**Introduction** Ms1 (also known as STARS and ABRA) has been shown to act as an early stress response gene in processes as different as hypertrophy in skeletal and cardiac muscle and growth of collateral blood vessels. It is important for cardiac development in zebrafish and is upregulated in mouse models for cardiac hypertrophy as well as in human failing hearts. Ms1 possesses actin binding sites at its C-terminus and is usually found in the cell bound to actin filaments in the cytosol or in sarcomeres. It is assumed that it activates SRF dependent gene expression in an indirect manner by shifting the equilibrium between cytosolic actin from G to F. This releases MRTFs which can then translocate to the nucleus to bind to SRF.

**Methods** We used NMR spectroscopy to determine the structure of the only folded domain of Ms1 and characterised its binding to F-actin and DNA using a combination of biochemical and biophysical assays. To identify a specific DNA sequence we used the SELEX approach *in vitro* in combination with EMSA assays. This was followed up by monitoring Ms1 subcellular localisation in neonatal rat cardiomyocytes using detection of endogenous protein with a new antibody or overexpression of wild type and mutated proteins.

**Results** We determined the NMR structure of the only folded domain of Ms1 comprising the second actin binding site called actin binding domain 2 (ABD2, residues 294–375), and found that it is similar to the winged helix-turn-helix fold adopted mainly by DNA binding domains of transcriptional factors. *In vitro* experiments show specific binding of this domain, in combination with a newly discovered AT-hook motif located N-terminally, to the sequence (A/C/G)AAA(C/A). NMR and fluorescence titration experiments confirm that this motif is indeed bound specifically by the recognition helix. In neonatal rat cardiomyocytes endogenous Ms1 is found in the nucleus in a spotted pattern, reminiscent of PML bodies. In adult rat cardiomyocytes Ms1 is exclusively found in the sarcomere. A nuclear localisation site (NLS) in the N-terminus of the protein is required for nuclear localisation as shown by the abolition of nuclear translocation of over expressed protein in which the NLS was mutated. A phosphorylation site immediately N-terminal of this NLS could provide regulatory control of nuclear transport.

**Conclusions** Our data suggest that Ms1 has the potential to act directly in the nucleus through specific interaction with DNA in development and potentially as a response to stress in adult tissues. Ms1 could act in parallel to the established MRTF/SRF pathway to regulate the expression of hypertrophic genes directly which would explain recent findings from studies in C1C12 cells where inhibition of SRF signalling did not lead to a change in myotube differentiation rate

## 198 HEART FAILURE INCREASES MITOCHONDRIAL S-NITROSYLATION

[1]Emma Radcliffe*, [2]Junhui Sun, [2]Angel Aponte, [1]David Eisner, [2]Elizabeth Murphy, [1]Andrew Trafford. [1]*University of Manchester*; [2]*National Heart, Lung and Blood Institute, NIH*

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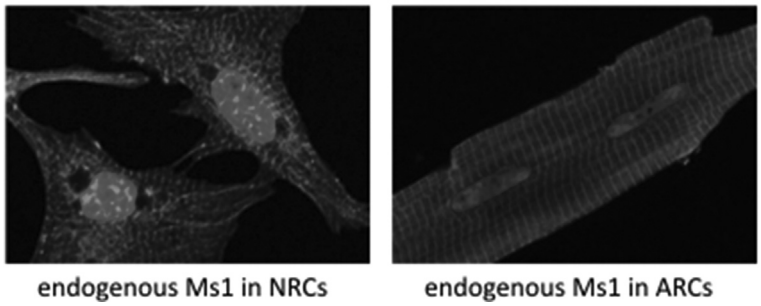
Heart failure affects over 5 50 000 people living in the United Kingdom (1) and roughly half of those present with reduced cardiac ejection fraction (2). The electron transport chain (ETC) within the mitochondria is the principle source of ATP within cardiac muscle. Consequently, the regulation of the ETC in heart failure represents a potential rate limiting step to cardiac contraction in the disease state. The post-translational modification of proteins by nitric oxide, S-nitrosylation (S-NO) has previously been shown to have inhibitor effects on complex I activity (3). The aim of this study is therefore to investigate the role of S-NO and how this may regulate ETC activity in an ovine tachypaced model of heart failure.

