ADIPOSE TISSUE DERIVED CERAMIDES REGULATE MYOCARDIAL REDOX STATE AND PREDICT CARDIOVASCULAR OUTCOMES


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Background Obesity is linked to both dysfunctional adipose tissue (AT) and heart failure, but the exact mechanisms mediating these associations are unknown. Although ceramides biosynthesis is dysregulated in obesity, their role as mediators of obesity-induced myocardial dysfunction is unclear.

Purpose We investigate the causal role of AT-derived ceramides in the regulation of myocardial redox state and explore their role in predicting cardiovascular outcomes.

Methods The study population included a total of 880 patients undergoing cardiac surgery. A panel of 20 sphingolipids was measured in plasma as well as in biopsies of subcutaneous AT (ScAT), thoracic AT (ThAT) and epicardial AT (EpAT) and their secretome, obtained from a subgroup of n=48. Myocardial redox state was measured using lucigenin chemiluminescence and the contribution of NOXs, uncoupled nitric oxide synthases and mitochondrial oxidases in O2- production was quantified. The cohort was followed up for a median of 8.3 years. Genome-wide genetic analysis was done using the UK Biobank array. A total of 99,524 SNPs within 50kb of 110 genes involved in sphingolipid biosynthesis were analysed to identify genetic variants that could predict CVD outcomes using cis-Mendelian Randomisation. The underlying mechanisms were then explored further, using differentiated H9c2 cardiomyocytes in vitro and human right atrial tissue ex vivo.

Results The production and secretion of C16:0-ceramide (CerC16) was higher in visceral AT (EpAT and ThAT) compared to ScAT (p<0.0001). Patients with high plasma levels of CerC16 and its derivative C16:0-glucosylceramide (GlcC16) had higher myocardial O2- production vs those with low/int. levels (p<0.05 for both) (A). To test the causality of this association, we performed a targeted single-SNP analysis for the genetic prediction of GlcC16 levels demonstrating that rs112572487, an intronic variant in UGCG (an enzyme that catalyses glucosylceramide formation from ceramides), was the top hit (B). Indeed, those with the rs112572487 minor allele (G) displayed significantly increased myocardial NOX-derived O2- (C) and plasma GlcC16 levels (D) vs those without. Exogenous CerC16 (20nM) induced NOX-derived O2- production in H9c2 cardiomyocytes, an effect prevented by the UGCG inhibitor D-PDMP (E), suggesting that GlcC16 is a modifiable regulator of myocardial NOX-O2-. Importantly, high plasma GlcC16 levels were associated with a higher risk of cardiac death and/or heart failure (adj. HR=2.128 [95%CI: 1.101, 4.115], p=0.025, for high vs low/int. levels), a relationship also seen with rs112572487 (F).

Abstract BS1 Figure 1
Conclusions We demonstrate for the first time, that AT-derived ceramides are causally related with dysregulated myocardial redox signalling and adverse cardiovascular disease outcomes in patients with advanced atherosclerosis. As such, GlcCer maybe an important therapeutic target for the prevention and treatment of cardiovascular complications in obesity and diabetes.

Introduction Autophagy and apoptosis are both essential for cardiomyocyte homeostasis and have been implicated in cardiac remodelling following myocardial infarction (MI). A thorough understanding of these processes is crucial for the development of novel therapeutic strategies that aim to promote cardiomyocyte survival and diminish myocardial injury post-MI. Microtubule-associated protein 1S (MAP1S) was recently identified as an interactor partner of a crucial autophagy marker, LC3. However, the role of MAP1S in the heart is poorly understood. Therefore, we aimed to investigate the role of MAP1S in regulating cardiomyocyte autophagy and apoptosis.

