Experimental Gerontology 111 (2018) 122–132

Contents lists available at ScienceDirect

Experimental Gerontology

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

Cardiac hypertrophy in sarcopenic obese C57BL/6J mice is independent of Akt/mTOR cellular signaling

Richard A. Perry Jr.[a], Lemuel A. Brown[a], Wesley S. Haynie[a], Jacob L. Brown[b], Megan E. Rosa-Caldwell[b], David E. Lee[b], Nicholas P. Greene[b], Tyrone A. Washington[a,*]

[a] Exercise Muscle Biology Laboratory, Exercise Science Research Center, Department of Health, Human Performance and Recreation, University of Arkansas, Fayetteville, AR 72701, United States of America

[b] Integrative Muscle Metabolism Laboratory, Exercise Science Research Center, Department of Health, Human Performance and Recreation, University of Arkansas, Fayetteville, AR 72701, United States of America

ARTICLE INFO

Section Editor: Werner Zwerschke

Keywords:
AMPK
MAPK
Cardiac remodeling pathological
TNFα

ABSTRACT

Sarcopenic obesity (SO) is the comorbidity of age-related muscle wasting and obesity. SO increases the risk of heart disease, but little is known about the cellular signaling in cardiac muscle of SO individuals.

Aim: The purpose of this study was to identify key cellular signaling alterations in cardiac muscle of sarcopenic obese mice.

Methods: Thirty-two, male C57BL/6J mice were randomly divided into lean and high-fat fed groups and raised to 3–4 months (young) or 20–22 months (aged) of age. Hearts were extracted and processed for Western blot and qRT-PCR analyses.

Results: Hearts of SO mice were 36–55% heavier than the young, obese or aged, lean groups. Markers downstream of Akt were not elevated in the SO group. p-p38:p38 MAPK was higher with age, and a 2-fold increase was observed in the obese vs. lean aged groups. pERK1/2:ERK1/2 MAPK was ~50–70% lower in the SO cardiac muscle compared to the young, obese group. pAMPK:AMPK was 50%–66% lower in the SO cardiac muscle compared to the obese and lean, aged groups. mRNA abundance of TNFα was ~2.5-fold higher in the SO group.

Conclusion: Cardiac hypertrophy in SO is likely pathogenic as evidenced by the alterations in MAPK and AMPK protein content and lack of activation in the Akt/mTOR pathway.

1. Introduction

In the United States and other well-developed countries, two ailments prevail as growing concerns: obesity and sarcopenia. Though the overall obesity rate in America has remained stable from 2003 to 2012, its prevalence remains a primary health concern (Ogden et al., 2012). Sarcopenia, an excessive loss of muscle mass associated with aging, is predicted to increase as the percentage of aged adults rises (Ethgen et al., 2016). Sarcopenia affects as many as 5–13% of persons aged 60 or older (Morley et al., 2014). Independently, obesity and sarcopenia are associated with an increase in morbidity, but the combination of these two ailments, a condition that has been termed "sarcopenic obesity", is reported to increase the risk of all-cause mortality (Tian and Xu, 2016; Atkins et al., 2014).

The sarcopenic obese population is sparsely studied, but several reports agree this group suffers from an increased risk of developing cardiovascular disease (CVD) resultant from pathological cardiac remodeling (Stephen and Janssen, 2009; Kim et al., 2015; Chung et al., 2013). Cardiac remodeling — alterations in dimension, shape, mass or function — can be physiological or pathological. In a mouse model of young (10–12 weeks of age) and middle-aged (40–44 weeks of age) mice fed a high-fat diet for 12 weeks, cardiac remodeling, expressed as physiological cardiac hypertrophy (an increase in heart mass with normal and/or enhanced cardiac function), has been reported to develop (Sung et al., 2011; Ooi et al., 2014). Cardiac hypertrophy is likely to also occur in aged, high-fat fed mice; however, this hypertrophy is expected to be pathological (an increase in heart mass with cardiac dysfunction) (Shimizu and Minamino, 2016). Pathological cardiac hypertrophy often leads to cardiac dysfunction via decreases in contractile strength as well as increases in apoptosis and/or fibrosis (Frey and Olson, 2003; Heineke and Molkentin, 2006). Pathological cardiac hypertrophy is often viewed as the first sign of CVD.