Tachypacing induced heart failure resulted in an increase in left ventricular diameter (3.10±0.06cm to 4.04±0.13cm, $p<0.01$, $n=5$), a thinning of the left ventricular free wall (81 ±10mm to 26±3mm, $p=0.01$, $n=5$) and a reduction in left ventricular contractility (0.47±0.01 to 0.20±0.03, $p<0.01$, $n=5$). Mass spectrometry in combination with either tandem mass tags or S-NO resin assisted capture, was used to assess protein expression or S-NO abundance respectively. The total number of S-NO proteins identified increased from 232±18 in control samples to 314±28.3 in heart failure ($p=0.02$, $n=6$). Of those identified in both groups 79% showed an increase in S-NO abundance in heart failure. Mitochondrial content per unit of protein was unaltered in heart failure ($p=0.93$, $n=5$). However, S-NO of several ETC complex subunits was increased in the disease state (eg. NDUFS1 ↑ 2.9 fold, SDHA ↑ 1.2 fold, UQCRH ↑ 1.1 fold, $n=6$) in the absence of corresponding protein expression changes (eg. NDUFS1 ↓ 0.2 fold, SDHA ↓ 0.07 fold, UQCRH†0.2 fold, $n=5$). In vitro S-NO of control mitochondria, using 0.2 mM S-nitrosoglutathione, oxidative phosphorylation by 52% ($p<0.01$, $n=5$).

This study demonstrates that in heart failure there is a gross increase in the level of myocardial S-NO. Within the mitochondria, S-NO of electron transport chain proteins is also increased, having an inhibitory effect on ATP production. This work therefore provides a novel insight into how S-NO may contribute to the deterioration of cardiac contractile function in heart failure.

### REFERENCES

1. Townsend NWJ, Bhatnagar P, Wickramasinghe K, Rayner M. Cardiovascular disease statistics 2014. London: British Heart Foundation; 2014.




**Abstract 197 Figure 1**

2. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, *et al*. Executive Summary: Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. *Circulation*. 2016;133(4):447-54. Epub 2016/01/27.
3. Chouchani ET, Methner C, Nadtochiy SM, Logan A, Pell VR, Ding SJ, *et al*. Cardioprotection by S-nitrosation of a cysteine switch on mitochondrial complex I. *Nat Med*. 2013;19(6):753.

## 199 TAKOTSUBO SYNDROME ASSOCIATED MIR-16 AND MIR-26A REDUCE CONTRACTILITY OF CARDIOMYOCYTES IN VITRO BY AN INHIBITORY G-PROTEIN DEPENDENT MECHANISM

[1]Liam Couch*, [2]Anselm A Derda, [2]Thomas Thum, [1]Cesare Terracciano, [1]Sian Harding. *[1]Imperial College London; [2]Hannover Medical School*

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**Introduction** Takotsubo syndrome (TTS) is a severe but reversible acute heart failure affecting predominantly post-menopausal women, where ventricular apical akinesis results from extreme adrenaline levels arising with stress. The pleiotropic $\beta^2$AR signals via stimulatory ($G_s$) and inhibitory ($G_i$) G-proteins, and whilst $G_s$ increases cardiac output, it concomitantly decreases survival. The duality of $\beta^2$AR is a homeostatic mechanism to limit cardiotoxicity by facilitating a switch to $G_i$, serving as cardioprotective despite being cardiodepressive. This is dysregulated in TTS where excess stimulus trafficking to $G_i$ results in profound negative inotropy. It is not understood what predisposes patients to TTS, but a microRNA (miR) profile of increased miR-16 and miR-26a has been identified. Given the importance of miRs in other cardiac diseases and that TTS is thought to be causally related to Î²2AR-$G_i$, we hypothesise that these miRs could predispose to the cardiodepression in TTS.

**Method** miRs were manipulated in adult rat apical cardiomyocytes with blinded transfection using Lipofectamine 3000. Percentage shortening was measured using an Ionoptix system, and pharmacological protocols applied. Calcium transients were obtained using Fluo-4-AM and sarcoplasmic reticulum (SR) calcium content measured with caffeine micro-application. N numbers displayed as n/N, where n/N=cells/rats.

