVF we have identified a ligand that is normally involved in wnt signalling in the adipose tissue, released in high amounts from the human perivascular adipose tissue in obesity/insulin resistance or type 2 diabetes. The release of that ligand is strongly related with vascular redox state in the patients included in the ox-HVF. Then using *in vivo* and *ex vivo* methodology we observed that this ligand controls vascular redox signalling, by activating specific pro-oxidant enzymatic systems in the human vascular smooth muscle cells and endothelial cells, promoting their pro-atherogenic phenotype. Despite our strong pilot data, the causal involvement of this novel aspect of the cross-talk between AT and the vascular wall is not yet documented, and the mechanisms mediating its vascular effects *in vivo* are unknown.

Hypothesis

The newly identified ligand is highly released from the human adipose tissue in obesity and type 2 diabetes and may exert paracrine or endocrine effects on the vascular wall in vivo. These effects result in the activation of pro-oxidant enzymes in the vascular wall (e.g. NADPH oxidases), and the identified ligand may serve as a master regulator of vascular redox signalling and vascular disease pathogenesis.

Aims

To investigate the causal links of a newly identified adipose tissue-derived ligand with vascular disease pathogenesis *in vivo,* and to explore its role as a trigger of cardiovascular complications in diabetes.

Description of the experimental work

Following our clinical/translational programme of work that identified the striking links between a novel adipose tissue-derived ligand and vascular redox state, we have recently generated a new Flox mouse model over-expressing that ligand when crossed with a mouse expressing Cre-recombinase in specific cells or tissues. The current project will offer the exciting opportunity to use this new animal model for the first time, in order to understand the causal role of this ligand in vascular disease pathogenesis. In the context of the project, we will generate global and tissue-specific mice overexpressing the transgene of interest in adipose tissue, in an inducible way using $CreER^{T2}$ and tamoxifen. The first data from our model suggest a striking phenotype in these animals, so the project will first characterise the metabolic consequences of this genetic modification, and will explore its implications in the expansion of the adipose tissue/adipocyte differentiation as well as its impact on the adipose tissue "secretome" and its inflammatory cell infiltration. Then by using state-of-the-art methodology, the project will explore the endocrine and paracrine effects of the new ligand on the regulation of vascular redox state and its impact on redox sensitive transcriptional pathways. As in the clinical studies, we have seen striking associations between this ligand's release from perivascular adipose tissue and endothelial function. This project will explore the causality of these associations and will characterise the underlying mechanisms of its vascular effects.

Finally, the project will use *ex vivo* adipose tissue explants from animals overexpressing the new ligand to test the paracrine effects on intact aortas as well as specific vascular cell populations (i.e. vascular smooth muscle cells) isolated from the aortas of wild type animals,[1] and will focus on studying the ability of this ligand to modify key features of the biology of these cells, involved in vascular disease pathogenesis (e.g. vascular redox signalling, cell migration and proliferation and others).

Value of the results

This will be the first study exploring the causal role of a recently identified ligand released from the human perivascular adipose tissue, in the regulation of vascular redox state and other key mechanisms of vascular disease pathogenesis, using novel animal models. Exploring the molecular basis of the cross-talk between adipose tissue and the vascular wall will allow better understanding of the role of adipose tissue in the vascular complications of obesity and diabetes, identifying novel therapeutic targets in vascular disease pathogenesis. As this study will be complemented by existing data from our clinical and translational programme of work, its translation to man will be immediate.

Recent Relevant Publications

1. Antonopoulos AS, Margaritis M, *et al*. (2015) Adiponectin as a Link Between Type 2 Diabetes Mellitus and Vascular NADPH-Oxidase Activity in the Human Arterial Wall: The Regulatory Role of Perivascular Adipose Tissue. *Diabetes* **64**: 2207-19.

2. Antonopoulos AS, Margaritis M, *et al*. (2016) Mutual Regulation of Epicardial Adipose Tissue and Myocardial Redox State by PPAR-γ/Adiponectin Signalling. *Circ Res* **118**: 842-55.

3. Antonopoulos AS, Margaritis M, *et al*. (2014) Reciprocal effects of systemic inflammation and brain natriuretic peptide on adiponectin biosynthesis in adipose tissue of patients with ischemic heart disease. *Arterioscler Thromb Vasc Biol* **34**: 2151-9.

4. McNeill E, Crabtree MJ, *et al*. (2015) Regulation of iNOS function and cellular redox state by macrophage Gch1 reveals specific requirements for tetrahydrobiopterin in NRF2 activation. *Free Radical Biology & Medicine* **79**: 206–216.

5. Margaritis M, Antonopoulos AS, *et al*. (2013) Interactions between vascular wall and perivascular adipose tissue reveal novel roles for adiponectin in the regulation of endothelial nitric oxide synthase function in human vessels. *Circulation* **127**: 2209-21.

6. Antoniades C, Bakogiannis C, *et al*. (2011) Rapid, direct effects of statin treatment on arterial redox state and nitric oxide bioavailability in human atherosclerosis via tetrahydrobiopterin-mediated endothelial nitric oxide synthase coupling. *Circulation* **124**: 335-45.