* Corresponding author at: University of Arkansas, Department of Health, Human Performance, and Recreation, 155 Stadium Dr. HPER 309, Fayetteville, AR 72701, United States of America.

E-mail address: tawashin@uark.edu (T.A. Washington).

https://doi.org/10.1016/j.exger.2018.06.023
Received 13 November 2017; Received in revised form 20 June 2018; Accepted 23 June 2018
Available online 26 June 2018
0531-5565/

Though both physiological and pathological cardiac hypertrophy result in greater heart mass, cellular signaling related to their progression differs. Sung et al. (2011) show that middle-aged, high-fat fed mice have higher phosphorylation of the Akt/mTOR pathway: mammalian target of rapamycin (mTOR), p70S6K and S6K. Additionally, these mice have lower relative phosphorylation of 5′ AMP-activated protein kinase (AMPK), an inhibitor of the Akt/mTOR pathway. These data indicate that the Akt/mTOR pathway may play a critical role in cardiac hypertrophy of high-fat fed, middle-aged mice. It is unknown if obesity in aged mice would promote the same alterations or if obesity in the aged mice would promote cellular pathways more indicative of pathological hypertrophy, such as the mitogen-activated protein kinase (MAPK) (p38 and extracellular signal-regulated kinase [ERK]) pathways (Wilkins et al., 2004; Bernardo et al., 2010).

In addition to the activation of alternate protein synthetic pathways, suppression of autophagy is another hallmark of pathological cardiac hypertrophy. Autophagy is a key process in the removal of long-lived or dysfunctional cellular components. In the heart, autophagy is often regarded as a cytoprotective mechanism especially for its role in preventing apoptosis (Nishida et al., 2009). Though the role of autophagy in cardiac hypertrophy is not fully elucidated, several studies show that autophagy is downregulated in pathological cardiac hypertrophy especially as it relates to aging (Hua et al., 2011; He et al., 2014). Specifically, these studies show AMPK downregulation and Akt upregulation to be key changes during pathological cardiac hypertrophy. Interestingly, common markers of autophagy (i.e. LC3) have proven to be inconsistent when aging models have been used (Linton et al., 2015).

The purpose of this study was to determine if aged obese mice develop cardiac hypertrophy and to identify the underlying cellular alterations associated with this response. Since the sarcopenic obese population has been observed to have a higher risk of CVD, we hypothesize that activation of the Akt/mTOR pathway would not be significant to the development of cardiac hypertrophy in the sarcopenic obese model; conversely, we hypothesize that targets of the MAPK pathway would be upregulated. We also hypothesize that autophagic and inflammatory responses would be downregulated and upregulated, respectively, in the sarcopenic obese mice.

2. Methods

2.1. Animals and housing

All animal use protocols complied with the NIH guide for the care and use of laboratory animals and were approved by the University of Arkansas Institutional Care and Use Committee. Thirty-two, male C57BL/6J mice were obtained from Rigel Pharmaceuticals and were housed in the University of Arkansas Central Laboratory Animal Facility. Animals were kept on a 12:12-hour light-dark cycle and given access to either normal (NC, 17% fat, Teklad 22/5 Rodent Diet, 86140, Teklad Diets, Madison, WI) or high-fat (HF, 60% kcals fat, D12492, Research Diets, Inc., New Brunswick, NJ) chow which they began at 4 weeks of age and maintained until time of tissue harvest. The study consisted of four groups: young lean (YL), young obese (YO), aged lean (AL), and aged obese (AO) (n = 8 per group). YO mice were on HFD for 8–12 weeks, and AO mice were on HFD for 21–23 months. Mice that were 3–4 months and 22–24 months old were considered young and aged, respectively. Mice were given access to water and chow ad libitum.

