Journal of Molecular and Cellular Cardiology 100 (2016) 64–71

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Preservation of myocardial fatty acid oxidation prevents diastolic dysfunction in mice subjected to angiotensin II infusion

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ARTICLE INFO

Article history:
Received 14 June 2016
Received in revised form 19 August 2016
Accepted 1 September 2016
Available online 28 September 2016

Subject codes:
[107] Biochemistry and metabolism
[140] energy metabolism
[145] genetically altered mice
[90] Lipid and lipoprotein metabolism
[105] Contractile function

Keywords:
Cardiac metabolism
Lipids
ACC2
Myocardium
Hypertrophy

ABSTRACT

Rationale: Diastolic dysfunction is a common feature in many heart failure patients with preserved ejection fraction and has been associated with altered myocardial metabolism in hypertensive and diabetic patients. Therefore, metabolic interventions to improve diastolic function are warranted. In mice with a germline cardiac-specific deletion of acetyl CoA carboxylase 2 (ACC2), systolic dysfunction induced by pressure-overload was prevented by maintaining cardiac fatty acid oxidation (FAO). However, it has not been evaluated whether this strategy would prevent the development of diastolic dysfunction in the adult heart.
Objective: To test the hypothesis that augmenting cardiac FAO is protective against angiotensin II (AngII)-induced diastolic dysfunction in an adult mouse heart.
Methods and results: We generated a mouse model to induce cardiac-specific deletion of ACC2 in adult mice. Tamoxifen treatment (20 mg/kg/day for 5 days) was sufficient to delete ACC2 protein and increase cardiac FAO by 50% in ACC2 flox/flox-MerCreMer^{+} mice (iKO). After 4 weeks of AngII (1.1 mg/kg/day), delivered by osmotic mini-pumps, iKO mice showed normalized E/E′ and E′/A′ ratios compared to AngII treated controls (CON). The prevention of diastolic dysfunction in iKO-AngII was accompanied by maintained FAO and reduced glycolysis and anaplerosis. Furthermore, iKO-AngII hearts had a ~ 50% attenuation of cardiac hypertrophy and fibrosis compared to CON. In addition, maintenance of FAO in iKO hearts suppressed AngII-associated increases in oxidative stress and sustained mitochondrial respiratory complex activities.
Conclusion: These data demonstrate that impaired FAO is a contributor to the development of diastolic dysfunction induced by AngII. Maintenance of FAO in this model leads to an attenuation of hypertrophy, reduces fibrosis, suppresses increases in oxidative stress, and maintains mitochondrial function. Therefore, targeting mitochondrial FAO is a promising therapeutic strategy for the treatment of diastolic dysfunction.

1. Introduction

Heart failure (HF) is a clinical syndrome that affects approximately 6 million Americans with a striking additional ~500,000 new diagnoses each year [1]. Although HF has been traditionally synonymous with reduced ejection fraction (HFrEF), approximately 50% of all HF patients have preserved ejection fraction (HFpEF) [2]. Although the risk factors (i.e. aging, hypertension, diabetes) and clinical symptoms overlap for HFrEF and HFpEF, the pathophysiological manifestations of the diseases can be quite distinct [3]. As a result, effective pharmacological interventions for patients with HFpEF remain elusive [4]. Despite a lack of consensus on whether diastolic abnormalities are a signature of HFpEF, nearly two-thirds of these patients present with diastolic dysfunction [5,6]. In this regard, further investigations into the pathology of diastolic dysfunction may identify underlying contributing factors and reveal new potential treatment strategies.

The development of diastolic dysfunction is a common feature in the aging population and is associated with an increased risk of HF, including HFpEF [7,8]. We recently demonstrated that acute treatment of rapamycin in aged mice increased fatty acid oxidation (FAO) and reversed diastolic dysfunction [9]. This finding led to the intriguing hypothesis that impaired FAO was an inciting factor in the declination of diastolic function and that restoring FAO in the diseased heart could correct the functional abnormality. Previously, we demonstrated that cardiac-specific deletion of ACC2 (ACC2H$^{-/-}$) in mice subjected to pressure-overload via transverse aortic constriction, had sustained systolic function and myocardial energetics, as a result of preserved cardiac

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http://dx.doi.org/10.1016/j.yjmcc.2016.09.001
0022-2828/

FAO [10]. These findings indicated that targeting FAO, specifically at the level of fatty acid entry into the mitochondria, was a potential metabolic therapy in which to prevent systolic dysfunction in the pathologically hypertrophied heart. However, it has not been determined whether targeting cardiac FAO, via ACC2 deletion, in an adult heart would be protective against the development of diastolic dysfunction.

To establish the therapeutic benefit of enhancing myocardial FAO in an adult heart with diastolic dysfunction, we generated a mouse model employing an inducible deletion strategy to target ACC2 in mice subjected to chronic angiotensin II (AngII) infusion. We selected the chronic AngII model based on its known systemic effects of hypertension as well as the predominant phenotype of diastolic dysfunction [11–14]. Our results show that deletion of ACC2 in an adult mouse is a safe and effective means of preserving myocardial FAO and preventing diastolic dysfunction that results from chronic exposure to AngII. Furthermore, sustained FAO during chronic AngII stimulation leads to an attenuation of pathological hypertrophy and fibrosis and is accompanied by suppressed elevations of oxidative stress and maintained mitochondrial function. These collective findings indicate that impaired myocardial FAO is a contributor to the development of diastolic dysfunction and that targeting of mitochondrial FAO is a viable therapy to restore diastolic function, and potentially, delay the transition to failure.