Methods and Results To overexpress MAP1S, primary neonatal rat cardiomyocytes (NRCM) were transfected with adenovirus construct carrying MAP1S coding sequence. GFP-LC3 expressing adenovirus was used to estimate autophagic flux. Following co-treatment with rapamycin (5 μM) and chloroquine (3 μM), MAP1S-overexpressing NRCM exhibited higher autophagy activity. Similarly, immunoblot analysis of endogenous LC3-II showed a trend towards higher autophagy activity in NRCM overexpressing MAP1S compared to controls (LC3-II turnover assay). Interestingly, overexpression of MAP1S significantly reduced apoptosis in response to oxidative stress (100 μM H2O2 treatment) as determined by TUNEL assay. Consistently, siRNA-mediated gene silencing of MAP1S in NRCM increased apoptosis level following H2O2-induced oxidative stress. We next analysed whether ablation of MAP1S in mice affects cardiac phenotype following MI. We subjected MAP1S-specific global knockout (KO) mice to coronary artery ligation and found significantly higher mortality in MAP1S-KO mice (60%) compared to wild-type counterparts (30%) 4 week post-MI. TUNEL assay demonstrated that cardiomyocyte apoptosis was elevated in MAP1S-KO mice at both 3 days and 4 weeks post-MI. These results triggered us to identify MAP1S interactor partners and associated pathways which could explain the involvement of MAP1S in apoptosis. In-silico analysis found 46 experimentally validated MAP1S interacting proteins expressed in the heart. Furthermore, KEGG and Reactome pathway analysis revealed that MAP1S interactor partners are primarily associated with the Hippo signalling pathway. Conclusion. Our findings suggested that MAP1S has a pivotal role in cardiomyocyte survival by regulating autophagy and apoptosis. MAP1S may be involved in apoptosis via the Hippo pathway.

Introduction Left ventricular diastolic dysfunction is a structural and functional condition that precedes the development of heart failure with preserved ejection fraction (HFrEF). The etiology of diastolic dysfunction includes alterations in fuel substrate metabolism that negatively impact cardiac bioenergetics, and may precipitate the eventual transition to heart failure. To date, the molecular mechanisms that regulate early changes in fuel metabolism leading to diastolic dysfunction remain unclear. However, recent work has suggested that changes in mitochondrial lysine acetylation may regulate this process in mouse models of HFrEF.

Methods We used a diet-induced obesity model and quantitative acetylproteomics in aged mice to examine the role played by mitochondrial lysine acetylation in the development of diastolic dysfunction. Wildtype and cardiac-specific GCN5L1 knockout mice (which are deficient in mitochondrial lysine acetylation) aged 5–7 months were placed on a low fat diet (10% fat) or high fat diet (60% fat) for 30 weeks. Echocardiography was performed after 30 weeks of diet to assess cardiac structure and function, followed by euthanasia. After rapid isolation, hearts were subject to quantitative acetylproteomics, respirometry measurements, and biochemical measurements of metabolic enzyme acetylation and activity. In addition, cell culture models of site-specific lysine acetylation were used to test the mechanism underlying bioenergetic changes in mouse hearts.

Results Cardiomyocyte-specific deletion of the mitochondrial lysine acetylation regulatory protein GCN5L1 prevented the development of diastolic dysfunction (measured as a change in E/e’ ratio) in response to a high fat diet. Quantitative acetylproteomics demonstrated that enzymes in the mitochondrial fatty acid oxidation and pyruvate utilization pathways were most affected by GCN5L1-dependent acetylation. Deletion of GCN5L1 prevented hyperacetylation of the pyruvate dehydrogenase complex subunit PDHA1, which increased its enzymatic activity, and allowed increased pyruvate utilization in hearts from obese, aged mice. Using a cell culture model of variable PDHA1 acetylation status, we confirmed that site-specific acetylation of five PDHA1 lysine residues significantly reduced its enzymatic activity in cardiac cells in vitro.

Conclusions Our findings suggest that changes in mitochondrial protein lysine acetylation represent a key metabolic component of diastolic dysfunction that precedes the development of heart failure. Our work suggests that manipulation of PDHA1 acetylation levels in vivo may represent a novel target for therapeutic intervention in the treatment of diastolic dysfunction.