Significant diastolic dysfunction was observed in WT STZ mice (E/A ratio: control 1.6±0.09 versus STZ 1.3±0.04). However, whilst Tg control mice demonstrated impaired diastolic function (E/A ratio: 1.6±0.09 vs 1.4±0.08), no further dysfunction was seen with experimental diabetes. Consistent with basal diastolic dysfunction, CTGF and MMP2 expression were increased in Tg control animals, without being further altered by STZ, whereas CTGF was increased in WT STZ animals versus controls. Interestingly, increased expression (compared to WT control animals) of SOD1 (WT 31% ±6.3%, Tg 78%±24%) and catalase (WT 24%±14%, Tg 85%±28%) seen in STZ diabetes was greater in Tg than in WT mice, which is likely to at least partly explain protection against further diastolic dysfunction.

Conclusion These data indicate that endothelial Nox4 NADPH oxidase may protect against adverse cardiac remodelling and dysfunction in experimental diabetes, thereby highlighting this major ROS source as a potential therapeutic target for CHF in diabetes.

10 AM2/IMD SECRETION FROM HUMAN PULMONARY SMOOTH MUSCLE CELLS AND PULMONARY FIBROBLASTS IS AUGMENTED IN RESPONSE TO MECHANICAL STRETCH

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Introduction Pulmonary hypertension (PHT) is a severe life-limiting condition resulting in progressive shortness of breath, exercise intolerance and heart failure. PHT is defined by increased mean pulmonary arterial pressure (PAP) ≥25 mmHg at rest, and has been attributed to an imbalance between vasodilator and vasoconstrictor influences in the pulmonary microcirculation. Assessment of the vasodilator AM2/IMD, a member of the CGRP/AM peptide family, may have potential application as novel disease biomarker.

Objective To quantify secretion of AM2/IMD from human pulmonary vascular cells cultured under basal, simulated normotensive and hypertensive conditions.

Methods Pulmonary fibroblasts (PF), pulmonary smooth muscle (PSM), human pulmonary artery endothelial cells (HPAEC) and human pulmonary microvascular endothelial cells (HPMEC) were cultured on silicone elastomer-bottomed Flexcell plates pre-coated with Matrigel at rest (un-flexed) or subjected to cyclic mechanical stretch (Flexcell Strain Unit) to simulate pulmonary normotensive (15 mmHg, 2.0 kPA) and hypertensive (40 mmHg, 5.3 kPA) conditions at a frequency of 1 Hz (60 cycles per minute) for 48 hour. AM2/IMD was extracted from the medium of cultured cells and quantified by ELISA (Phoenix Pharmaceuticals Inc. Karlsruhe, Germany).

Results Concentrations of AM2/IMD in culture medium from cells incubated under various conditions were as follows: ng. $ml^{-1,}$ mean ±SE, n=2–12; *difference relative to un-flexed, $p<0.05$; +difference between normotensive and hypertensive condition.

Conclusion Cyclic stretch enhanced secretion of AM2/IMD from PF and PSM, indicating that these cells may be an important source of this vasodilator peptide in the pulmonary microcirculation under physiological conditions. Secretion was not augmented in hypertension relative to normotensive conditions. AM2/IMD is unlikely therefore to be a suitable diagnostic or prognostic biomarker in PHT.

Abstract 10 Table 1

	PF	PSM	HPAEC	HPMEC
un-flexed	6.98±2.01	0.40±0.06	1.28±0.24	12.63±1.38
normotensive	51.49 ±11.25*	106.81 ±59.22	0.48±0.06*	13.53±1.67
hypertensive	41.58±8.57*	83.65 ±20.53*	0.82±0.07+	18.96 ±2.43*

11 THE ROLE OF N-GLYCOSYLATION OF THE NOTCH1 RECEPTOR IN JAGGED1-STIMULATED MYOGENIC DIFFERENTIATION *IN VITRO*

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Resident stem cell fate decisions within the vasculature are crucial to the pathogenesis of vascular diseases, including, arteriosclerosis, atherosclerosis and in-stent restenosis after angioplasty. The Notch signalling pathway regulates stem cell fate and is highly regulated by a number of mechanisms including glycosylation, a post-translational modification.

Our main objective was to define a putative role for N-glycosylation of Notch1 receptor in controlling resident vascular stem cell fate *in vitro*. Utilising ligand-induced Notch signalling assay with Jagged1, qRT-PCR, immunocytochemistry, ectopic expression of Notch1 receptor, siRNA knockdown, pharmacological inhibition and enzyme linked lectin assay (ELLA), alterations in N-glycan decoration of the Notch1 receptor were assessed before evaluation of their effects on Notch signalling and Notch ligand promotion of myogenic differentiation.

N-glycosylation of the Notch1 receptor was assessed using a combination of the HPLC and ELLA assays and confirmed the presence of N-glycans on the receptor, an effect that was abrogated following inhibition of glycosyltransferase activity with tunicamycin and lunatic fringe (Lfng) knockdown. Jagged1- induced Notch activation increased Notch target gene expression and promoted myogenic differentiation of bone-marrow derived mesenchymal stem cells and resident vascular stem cells. Selective knockdown of the Notch1 receptor in stem cells resulted in a significant decrease in Jagged1 stimulated Hey1 expression, a Notch1 target gene, concomitant with a reduction in myogenic differentiation due to decreased smooth muscle differentiation marker expression (CNN1 and MYH11 mRNA and protein levels). Inhibition of N-glycosylation with tunicamycin lead to a down regulation of smooth muscle differentiation markers, CNN1 and MYH11 independent of a reduction in Notch target gene expression. Lfng knockdown lead to a similar significant reduction in Jagged1 induced myogenic differentiation (reduced CNN1 expression). Collectively, these results suggest that N-glycosylation of Notch1 receptor is involved in Notch signalling leading to altered resident vascular stem cell fate.

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