relationships between metabolic flux changes and the functional decline observed.

P17 CYCLOSPORIN A MEDIATED INHIBITION OF THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE (MPTP) ATTENUATES TIOTROPIUM BROMIDE MEDIATED CARDIOTOXICITY

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10.1136/heartjnl-2018-BSCR.22

Muscarinic antagonists relieve bronchoconstriction due to the progressive condition of chronic obstructive pulmonary disease (COPD). Recent meta-analyses have highlighted increased stroke and myocardial infarction with the long acting muscarinic receptor antagonist, Tiotropium bromide. Opening of the mitochondrial permeability transition pore (mPTP) triggers cardiomyocyte death, therefore modulation of the pore could promote cardiomyocyte survival.

Isolated perfused rat hearts were subjected to ischaemia/reperfusion (I/R) or normoxic protocols. Hearts were subjected to stabilisation, and perfusion ±Tiotropium (10 nM – 0.1 nM), Cyclosporin A (CsA) (200 nM) or Tiotropium (1 nM)±CsA. For I/R, regional ischaemia was induced following stabilisation. Hearts were stained using triphenyl-tetrazolium chloride (TTC) to determine infarct/risk ratios (%). Data was analysed using one-way ANOVA and LSD, presented as mean ±SEM.

All concentrations of Tiotropium significantly increased infarct/risk ratio compared with controls. CsA decreased infarct/risk with respect to controls (Normoxia: 5.1±1.0% vs 10.3±1.9%, $p<0.05$; I/R: 7.2±1.2% vs 50.9±3.9% and 10.3%±1.9%, $p<0.0001$), co-administration maintained this, with respect to Tiotropium (1 nM) in normoxia, and also with control in I/R (Normoxia: 8.4±2.1% vs 18.7±1.8%, $p<0.0001$; I/R: 16.3 ±0.8% vs 65.4±3.0% and 50.9%±3.9%, $p<0.0001$).

This is the first pre-clinical study to suggest that Tiotropium increases infarct/risk ratio in an isolated perfused heart model via mPTP opening, as CsA decreases Tiotropium- and ischaemia/reperfusion-mediated myocardial injury. These findings suggest for a role of the mitochondria in mediating the adverse cardiac side-effects seen clinically.

P18 ROLE OF cAMP in the regulation of Parkin-dependent mitophagy

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10.1136/heartjnl-2018-BSCR.23

Parkinson's disease (PD) is characterised by compromised mitophagy, a highly specialised quality control process that removes dysfunctional mitochondria through a macroautophagy pathway. The proteins Parkin and PINK1 are key players in the mitophagic process. In healthy mitochondria with normal membrane potential, Parkin is located mainly in the cytosol, where its ubiquitin ligase activity is inhibited while PINK1 is imported into the mitochondria and becomes degraded by proteolysis. Following cellular stress, the depolarization of the mitochondrial membrane potential allows the stabilisation of PINK1 at the outer mitochondrial membrane (OMM), where it phosphorylates ubiquitin. This induces activation of Parkin and its translocation to damaged mitochondria, followed by mitophagy. Recent advances revealed that Parkin recruitment to depolarized mitochondria is severely inhibited by treatment with cAMP raising agents. cAMP-dependent activation of PKA has been shown to reduce PINK1 protein levels at the OMM through phosphorylation of MICOS (mitochondrial contact site and cristae organising system). Here we show that phosphodiesterase 2A2 (PDE2A2), a cAMP-degrading enzyme, interacts with components of the MICOS complex and regulates cAMP levels selectively at the mitochondria. Furthermore, our preliminary data show that in mouse embryonic fibroblasts deleted of PDE2A2 (MEF)PDE2A-/- the amount of Parkin recruited to the mitochondria is reduced compared to MEFWT under basal conditions. In agreement with these results, treatment with BAY 60–7550, a selective PDE2A inhibitor, promotes PKA-dependent phosphorylation of Mitofilin. In conclusion, we propose that PDE2A2 regulates a local cAMP pool at the mitochondria that leads to PKA-dependent phosphorylation of MICOS and Parkin recruitment to damaged mitochondria.

P19 LOW LEVELS OF THE A3243G MTDNA MUTATION IN HUMAN INDUCED PLURIPOTENT STEM CELL-CARDIOMYOCYTES DO NOT CAUSE FUNCTIONAL OR METABOLIC DISTURBANCES BUT INCREASE WITH FURTHER PASSAGING

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10.1136/heartjnl-2018-BSCR.24

The heteroplasmic mtDNA mutation A3243G can cause the mitochondrial condition MELAS. Mitochondrial replacement therapy can prevent transmission of mtDNA mutations to offspring but to maintain nuclear integrity, a certain amount of cytoplasm and mutated mtDNA is carried over (<3%). It is unknown whether this will increase with age and this is particularly relevant in the heart, where mutations accumulate over time. We applied small molecule modulation of the Wnt/β-catenin signalling pathway to generate pure populations of cardiomyocytes (CMs) from human induced pluripotent stem cells (hiPSCs) from a patient with 20% heteroplasmy for the A3243G mtDNA mutation No changes in the basal beating rate or time to peak and time to 50% relaxation were found. No differences in the response to β-adrenergic stimulation by isoprenaline or muscarinic inhibition by carbachol. A3243G hiPSC-CMs showed reduced excitability (18.85±3.045 ms for control and 38.08±6.126 ms for A3243G, Mean ±SEM, $p=0.0084$) but there were no changes in other calcium handling properties. Mitochondrial DNA copy number and both mitochondrial respiration and basal glycolysis were unaffected. We have seen a gradual increase in A3243G hiPSCs and derived CMs heteroplasmy with passaging (26.4% to 38.7% over 6 passages). We conclude that A3243G heteroplasmy <40% is not sufficient to affect the generation of hiPSC-CMs and their beating, calcium handling and metabolic properties. Having observed an increase in heteroplasmy with

passaging, these results provide useful insights into changes that might happen with age in children resulting from mitochondrial replacement therapy.