2. Materials and methods

2.1. Animal model

Studies were approved by the University of Washington Institutional Animal Care and Use Committee. Mice were kept on a 12-hour light/dark cycle with water and food ad libitum. ACC2 flox/flox-MerCreMer + ($ACC2^{-f/f\text{-}MCM+}$) mice were mated with $ACC2^{f/f}$ to produce both study and control littermates (see Online Supplement for additional details). At 12–14 weeks of age, both $ACC2^{f/f\text{-}MCM+}$ and $ACC2^{f/f}$ (CON) mice received an intraperitoneal injection of tamoxifen (20 mg/kg) for 5 days, which was sufficient to cause ACC2 deletion in $ACC2^{f/f\text{-}MCM+}$ (iKO). Four weeks after the last injection of tamoxifen, male CON and iKO mice were implanted with an osmotic mini-pump (Alzet, Durect Corporation, #2004) containing angiotensin II (AngII; 1.1 mg/kg/day) or saline (vehicle) for 4 weeks, which resulted in similar increases in both systolic and diastolic blood pressures in both groups (Fig. S1).

2.2. Transthoracic echocardiography

Murine trans-thoracic echocardiography was conducted in mice using a Vevo 770 High-Resolution Imaging System (VisualSonics, Toronto, Ontario, Canada) machine and a 30 mHz probe [15]. Isoflurane (1%) was administered during the procedure. Serial echocardiography was performed on mice at baseline (before tamoxifen) and at 4 and 12 weeks post tamoxifen injections. At the end of the 4 week AngII treatment, echocardiography was performed and diastolic function was assessed via Tissue Doppler Imaging (TDI).

2.3. Isolated heart perfusion and nuclear magnetic resonance (NMR) spectroscopy

Langendorff, isolated mouse heart experiments were conducted as previously described [10,16]. Hearts were perfused with ^{13}C-labeled substrates (1,6-^{13}C glucose and U-^{13}C fatty acids) to determine substrate utilization. ^{13}C NMR spectroscopy was performed on lyophilized heart extracts [10,17]. Substrate utilization, glycolysis, and anaplerosis were calculated by isotopomer analysis. (See Online Supplement for expanded methods.)

2.4. Electron microscopy

Myocardial tissue sections were prepared and imaged on an electron microscope (JEOL USA 1230, Peabody, MA). Mitochondrial area was measured in 5 random fields from each heart by imaging software (Image J, NIH). (See Online Supplement for expanded methods.)

2.5. Organ weight and histological assessment

Body weight, tibia length (TL), and heart weight (HW) were measured in mice at 4 weeks post-mini-pump implantation. Hearts were harvested, rinsed briefly in 1× PBS, blotted dry, and weighed. HW was normalized to TL to assess changes in hypertrophy. For histology, hearts were arrested in diastole by KCl (30 mmol/L) and perfusion fixed with 10% neutral buffered formalin. Longitudinal sections of hearts were stained with Masson's Trichrome. Percentage of fibrosis was determined using an imaging processing software (Image J, NIH).

2.6. RNA extraction and quantitative real-time polymerase chain reaction

Total RNA was isolated from frozen LV tissue using the RNeasy Kit (Qiagen, Valencia, CA). Omniscript reverse synthase and random hexamers were used for cDNA synthesis according to manufacturer's guidelines. Real Time PCR was performed using SYBR green (Bio-Rad, Hercules, CA). Results of mRNA levels were normalized to 18S rRNA levels and reported as fold-change over control. Primer sequences appear in Supplemental Table 1.

2.7. Biochemical assays and immunoblotting

Protein carbonyls were measured in heart lysates using the Protein Carbonyl ELISA kit (Cell BioLabs, Inc., San Diego, CA). Hydrogen peroxide was measured in frozen heart lysates using the Amplex Red Assay kit (Invitrogen, Grand Island, NY). Aconitase activity was measured in frozen heart lysates using a commercially available kit (Cayman Chemical, Ann Arbor, MI). Western blotting was performed on heart lysates. Protein extracts were separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes and probed with the following antibodies: anti-ACC (Cell Signaling Technology, Danvers, MA), anti-sarcomeric actin (Sigma Aldrich, St. Louis, MO).

2.8. Respiratory complex activity

Respiratory complex activity (Complex I, II, III, IV and citrate synthase) were assessed in lysates from frozen cardiac tissue according to previously published methods using a spectrophotometer (Evolution 220, Thermo Scientific, Waltham, MA) [18]. All assays were performed at 37 °C. Activity was calculated by the difference in the change of absorbance in the presence and absence of specific inhibitors and normalized to protein concentration.

2.9. Statistical analysis

All data are presented as means ± standard error (SEM). Statistical analysis was tested with a one-way analysis of variance (ANOVA) or two-way ANOVA with a Bonferroni's post hoc. Two group comparisons were made using Student's *t*-test. All analyses were performed using GraphPad Prism 6.0. Statistical significance was considered at $P < 0.05$ level.