prolongation and increased incidence of pro-arrhythmic events in isolated cardiomyocytes which was prevented by pharmacological inhibition of the late sodium current. Our current data suggests a direct effect of the mutation on cardiac function rather than the observed phenotypes resulting from an accumulation of BCAAs. Thus we have identified a novel model of sudden cardiac death resulting from abnormal BCAA metabolism.

T4 ERK5 DEGRADATION: A TURNING POINT FROM COMPENSATED METABOLIC CARDIOMYOPATHY TO HEART FAILURE

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Rationale The accumulated prevalence of obesity, diabetes, and metabolic syndrome is more than 25% of the world's population. These are all conditions that have been repeatedly related to a higher risk of heart failure, and effective treatment has not been found. It remains essential to continue deciphering the underlying molecular mechanism to discover novel treatment strategies.
Methodology Initial screening was performed on myocardium samples from ob/ob mice, db/db mice, rhesus monkey with spontaneous metabolic syndrome, and mice fed for 25 weeks with high-fat diet (HFD). In subsequent studies, extracellular signal-regulated protein kinase 5 (ERK5) cardiomyocyte-specific knockout mice (ERK5-cko) were evaluated up until 16 weeks of HFD feeding. *In vitro* experiments were performed on rat ventricular myocytes treatd with saturated fatty acids.
Results The screening of obese and diabetic models showed that ERK5 was selectively lost in the myocardium. ERK5-cko presented cardiac dysfunction after only 16 weeks of HFD. Further studies showed the loss of contractility was accompanied by augmented oxidative stress, increased lipid accumulation, and severe mitochondrial dysfunction. Mechanistic studies revealed ERK5 to act upstream of the mitochondrial regulator peroxisome proliferator-activated receptor γ co-activator-1α (PGC-1α). Moreover, it was observed that ERK5 degradation after saturated fatty acid treatment was mediated by calpain-1, while the inhibition of this degradation could prevent the mitochondrial dysfunction.
Conclusion The calpain-mediated degradation of ERK5 blunts the compensatory response that would usually maintain mitochondrial integrity when facing metabolic stress.

T5 VERY LOW CALORIE DIET IN OBESITY IMPROVES METABOLIC RISK FACTORS AT THE INITIAL COST OF VENTRICULAR FUNCTION AND STEATOSIS

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Background Very Low Calorie Diets (VLCD) in obesity are an effective weight loss intervention that rapidly reduce liver fat and improve peripheral insulin resistance. We hypothesised that improved peripheral glycaemic control would be accompanied by initial impairment of cardiac function as hepatic fat stores are mobilised and taken up by the myocardium.
Methods 14 obese volunteers (4 male, 49±15 years, BMI 36.2±5.9 kg m-2) underwent body composition analysis and MR scanning for abdominal visceral and liver fat, LV structure and function, 1H-MRS to measure myocardial triglyceride content (MTGC), and echocardiography for diastolic function (E/E'), before and one week into a VLCD (800 kcal/day).
Results 7 days of VLCD led to significant reductions in total body fat, visceral and hepatic fat, and insulin resistance. However, MTGC rose from 1.74±0.99% to 3.02±1.70% (p=0.030), and there was a reduction in both systolic function (LVEF 67±3% to 62±5%, p=0.014; peak radial strain 51 ±8% to 42±9%, p=0.005) and diastolic function (e/e' 8.5 ±1.6 to 10.3±3.5, p=0.034). The change in MTGC at one week correlated with change in diastolic function (r=0.729, p=0.017). However at 8 weeks (n=6), changes in MGTC as well as cardiac function had returned to normal.
Conclusions We demonstrate for the first time in healthy obese individuals that a 7 day period of severe caloric restriction results in accumulation of myocardial fat which is associated with impairment of both systolic and diastolic LV function at this timepoint, despite a significant improvement in hepatic fat and whole body insulin sensitivity.

P1 CAUSAL LINK BETWEEN INTRACELLULAR SODIUM OVERLOAD AND METABOLIC REMODELLING IN THE HEART: UNCOUPLING ATP SUPPLY AND DEMAND?

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Rationale Intracellular Na elevation is a hallmark of the ischaemic and failing heart – pathologies in which both acute and chronic metabolic remodelling occur.
Objective To assess whether acute (75 μM ouabain 100 nM blebbistatin) and chronic myocardial Nai load (PLM3SA mouse) are causally linked to metabolic remodelling and if the failing heart shares a common Na-mediated metabolic 'fingerprint'.
Methods 23Na, 31P and 13C NMRS were performed in normal and hypertrophied (pressure overload) Langendorff perfused mouse hearts followed by 1 hour NMRS metabolomic profiling, mass spec and *in silico* modelling.
Results Na overload (acute, chronic (PLM3SA), and hypertrophy 2, 1.3 and 1.4-fold respectively) resulted in common metabolic perturbations: substrate switch (palmitate 35% reduction, glucose 58% increase), flux (TCA cycle, OXPHOS, glycolysis) and metabolomic profile (TCA cycle, glycolysis, anaplerosis) without energetic impairment (PCr/ATP 1.5 ±0.1 vs control 1.3±0.1). Inhibition of mitochondrial Na/Ca exchanger by CGP37157 during both acute and chronic Na load ameliorated the metabolic changes.

Conclusion Elevated Na leads to complex metabolic alterations preceding any energetic and functional impairment. Early prevention of Na overload and inhibition of Na/Ca_{mito} could ameliorate metabolic dysregulation in hypertrophy and failure.

