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, GlcC16 may be 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 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 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). Importantly, 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-KO mice to coronary artery ligation and assessed cardiac phenotype following MI. We subjected MAP1S-KO mice to coronary artery ligation and assessed cardiac phenotype following MI.

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 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.

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.

Abstracts

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Introduction Left ventricular diastolic dysfunction is a structural and functional condition that precedes the development of heart failure with preserved ejection fraction (HFpEF). 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 HFpEF.

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.