P20 STUDY OF INSULIN CARDIOVASCULAR RESISTANCE IN AN ANIMAL MODEL OF CHILDHOOD OBESITY BY OVERFEEDING DURING LACTATION

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10.1136/heartjnl-2018-BSCR.25

Childhood obesity is a pandemic that has become one of the major public health problems of the 21st century. The aim of this work was to analyse cardiovascular insulin sensitivity in an experimental model of early overnutrition.

Male 21-day-old Sprague-Dawley rats were used. On the day of weaning offspring were weighed and sacrificed by decapitation. Hearts were mounted in a Langendorff system whereby increasing doses of insulin were administered (10–10–10–7M). In addition, 2mm rings of aorta were mounted in an organ bath and precontracted with phenylephrine (10–7.5 M). Changes in isometric tension were recorded after addition of cumulative insulin doses (10–8–10–5.5 M) in the presence or absence of L-NAME (10–4M).

On the day of weaning, the body weight of offspring raised in reduced litters was significantly higher than that of control pups. Hearts from overfed offspring showed lower contractility (dp/dt) both at baseline and in response to increasing doses of insulin than controls. Insulin induced greater vasodilation of the coronary arteries in the hearts of offspring from C12 than in offspring from C3. Similarly, the aortic rings from overfed offspring showed decreased insulin relaxation in the aortic rings compared to controls, an effect that was blocked by the preincubation of the arterial rings with the N-oxide synthase inhibitor L-NAME.

Overfeeding during lactation in rats is associated with cardiovascular insulin resistance both in the heart and in the aorta. This fact could be associated, at least in part, with the higher incidence of cardiovascular diseases in individuals with long-standing obesity.

P21 CARDIAC METABOLISM IN PATIENTS WITH HEART FAILURE WITH MID-RANGE EJECTION FRACTION

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10.1136/heartjnl-2018-BSCR.26

Rationale Disturbance in cardiac energy metabolism is a key feature of heart failure (HF). It is unknown whether HF with mid-range left ventricular (LV) ejection fraction (HFmrEF), defined by LVEF 40%–49%, represents a separate clinical entity and whether there are distinct cardio-metabolic changes compared with HF patients with more severely reduced EF (HFrEF – LVEF <40%). We sought to investigate this by assessing myocardial energetics (phosphocreatine-to-adenosine triphosphate – PCr/ATP) and myocardial triglyceride (MTG) content in both HFmrEF and HFrEF.

Methods 53 subjects (13 HFmrEF, 13 HFrEF and 25 age, sex and BMI-matched controls) underwent cardiovascular magnetic (MR) resonance at 3 Tesla (3T) to assess left ventricular (LV) volumes and function, 31P-MR spectroscopy (MRS) to measure myocardial PCr/ATP, and 1H-MRS to measure MTG content.

Results There were no significant differences in PCr/ATP ratios between the two HF groups (HFmrEF 1.69±0.39 vs HFrEF 1.64±0.41 HFrEF, $p<0.05$), and both were significantly lower than controls (1.96±0.27, $p<0.05$). Similarly, MTG was not significantly different between HFmrEF (0.61%±0.39%) and HFrEF (0.71%±0.49%), both significantly higher than in controls (0.42%±0.16%, $p<0.05$). Interestingly, similar levels of PCr/ATP and MTG changes were observed in both HF groups despite significant differences in LV volumes (HFmrEF 192 ±45 vs HFrEF 270±92, $p<0.001$) and EF (HFmrEF 46±3 vs HFrEF 31±6, $p<0.001$).

Conclusion Our results indicate no differences in energetic and lipid derangement between HFmrEF and HFrEF. This emphasises the need for a new phenotyping model for HF patients based on factors other than LVEF.

P22 CARDIAC ENERGY METABOLISM INCREASES WITH KETONE OXIDATION

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10.1136/heartjnl-2018-BSCR.27

Rationale The oxidation of ketone bodies plus glucose improves the efficiency of the working rat heart by ~30% compared to glucose alone.[1] However, perfusions of hearts with ketones alone led to gradual functional decline with reduced flux through α-ketoglutarate dehydrogenase.[2] We have shown that that the rate of d-β-hydroxybutyrate (βHB) oxidation in isolated perfused rat heart increases with the glycogen content via replenishing anaplerotic substrates. Therefore, we aimed to determine whether the availability of glycogen increases the oxidation of ketones and improves myocardial energetics.

Methods Hearts from male Wistar rats (320–360 g) were isolated and perfused with buffer to alter the glycogen content, before perfusion with either 11 mM glucose or 11 mM glucose plus 4 mM βHB. Fully relaxed 31P-MR spectra were acquired at each stage of the protocol to measure pH, PCr, Pi and ATP concentrations ([PCr], [Pi], [ATP]). Results were analysed using 2-way ANOVA with Bonferroni's correction.

Results [ATP] remained constant under all perfusion conditions, irrespective of the cardiac glycogen content. In hearts with high glycogen, addition of βHB increased [PCr] by 41%, from 15.4±0.5 to 21.7±0.5 mM, and △GATP by −6.3 kJ/mol (both $p<0.001$). In low glycogen hearts, addition of βHB did not alter [PCr] or △GATP.

Conclusion High glycogen concentrations increased the oxidation of βHB, thereby improving cardiac energetics.

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