P2 FATTY ACID TRANSPORTER1 (FAT/CD36) AND GUANINE NUCLEOTIDE-BINDING PROTEIN G(I) SUBUNIT ALPHA-2 (GALPHAI2) ARE NOVEL SUBSTRATES OF THE CELL SURFACE LOCALISED PALMITOYL TRANSFERASE DHHC5

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Palmitoylation is the reversible addition of a 16 carbon fatty acid group to cysteine residues on proteins. The forward reaction is catalysed by DHHC palmitoyl acyl transferase (PAT) enzymes and there are 23 human isoforms. DHHC5 is a cell surface localised PAT proposed to contribute to cardiac reperfusion injury. A peptide array based on the DHHC5 extended C tail was used to screen rat cardiac lysates for DHHC5 interactors, which were subsequently identified by mass spectrometry. Biotinylated peptides covering the C-tail of DHHC5 were incubated with cardiac lysates and captured on streptavidin sepharose beads. Following the removal of contaminants, targets were identified as potential substrates of DHHC5 if they appear in the cardiac palmitoyl proteome or the SwissPalm database. Included are several kinases, phosphatases, ion transporters, G protein alpha subunits (Galphai2) as well as GLUT4 and FAT/CD36. DHHC5 interactors identified in the screen were expressed with an N terminal GFP tag for detection in both HEK293 and Crispr-engineered FT293 DHHC5 KO cells. Acyl resin assisted capture (AcylRAC) was used to assess their palmitoylation. Both FAT/CD36 and Galphai2 were less palmitoylated in DHHC5 KO cells, and this was restored on over expression of DHHC5 (but not DHHC17), indicating these are DHHC5 substrates. Further work will investigate the functional consequences of DHHC5 palmitoylation of CD36 and Galphai2, as well as validating other putative DHHC5 candidate substrates.

P3 CROSSTALK BETWEEN GLUCOSE AND CREATINE IN THE HEART

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Background Diabetic cardiomyopathy is characterised by metabolic remodelling, impaired glucose and high-energy phosphate metabolism. Transgenic mice overexpressing the creatine transporter in the heart (CrT-OE) have high intracellular creatine and elevated glucose, suggesting changes in substrate utilisation.

Objective We aimed at investigating the unexplored glucose-creatine link, in addition to the involvement of shared molecules in glucose and creatine metabolism.

Methods and results The correlation between myocardial creatine and glucose ($r2=0.67$; $p=0.002$) is accompanied by increased Glucose transporter 4 (Glut4) ($p<0.05$) and sodium-glucose co-transporter SGLT1 gene expression. Thioredoxin interacting protein (Txnip), that inhibits glucose and creatine transport (via Glut4 and CrT, respectively) is elevated in CrT-OE ($p<0.05$), indicating activation of both metabolic pathways.

To test if modulating creatine *in vivo* alters glucose uptake, we measured 3H-2-deoxyglucose incorporation in isolated cardiomyocytes from CrT-OE and WT hearts. In WT, insulin caused a 2-fold increase ($p<0.05$) in glucose uptake, unaffected by exogenous creatine pre-exposure. CrT-OE cells showed blunted glucose uptake vs WT in response to insulin ($p>0.05$) and responded to creatine ($p<0.01$).

In a cohort of samples taken during coronary artery bypass surgery from diabetic and non-diabetic patients ($n=8$ each; blood glucose 90 ± 7.1 and 138 ± 16.5, respectively), there was a negative correlation between CrT and Glut4 transcript ($r2=-0.5$; $p=0.028$) further supporting the glucose-creatine relationship in the clinical setting.

Conclusion Our observations suggest that changing myocardial creatine can regulate glucose uptake. Further studies will explore the potential use of creatine as a biochemical 'switch' to correct impaired glucose uptake and potentially insulin resistance.

P4 α-TOCOPHEROL DOES NOT INHIBIT LOW DESNSITY LIPOPROTEIN OXIDATION AT LYSOSOMAL PH

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The oxidation of low density lipoprotein (LDL) was considered to be important in atherogenesis. It is well known that α-tocopherol protects against the oxidation of LDL by cells or copper ions, but α-tocopherol did not protect against cardiovascular disease in large clinical trials, leading some to doubt the importance of oxidised LDL. We have previously shown that LDL is oxidised in the lysosomes of macrophages, due to their acidic pH and presence of redox-active iron, raising the possibility that this is the main site of LDL oxidation, rather than the extracellular space of the arterial intima. We have now enriched LDL with α-tocopherol (by adding α-tocopherol to human plasma followed by the isolation of LDL by ultracentrifugation) and measured its oxidation by monitoring conjugated diene formation in a spectrophotometer set to 37°C and 234 nm. α-Tocopherol-enriched LDL was oxidised much slower by Cu2+ (2, 5 or 20 μM) at pH 7.4, as expected, but was not protected against oxidation by these concentrations of Cu2+, Fe2+ or Fe3+ at pH 4.5 (lysosomal pH). The lack of protection of cardiovascular disease by α-tocopherol is therefore not proof that oxidised LDL is not important in atherosclerosis, if the oxidation of LDL occurs in lysosomes. We therefore need to retest the oxidised LDL hypothesis using antioxidatnts that accumulate in lysosomes and inhibit the oxidation of LDL at acidic pH.

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