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Mitochondrial remodeling in mice with cardiomyocyte-specific lipid overload

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ABSTRACT

Background: Obesity leads to metabolic heart disease (MHD) that is associated with a pathologic increase in myocardial fatty acid (FA) uptake and impairment of mitochondrial function. The mechanism of mitochondrial dysfunction in MHD, which results in oxidant production and decreased energetics, is poorly understood but may be related to excess FAs. Determining the effects of cardiac FA excess on mitochondria can be hindered by the systemic sequelae of obesity. Mice with cardiomyocyte-specific overexpression of the fatty acid transport protein FATP1 have increased cardiomyocyte FA uptake and develop MHD in the absence of systemic lipotoxicity, obesity or diabetes. We utilized this model to assess 1) the effect of cardiomyocyte lipid accumulation on mitochondrial structure and energetic function and 2) the role of lipid-driven transcriptional regulation, signaling, toxic metabolite accumulation, and mitochondrial oxidative stress in lipid-induced MHD.
Methods: Cardiac lipid species, lipid-dependent signaling, and mitochondrial structure/function were examined from FATP1 mice. Cardiac structure and function were assessed in mice overexpressing both FATP1 and mitochondrial-targeted catalase.
Results: FATP1 hearts exhibited a net increase (+12%) in diacylglycerol, with increases in several very long-chain diacylglycerol species (+160–212%, $p < 0.001$) and no change in ceramide, sphingomyelin, or acylcarnitine content. This was associated with an increase in phosphorylation of PKCα and PKCδ, and a decrease in phosphorylation of AKT and expression of CREB, PGC1α, PPARα and the mitochondrial fusion genes MFN1, MFN2 and OPA1. FATP1 overexpression also led to marked decreases in mitochondrial size (−49%, $p < 0.01$), complex II-driven respiration (−28.6%, $p < 0.05$), activity of isolated complex II (−62%, $p = 0.05$), and expression of complex II subunit B (SDHB) (−60% and −31%, $p < 0.01$) in the absence of change in ATP synthesis. Hydrogen peroxide production was not increased in FATP1 mitochondria, and cardiac hypertrophy and diastolic dysfunction were not attenuated by overexpression of catalase in mitochondria in FATP1 mice.
Conclusions: Excessive delivery of FAs to the cardiac myocyte in the absence of systemic disorders leads to activation of lipid-driven signaling and remodeling of mitochondrial structure and function.

1. Introduction

Metabolic heart disease (MHD) in patients with obesity and diabetes is characterized by cardiac hypertrophy and diastolic dysfunction. In MHD, increased cardiomyocyte uptake of circulating fatty acids (FAs) [1] through cell surface transporters such as FATP1 [2] leads to lipid accumulation in the myocardium [3]. Cardiomyocyte lipid accumulation correlates with diastolic dysfunction [1], impaired mitochondrial energetics [4], and increased oxidative stress [5]. The underlying mechanisms of MHD, including the direct effects of cardiomyocyte FAs on mitochondria, are not well described. It has been hypothesized that increased cardiomyocyte FA uptake could cause mitochondrial dysfunction [5–7]. Possible mechanisms of mitochondrial dysfunction in MHD include respiratory chain uncoupling from ATP synthesis [8], increased reactive oxygen species (ROS) production [6], altered biogenesis [9,10], and/or impaired quality control [11]. However, elucidating the specific role of cardiomyocyte lipid excess in the pathogenesis of MHD can be confounded by systemic metabolic changes that occur with

Abbreviations: FATP1, Fatty acid transport protein 1; FA, Fatty acids; MHD, Metabolic heart disease; ROS, Reactive oxygen species; PPARα, Peroxisome proliferator-activated receptor α; PGC1α, PPARγ coactivator-1 α; DG, Diacylglycerol; CREB, Cyclic AMP responsive element binding protein; PKC, Protein kinase C; ETC, Electron transport chain; ATP, Adenosine triphosphate; ADP, Adenosine diphosphate; SM, Sphingomyelin; CER, Ceramide; AC, Acyl carnitine; EM, Electron micrographs; SDHB, Succinate dehydrogenase subunit B; mCAT, Mitochondrial catalase; ACS, Acyl-CoA synthetase.

