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Deficiency of cardiac Acyl-CoA synthetase-1 induces diastolic dysfunction, but pathologic hypertrophy is reversed by rapamycin

David S. Paul [b], Trisha J. Grevengoed [a], Florencia Pascual [a], Jessica M. Ellis [a], Monte S. Willis [b,c], Rosalind A. Coleman [a,b,*]

[a] Department of Nutrition, University of NC at Chapel Hill, 27599, USA
[b] McAllister Heart Institute, University of NC at Chapel Hill, 27599, USA
[c] Department of Pathology and Laboratory Medicine, University of NC at Chapel Hill, 27599, USA

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ABSTRACT

In mice with temporally-induced cardiac-specific deficiency of acyl-CoA synthetase-1 (*Acsl1*$^{H-/-}$), the heart is unable to oxidize long-chain fatty acids and relies primarily on glucose for energy. These metabolic changes result in the development of both a spontaneous cardiac hypertrophy and increased phosphorylated S6 kinase (S6K), a substrate of the mechanistic target of rapamycin, mTOR. Doppler echocardiography revealed evidence of significant diastolic dysfunction, indicated by a reduced E/A ratio and increased mean performance index, although the deceleration time and the expression of sarco/endoplasmic reticulum calcium ATPase and phospholamban showed no difference between genotypes. To determine the role of mTOR in the development of cardiac hypertrophy, we treated *Acsl1*$^{H-/-}$ mice with rapamycin. Six to eight week old *Acsl1*$^{H-/-}$ mice and their littermate controls were given i.p. tamoxifen to eliminate cardiac *Acsl1*, then concomitantly treated for 10 weeks with i.p. rapamycin or vehicle alone. Rapamycin completely blocked the enhanced ventricular S6K phosphorylation and cardiac hypertrophy and attenuated the expression of hypertrophy-associated fetal genes, including α-skeletal actin and B-type natriuretic peptide. mTOR activation of the related *Acsl3* gene, usually associated with pathologic hypertrophy, was also attenuated in the *Acsl1*$^{H-/-}$ hearts, indicating that alternative pathways of fatty acid activation did not compensate for the loss of Acsl1. Compared to controls, *Acsl1*$^{H-/-}$ hearts exhibited an 8-fold higher uptake of 2-deoxy[1-^{14}C]glucose and a 35% lower uptake of the fatty acid analog 2-bromo[1-^{14}C]palmitate. These data indicate that Acsl1-deficiency causes diastolic dysfunction and that mTOR activation is linked to the development of cardiac hypertrophy in *Acsl1*$^{H-/-}$ mice.

1. Introduction

It has been a recurring question as to whether altered cardiomyocyte metabolism activates pathways that lead to hypertrophy. Hypertrophied and stressed hearts increase their reliance on glucose as a major fuel, and it has been hypothesized that this alteration in glucose homeostasis underlies the heart's conversion, during pathologic hypertrophy, to a fetal pattern of gene expression [1]. However, it is unclear whether hypertrophy leads to increased glucose use or vice versa, or whether cardiac hypertrophy is an essential adaptation that is required to maintain normal heart function.

Long-chain acyl-CoA synthetase-1 (Acsl1), one of 5 independent Acsl isoforms, catalyzes the activation of long-chain fatty acids to acyl-CoAs that are directed towards mitochondrial β-oxidation [2,3]. The Acsl1 isoform is specifically required for fatty acid oxidation in highly oxidative tissues like heart [4], skeletal muscle [4], and brown adipose [5]. Normally, β-oxidation provides 60–90% of the ATP required for myocardial contraction [6–8], but in hearts from temporally-induced, multi-tissue Acsl1 deficient mice (*Acsl1*$^{T-/-}$) or from mice with a cardiomyocyte-specific Acsl1 deficiency (*Acsl1*$^{H-/-}$), fatty acid oxidation in ventricles is 90% lower than in littermate controls, and the uptake of [^{14}C]glucose into the heart increases 8-fold [4]. Although hypertension is not present, both *Acsl1*$^{T-/-}$ and *Acsl1*$^{H-/-}$ cardiomyocytes become hypertrophied within 10 weeks of the temporal initiation of the knockout [4]. In these mouse models, the altered fuel use is primary, rather than the consequence of hypertension or hypertrophy.