**Results** Up-regulation of miR-16 and miR-26a significantly reduced basal contractility (miR-16=3.52±0.34% versus control=4.91±0.46%; n/N=30/6; $p<0.05$ and miR-26a=2.77 ±0.21% versus control=4.30±0.43%; n/N=50/10; $p<0.01$), whereas down-regulation had no effect. miR-16/-26a manipulation did not alter Î²2AR response. Inhibiting Gi with pertussis toxin (PTX) prevented this (miR-16 untreated=5.08±0.49%, n=22; versus miR-16 PTX-treated=8.82±0.63%, n=20; $p<0.001$; and miR-26a untreated=3.20±0.29% versus miR-26a PTX-treated=5.05±0.48%; n/N=30/6; $p<0.05$ respectively). PTX-treatment did not change contractility of control transfected cells. No synergism was observed with dual miR-16/-26a transfection possibly suggesting a unified mechanism. Calcium transient amplitude was decreased with miR-16/-26a up-regulation (F/F0 for control=1.85±0.04, n/N=66/4 versus miR-16=1.51±0.04, n/N=37/4 and miR-26a=1.57±0.04, n/N=26/4; $p<0.001$), along with a concomitant decrease in SR calcium content (caffeine-induced F/F0 for control=3.13 ±0.14, n/N=32/4 versus miR-16=2.13±0.13, n/N=22/4 and miR-26a=2.57±0.19, n/N=17/4; $p<0.001$ and $p<0.05$ respectively).

**Conclusion/implication** Increased miR-16/−26a reduce basal contractility of cardiomyocytes *in vitro*, possibly through a shared $G_i$-dependent mechanism. Decreased calcium transient amplitude is also likely to contribute. This suggests these miRs may be mechanistically involved in TTS, but further work is needed to investigate their specific mechanistic and spatiotemporal involvement.

## 200 NITRIC OXIDE PROMOTES INSULIN-INDEPENDENT GLUCOSE UPTAKE AND PRESERVES CARDIAC FUNCTION AND ENERGETICS IN DIABETES

Klemen Ziberna*, [1]Drew Duglan, [2]Simona Mafrici, [1]Jillian Simon, [1]Craig Lygate, [1]Keith Channon, [1]Barbara Casadei, [1]Ricardo Carnicer. *[1]University of Oxford; [2]University Magna Graecia of Catanzaro*

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**Introduction** In the presence of diabetes (DM), myocardial glucose uptake and glycolysis are impaired and the heart rapidly adapts to use exclusively fatty acids (FA) for ATP generation. This maladaptation is believed to play a key role in the development of a cardiomyopathy over time. Here, we show that stimulating myocardial nitric oxide synthase (NOS) activity is sufficient to alleviate myocardial metabolic inflexibility, improve energy metabolism and prevent LV dysfunction in DM by increasing myocardial insulin-independent glucose transport.

**Methods** Myocardial-specific overexpression of GTP cyclohydrolase I (mGCH1) was used to increase both tetrahydrobiopterin (BH4) and NOS activity in cardiomyocytes. Diabetes mellitus (DM) was induced by multiple low-dose streptozotocin injections (vs sham). PCr/ATP ratio was measured in perfused hearts using $^{31}$P-MRS, glucose transport estimated by deoxy-glucose uptake, and oxygen consumption rate (OCR) of intact cardiomyocytes using a phosphorescent probe.

**Results** As expected, sham-injected mGCH1 transgenic hearts had higher BH4 levels and constitutive NOS activity compared with WT. 12 weeks after DM induction, LV dysfunction developed in WT mice but not in mGCH1 mice, in the absence of changes in myocardial BH4 content and NOS activity in either group. WT diabetic hearts had a lower PCr/ATP ratio (1.32±0.1 vs 1.73±0.1, $p<0.05$, n=11 per group) and mitochondrial creatine kinase (CK) activity (1.56±0.1 AU vs 1.98 ±0.1 AU, $p<0.005$, n=10 per group) when compared with non-diabetic WT mice, consistent with impaired cardiac energetics. By contrast, PCr/ATP and CK activity were preserved in diabetic mGCH1 hearts in the absence of differences in myocardial mitochondrial content.

Myocardial GCH1 overexpression was associated with a higher protein levels of the insulin-independent glucose transporter, GLUT-1 ($p<0.05$, n=12 per group), but no changes in GLUT-4 protein. Myocardial glucose transport was 40% higher in LV myocytes from mGCH1 diabetic mice when compared with WT diabetic mice. This was accompanied by increased myocardial glucose oxidation, as determined by OCR. Pre-incubation of myocytes with inhibitors of NOS-PKG signalling (L-NAME, 1 mmol/L or Rp8pCPT PET cGMP 10 µmol/L) or GLUT-1 (STF-31, 10 µmol/L,) abolished all differences between mGCH and WT diabetic hearts.

**Conclusions** Our study reveals that a myocardial increase in BH4 and NOS activity is sufficient to maintain a favourable substrate utilisation and preserve cardiac mitochondrial function in the presence of DM. This work provides new insight into the potential metabolic triggers of diabetic