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obesity (inflammation, insulin resistance, circulating factors) [12,13]. Thus, this study sought to test the effect of increased cardiomyocyte FA accumulation on mitochondrial structure, function, and oxidant production in the absence of systemic metabolic perturbations.

The hypothesis of this study was that excess cardiomyocyte FA uptake causes mitochondrial dysfunction due to lipid-mediated inhibition of mitochondrial biogenesis and/or energetic function. To address this hypothesis, mice with cardiomyocyte-specific overexpression of the fatty acid transport protein (FATP1) were used as a model of increased cardiomyocyte FA content in the absence of alterations in systemic metabolism. FATP1 mice exhibit increased cardiac FA uptake (4-fold) and utilization (2-fold) and decreased glucose utilization (−50%) [14]. Their cardiac phenotype has been previously described [14]; they develop cardiac hypertrophy and diastolic dysfunction, and thus provide a model of MHD due to cardiac lipid overload. The goals of this study were to 1) determine the effects of FATP1-driven lipid excess on cardiomyocyte mitochondrial structure, function, and oxidant production, and 2) elucidate the mechanisms responsible for MHD in FATP1 hearts by examining toxic lipid accumulation, lipid-driven transcriptional regulation, and oxidative stress on mitochondrial structure and energetic function.

2. Materials and methods

2.1. Experimental animals

Cardiomyocyte-specific FATP1 [14] and littermate control mice 5–8 weeks of age were fed normal chow diet and used for all animal studies. Mice with systemic overexpression of catalase in mitochondria (mCAT) [15] were crossed with FATP1 mice and echocardiographic studies were performed at 2–4 months of age. The Institutional Animal Care and Use Committee at Boston University approved this study.

2.2. Lipidomic analysis

Plasma and hearts from FATP1 and control mice were harvested and flash frozen. Hearts were homogenized in PBS and protein concentration quantified by BCA protein assay. 50 μl of the homogenate or plasma was mixed 1:1 with methanol for protein precipitation. The supernatant was spiked with internal standards and was adjusted to 1 ml with 1:1 methanol/water solution for sphingomyelin (SM), ceramide (CER), diacylglycerol (DG) and acylcarnitine (AC). The samples were dried down and reconstituted in the 1:1 methanol/water solution. Samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as previously described [16] at the Metabolomics Facility of the Diabetic Cardiovascular Disease Center at Washington University.

2.3. Immunoblotting

Whole-heart protein lysates made from freeze-clamped hearts using lysis buffer (Hepes pH 7.4 (20 mM), β-glycerol phosphate (50 mM), EGTA (2 mM), DTT (1 mM), NaF (10 mM), NaVO4 (1 mM), Triton-X 100 (1%), glycerol (10%,) and 1 protease inhibitor complete mini tablet-EDTA free/20 ml (Roche)) were subjected to SDS-PAGE with protein content quantified using the Bradford method (Biorad). Following transfer, nitrocellulose membranes were probed with primary antibodies to MFN1 (Neuromab), MFN2 (Sigma-Aldrich AV42420), OPA1 (BD Transduction 612606), phospho-PKCpan (Cell Signaling 9371), phospho-PKCα (Cell Signaling 06-822), total PKCα (EMD 05-154), phospho-PKCδ (Cell Signaling 9376), total PKCδ (Santa Cruz 937), phospho-PKCε (Santa Cruz sc12355), total PKCε (EMD 06-991), SDHB (Abcam ab110413), CREB (Pierce MA1-083), GAPDH (Abcam ab9485), and visualized using horseradish peroxidase secondary antibodies with SuperSignal West chemiluminescence substrate and an enhanced chemiluminescence Fujifilm LAS-4000 imager or LI-COR secondary antibodies and the LICOR Odyssey IR imager. Densitometry was quantified using Fujifilm Multigauge or Odyssey software.