Left ventricular hypertrophy (LVH) results from a variety of stresses, including ischemia, infarction, valvular insufficiency, diabetes, and hypertension [9]. As a response to systemic hypertension, LVH is considered to be maladaptive and pathologic because it is a stronger predictor of

Abbreviations: Acsl, long-chain acyl-CoA synthetase; FA, fatty acid; mTOR, mechanistic target of rapamycin; mTORC1, mTOR complex-1; MPI, mean performance index

* Corresponding author at: 135 Dauer Drive, MHRC 2301, Department of Nutrition, CB# 7461, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA. Tel.: +1 919 966 7213; fax: +1 919 843 8555.
E-mail addresses: david_paul@med.unc.edu (D.S. Paul), tgrevengoed@gmail.com (T.J. Grevengoed), fpascual@live.unc.edu (F. Pascual), jessie.ellis@gmail.com (J.M. Ellis), monte_willis@med.unc.edu (M.S. Willis), rcoleman@unc.edu (R.A. Coleman).

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morbidity and mortality than is hypertension itself [10], and hypertrophy may ultimately result in heart failure and sudden death [9]. Pathologic hypertrophy is believed to result from events that involve biomechanical deformation signals, activation of G-protein-coupled receptors and their intracellular signaling pathways, and activation of the PI3K–Akt pathway initiated by insulin and other growth signals [9]. The growth-promoting mechanistic target of rapamycin (mTOR) kinase may be involved, because when rapamycin is used to inhibit mTOR in several mouse models, myocardial dysfunction improves [11–13], and the hypertrophy associated with excess thyroid hormone, constitutively active Akt, pressure overload, or over-expressed focal adhesion kinase, diminishes [12,14–16]. In contrast, in a mouse model with cardiac-specific deficiency of mTOR's partner Raptor (Regulatory-associated protein of mTOR), a dilated cardiomyopathy ensues, with death occurring within 6 weeks [17]. When cardiac-specific Raptor$^{-/-}$ hearts are challenged with pressure overload-induced hypertrophy, dilated cardiomyopathy ensues within 1 week, adaptive cardiomyocyte growth does not occur, and apoptosis and autophagy are enhanced [17]. Compared to isolated working wildtype hearts, palmitate oxidation by the Raptor knockout hearts decreased 51% and glucose oxidation increased 24%. These changes in fuel use were attributed to declines in PPARα and PGC1α expression, paralleling decreases in their target genes, including CPT1β, MCD-1, and SCOT, and a concomitant 2.2-fold higher expression of GLUT1 [17].

We previously showed in hearts lacking Acsl1 that mTOR complex-1 (mTORC1) is activated, as indicated by a 5-fold increase in phosphorylation of the mTORC1 target, p70 S6 kinase (S6K) [4], and we hypothesized that the inability to activate long-chain fatty acids for mitochondrial oxidation initiates metabolic shifts that activate mTOR. In *Acsl1*-deficient hearts, these shifts result in multiple inputs that signal the enhanced availability of glucose and other nutrients, demonstrated by the 8-fold increase in glucose flux and increased glucose metabolism and the higher ventricle content of amino acids. Although both Acsl1-deficient models have activated mTORC1, only the multitissue *Acsl1*$^{T-/-}$ mice have diminished phosphorylation of AMP-activated kinase (AMPK) [4], an inhibitor of mTORC1 signaling [18]. Cardiac hypertrophy was judged to be pathologic in both *Acsl1*$^{T-/-}$ and *Acsl1*$^{H-/-}$ hearts because of the upregulation of the fetal genes, α-skeletal actin, brain natriuretic peptide, and *Acsl3*. We hypothesized that the cardiomyocyte hypertrophy that develops in *Acsl1*-deficient hearts results from the promotion of cardiac remodeling and hypertrophy by mTORC1. We treated *Acsl1*$^{H-/-}$ mice and their littermate controls with rapamycin to determine whether hypertrophy in this metabolic model resulted from mTOR activation, and, if so, whether the hypertrophy was required to maintain normal systolic function when *Acsl1* deficiency impaired the use of fatty acids for energy production.

