technology and ELISA. AoEMPs were also subjected to proteomic and miR screening to identify relevant molecules and pathways.

AoEMP-treated HCASMCs showed enhanced calcification after 21 days, by both alizarin red staining ($p < 0.005$) and calcium deposition assays ($p < 0.05$). In addition, secreted osteocalcin and osteoprotegerin levels were elevated in AoEMP-treated cells after 7 and 21 days respectively. ELISA determined that HGF, a key protein in vascular calcification, was elevated in media from the AoEMP-treated group compared to controls at 14 and 21 days. HGF levels were also higher in SLE patient plasma ($p < 0.05$) compared to healthy controls, suggesting that HGF may be involved in the vascular calcification observed in SLE patients. Furthermore, proteomic screening identified HGF in AoEMPs, whilst miR screening highlighted miR-3148 in both AoEMPs and SLE plasma and is predicted to target osteoprotegerin gene expression.

We conclude that AoEMPs enhance calcification and the reprogramming of HCASMCs to an osteogenic pathway *in vitro*, which may be in part, linked with HGF and miR-3148, supporting their role in vascular calcification in SLE and carotid artery disease. Further studies are required to determine how AoEMPs contribute to pathophysiological mechanisms *in vivo* and whether the circulating property of AoEMPs may represent a new biomarker in vascular calcification.

178 CAVEOLIN-3 A NOVEL REGULATOR OF RYANODINE RECEPTOR; A RELATIONSHIP THAT IS PERTURBED IN THE OBESE HEART

Mohammad Monib Iqbal*, Hayley J Bennett, Bernard Davenport, Charlotte Bussey, Elizabeth Cartwright, Ashraf Kitmitto. *University of Manchester*; **Presenting Author*

10.1136/heartjnl-2016-309890.178

Background Obesity is the leading cause of insulin resistance and a key factor underlying the development of type 2 diabetes.[1] Impaired excitation-contraction (EC) coupling is a hallmark of both type 1 and type 2 diabetes.[2] EC coupling is maintained by the finely tuned process termed calcium induced calcium release involving a number of proteins including the ryanodine receptor (RyR2). Our group has previously reported an interaction between caveolin-3 (Cav3) and RyR,[3] however, the functional consequences of this interaction are unknown. Cav3, a small integral membrane protein is involved in orchestrating an array of signalling pathways and is a negative regulator of nitric oxide synthase (NOS) and thus nitric oxide (NO) production. Significantly, post-translational modification of RyRs, S-nitrosylation, the addition of nitroso group, has been linked to 'leaky' channels. We hypothesize that the formation of a Cav3-RyR2 complex has a cardioprotective role regulating NOS activity within cardiac myocytes by maintaining the nitroso-redox state of the receptor and that this relationship is perturbed in the obese heart.

Methods Tissue was lysed from the left ventricle fromrats fed a high fat diet (40% HFD) and age-matched control rats on normal chow (10% diet) and probed for protein expression levels using western blotting. The SPRY domain of RyR2 was recombinantly expressed and purified from *E.coli*, and recombinant full length Cav3 was expressed and purified from yeast (*Pischia Pastoris*). Interaction between full-length recombinant Cav3 and SPRY domain was analysed by microscale thermophoresis (MST).

Results In the pre-diabetic heart (obesity) we have determined a down-regulation of Cav3 expression ($P < 0.05$), elevated levels of NOS3 ($P < 0.01$) but no change to RyR2 levels in the left ventricle. Our bioinformatics analyses identified multiple putative caveolin binding motifs (CBMs) within the RyR1 and RyR2 primary sequences that are conserved. The recent publication of a high resolution 3-D structure of skeletal RyR1 allowed us to determine that one of the CBMs is localised to a SPRY domain and sequence alignment with RyR2 found this region is conserved. We have successfully purified the RyR2 SPRY domain and full-length Cav3. Preliminary data indicate a possible interaction between recombinant Cav3 and the SPRY domain using microscale thermophoresis.

Conclusion Our study shows down-regulation of Cav3 and upregulation of NOS3 with no change in RyR2 expression level in the obese rat model. Functional studies are now underway to investigate the implications of an imbalance between NOS3 and Cav3 in terms of RyR2 calcium release. Further, biophysical and structural studies are now necessary to investigate the putative SPRY-Cav3 interaction and determine if other regions of RyR2 mediate Cav3-RyR2 binding.

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179 NEUROVASCULAR FUNCTION IN ATHEROSCLEROSIS

Lewis Wong*, Paul Sharp, Clare Howarth, Nigel Hoggard, Jason Berwick, Sheila Francis. *University of Sheffield*; **Presenting Author*

10.1136/heartjnl-2016-309890.179

Cardiovascular and neuronal dysfunction have to a large extent been treated as separate disease categories from both research and clinical perspectives. However, there is now growing evidence that pathological changes in the shared circulatory system may be key drivers of both cardiovascular and neuronal dysfunction. It is speculated that compromised circulatory function, as can be seen in inflammatory vascular conditions such as atherosclerosis, may impact the regulation of cerebrovascular blood flow in response to dynamically changing neuronal metabolic demands also known as neurovascular coupling. With impaired neurovascular function being a pathogenic factor underlying cerebrovascular pathology, here we aim to establish if atherosclerosis elicits any alterations in the neurovascular function.

Paigen Diet fed ApoE -/- mice fitted with a stable cranial window over the right somatosensory cortex combined with state of the art multi-modal neurovascular imaging comprised of 2D-optical imaging spectroscopy (OIS) to measure evoked blood flow, volume, and oxygenation changes and electrophysiology to record neuronal activity. Any neurovascular breakdown will then be further investigated using high resolution multi-photon microscopy and immunohistochemistry to identify cellular deficits and potential molecular targets. Functional magnetic resonance imaging (fMRI) will also be used later to assess any sub-cortical effects that 2D-OIS and electrophysiology cannot detect, which will assist in combining highly