2.4. PCR array and RT-PCR

Frozen hearts were ground under liquid nitrogen and total RNA was extracted with the mirVana miRNA Isolation Kit (Applied Biosystems). Total RNA was treated with DNAse before cDNA synthesis with the High Capacity RNA-to-cDNA Kit (Applied Biosystems). RT^2 Profiler PCR arrays were performed per manufacturer instructions (SABiosciences) using the cataloged Mitochondria (PAMM-087Z), Mitochondrial Metabolism (PAMM-008Z) and PPAR Targets (PAMM-149Z) array configurations. Quanitative PCR was performed with TaqMan Universal PCR Master Mix and TaqMan primers (Applied Biosystems) specific for mouse PPARα (Mm00440939_m1), PGC1α (Mm01208835_m1), SDHB (Mm00458272_m1), MFN1 (Mm01289369_m1), MFN2 (Mm00500120_m1), OPA1 (Mm00453873_m1), CREB1 (Mm00501604_m1), HSL (Mm00495359_m1), ATGL (Mm00503040_m1), DGAT1 (Mm00515643_m1), DGAT2 (Mm00499536_m1), and GAPDH (4352339E) using the Applied Biosystems Step One Plus Real Time PCR Systems. Relative mRNA expression was normalized to GAPDH expression using the ΔΔCT method.

2.5. Electron micrographs

Hearts were harvested, minced (4 × 4 × 1 mm), and placed in 2.5% glutaraldehyde/2.0% paraformaldehyde in 0.1 M cacodylate buffer fixative solution overnight. Specimens were then post-fixed in 1.0% osmium tetroxide in 0.15 M cacodylate buffer, dehydrated using a graded acetone series, and infiltrated and embedded with an epoxy resin at 65° overnight. Specimens were thick sectioned (200 nm) to search for areas of longitudinal fiber orientation, thin sectioned (75 nm) using a diamond knife onto a copper grid, stained with 4% aqueous uranyl acetate and lead citrate, and visualized using a CCD camera at 4000× magnification. Image analysis was performed blinded to genotype with free-hand tracing of mitochondria to estimate size using ImageJ and grid counting to estimate mitochondrial number per visual field.

2.6. Mitochondrial isolation

Cardiac mitochondria were isolated as previously described [17]. In brief, hearts were harvested, washed and minced in ice-cold relaxation buffer (KCl 100 mM, EGTA 5 mM, HEPES 5 mM pH 7.0 with KOH). Heart pieces were then homogenized in 2 ml of HES buffer (HEPES 5 mM, EDTA 1 mM, Sucrose 250 mM pH 7.4) in a Teflon motor-drive homogenizer. The homogenized solution was centrifuged at 500 ×*g* for 10 min at 4 °C. The pellet was discarded and the supernatant was centrifuged at 9000 ×*g* for 15 min at 4 °C. The mitochondrial pellet was resuspended in 50–100 μl of HES buffer. Protein was quantified using bicinchoninic acid (BCA, Thermo Scientific) assay.

2.7. Mitochondrial oxygen consumption rate

Oxygen consumption rates were monitored using a Seahorse XF24 oxygen flux analyzer as previously described [17]. Isolated mitochondria were loaded in a 24-well Seahorse plate on ice (5–10 μg per well) and 440 μl of ice-cold mitochondrial assay solution (MAS: 70 mM sucrose, 220 mM mannitol, 5 mM KH_2PO_4, 5 mM $MgCl_2$, 2 mM HEPES, 1 mM EGTA, 0.2%BSA fatty acid-free, pH 7.4) plus 10x substrates (complex I: 50 mM pyruvate and 50 mM malate; complex II: 50 mM succinate and 20 μM rotenone in MAS) were added on top. The 4 sequential injection ports of the Seahorse cartridge contained the following: Port A—50 μl of 10X substrate and 2.5 mM ADP, Port B—55 μl of 20 μM oligomycin, Port C—60 μl of 40 mM FCCP, and Port D—65 μl of