2. Materials and methods

2.1. Animal treatment

Mouse protocols were approved by the University of North Carolina Institutional Animal Care and Use Committee and conformed to the NIH Guide for the Care and Use of Laboratory Animals. Mice were housed in a pathogen-free barrier facility (12 h light/dark cycle) with free access to water and food (Prolab RMH 3000 SP76 chow). Mice with *Loxp* sequences inserted on either side of exon 2 in the *Acsl1* gene were backcrossed to the C57Bl/6J strain six times and then interbred with mice in which *Cre* expression is driven by an α-myosin heavy chain promoter that is induced by tamoxifen (B6.Cg-Tg(Myh6-cre/Esr1) 1Jmk/J, Jackson Labs) to generate tamoxifen-inducible, heart-specific *Acsl1*$^{H-/-}$ knockout mice. Six to 8 week old *Acsl1*$^{H-/-}$ and littermate *Acsl1*$^{flox/flox}$ control male mice were injected i.p. with tamoxifen (Sigma, St. Louis, MO) (75 mg/kg BW), dissolved in corn oil (20 mg/ml) for 4 consecutive days (3 mg/40 g body weight). Starting on the next day, subgroups of mice were injected daily i.p. for either 2 weeks or 10 weeks with rapamycin (1 mg/kg in PBS/8% ethanol, 10% Tween 20/10% PEG-400) or with the vehicle alone. Ten weeks after tamoxifen induction, the mice were anesthetized with avertin and heart ventricles were removed and snap frozen in liquid nitrogen. To isolate total membranes, hearts were homogenized with 10 up-and-down strokes, using a motor-driven Teflon pestle and glass mortar in ice-cold buffer (10 mM Tris [pH 7.4], 1 mM EDTA, 0.25 M sucrose, 1 mM dithiothreitol). Homogenates were centrifuged at 100,000 ×*g* for 1 h at 4 °C. The membrane pellet was then resuspended in buffer. Protein content was determined by the BCA assay (Pierce, Rockford, IL) with BSA as the standard. Plasma was collected from mice in 10% 0.5 M EDTA. Glucose tolerance tests were performed by i.p. injection with D-glucose (2.5 g/kg body wt), and tail blood glucose was measured at baseline, 15, 30, 60, and 120 min using a One Touch Ultra glucometer (Lifescan, Inc., Milpitas, CA).

2.2. Echocardiography and Doppler analysis

Cardiac echocardiography was performed (blinded to mouse type and treatment) on conscious mice at the indicated time points using a VisualSonics Vevo 770 or Vevo 2100 ultrasound biomicroscopy system (VisualSonics, Inc., Toronto, Ontario, Canada). A model 707B (30 MHz) or model MS-550D (22–55 MHz) scan head was used on the Vevo 770 and Vevo 2100, respectively, as previously described [19]. Two dimensional guided M-mode echocardiography was performed in the parasternal long-axis view at the level of the papillary muscle on loosely restrained conscious mice. Wall thickness was then determined by measurements of epicardial to endocardial leading edges. Doppler analysis of the mitral valve to determine the mitral inflow velocity was performed on lightly anesthetized mice (2% (vol/vol) isoflurane/100% oxygen) as previously described [4,20,21]. Mitral valve flow Doppler was acquired by positioning the transducer angled cranially in a supine mouse at 45° in an epigastric position to achieve an apical four-chamber view. Peak E and A heights were determined on mitral valve sequential waveforms in at least 5 waveforms. The mean performance index (ICT + IRT / ET) is calculated as the isovolumetric contraction time (ICT) plus the isovolumetric relaxation time (IRT) divided by the ejection time [22,23]. E-wave deceleration time (DT) was also measured. These measures were determined by Doppler waveform analyses of the mitral valve (as described above) and aortic valve along with EKG measurements to ensure the correct waveform times. The mean performance index (MPI; (ICT + ET + IRT) − ET / ET) can determine if either systolic or diastolic dysfunction is present [24,25] and has been successfully used in mice [26]. M-mode and Doppler measurement data represent 3–6 averaged cardiac cycles from at least 2 scans per mouse.

2.3. Acsl assay

Acsl initial rates were measured with 50 μM [1-^{14}C]palmitic acid (Perkin Elmer, Waltham, MA), 10 mM ATP, and 0.25 mM CoA in total membrane fractions from ventricles (2–6 μg protein) [27]. This assay measures the total ATP-mediated activation of long-chain fatty acids by long-chain Acsl isoforms plus very-long-chain Acsl isoforms (ACSVL, also called FATP). No Acsl activity was measurable in the soluble (cytosolic) fraction.

2.4. RT-PCR

Total RNA was isolated from heart ventricles (RNeasy Fibrous Tissues Kit, Qiagen, Alameda, CA). cDNA was amplified by real-time PCR using SYBR Green (Applied Biosystems, Foster City, CA) detection with specific primers for the gene of interest and normalized to *Gapdh or Tubulin* and expressed as arbitrary units of $2^{-\Delta CT}$ relative to the control group (Table 2).