2.2. Heart and tibia extraction

Heart and tibia extraction were performed as previously described (Brown et al., 2015). At either 3–4 months or 22–24 months of age, mice were anesthetized with a subcutaneous injection of a cocktail containing ketamine hydrochloride (90 mg/kg body weight), xylazine (3 mg/kg body weight) and acepromazine (1 mg/kg body weight). The heart was excised, weighed and then powdered in a liquid nitrogen-cooled container. The powdered tissue was stored at −80 °C for protein and gene expression analysis. After removal of the heart, the tibias were removed and measured using a digital caliper (VWR, Radnor, PA, USA). Tibia length was used to normalize tissue weight to body size in a manner independent of HFD and aging induced changes in total body weight. Cardiac hypertrophy was evaluated by determining the heart weight:tibia length (HW:TL) ratio as well as the heart weight:body weight (HW:BW) ratio (Sung et al., 2011).

2.3. Western blotting

Western blotting was performed as previously described (Brown et al., 2015). Cardiac homogenate was fractionated in 8–12% SDS-polyacrylamide gels. Gels were transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were stained with *Ponceau S* before blotting to verify equal loading of the gels. Membranes were blocked in 5% milk, in Tris-buffered saline (TBS) with 0.1% Tween-20 (TBST), for 2 h. Primary antibodies for p-Akt (Ser473) (#9271), Akt (#2920), p-mTOR (Ser2448) (#5536), mTOR (#2983), p-4EBP1 (Thr37/46) (#2855), 4EBP1 (#9644), p-p70S6K (Thr389) (#9234), p70S6K (#9202), p-AMPK (Thr172) (#2535), AMPK (#5832), p-MAPK p38 (Thr180/Tyr182) (#4511), MAPK p38 (#8690), p-MAPK ERK1/2 (Thr202/Tyr204) (#4370), MAPK ERK1/2 (#9102), NFκB p65 (#8242) and LC3A/B (#12741) were obtained from Cell Signaling. SQSTM1/p62 (#P0067) primary antibody was obtained from Sigma. Primary antibodies were diluted 1:500 to 1:1000 in 5% milk, in TBST, and incubated at 4 °C overnight. Anti-rabbit and anti-mouse monoclonal secondary antibodies (Cell Signaling Technologies, Danvers, MA) were diluted 1:2000 in 5% milk, in TBST, and incubated at room temperature for 1 h. Enhanced Chemiluminescence (ECL) was performed using Fluorochem M imager (Protein Simple, Santa Clara, California) to visualize antibody-antigen interaction. Blotting images were quantified by densitometry using Alphaview software (Protein Simple). The Ponceau-stained membranes were digitally scanned, and the 45-kDa actin bands were quantified by densitometry and used as a protein loading correction factor for each lane.

2.4. RNA isolation, cDNA synthesis, and quantitative RT-PCR

The following procedures were completed as previously described (Brown et al., 2015). RNA was extracted with Trizol reagent (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA was isolated using the Purelink mRNA mini kit (Thermo Fisher Scientific, Waltham, MA, USA). cDNA was reversed transcribed from 1 μg of total RNA using the Superscript Vilo cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA) for a final result of a 1:20 ratio of RNA to total volume. This final volume was then brought to a 1:100 dilution factor. Real-time PCR was performed, and results were analyzed by using the StepOne Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). cDNA was amplified in a 25 μL reaction containing appropriate probes and Taqman Gene Expression Mastermix (Thermo Fisher Scientific). Samples were incubated at 95 °C for 4 min, followed by 40 cycles of denaturation, annealing and extension at 95 °C, 55 °C and 72 °C, respectively. TaqMan fluorescence was measured at the end of the extension step each cycle. Fluorescence labeled probes for 18s, TNF-R, IL-6, Collagen I, Collagen III, TGF-β, MMP-2, MMP-9, TIMP-1, LDH-A, LDH-B and TNF-*α* were purchased from ThermoFisher Scientific and quantified with TaqMan Gene Expression Mastermix. Cycle Threshold (Ct) was determined, and the ΔCt value was calculated as the difference between the Ct value and the 18s Ct value. Final quantification of gene expression was calculated using the ΔΔCt method Ct = [ΔCt(calibrator) − ΔCt (sample)]. Relative quantification was then calculated as $2^{-\Delta\Delta Ct}$. Ct values of the 18S control gene did not differ among experimental conditions.