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MOLECULAR METABOLISM

Muscle-specific knockout of general control of amino acid synthesis 5 (GCN5) does not enhance basal or endurance exercise-induced mitochondrial adaptation

Jessica R. Dent [1], Vitor F. Martins [2,5], Kristoffer Svensson [2], Samuel A. LaBarge [2], Noah C. Schlenk [2], Mary C. Esparza [2], Elisa H. Buckner [2], Gretchen A. Meyer [3], D. Lee. Hamilton [4], Simon Schenk [2,5,], Andrew Philp [1,*]**

ABSTRACT

Objective: Lysine acetylation is an important post-translational modification that regulates metabolic function in skeletal muscle. The acetyltransferase, general control of amino acid synthesis 5 (GCN5), has been proposed as a regulator of mitochondrial biogenesis via its inhibitory action on peroxisome proliferator activated receptor-γ coactivator-1α (PGC-1α). However, the specific contribution of GCN5 to skeletal muscle metabolism and mitochondrial adaptations to endurance exercise *in vivo* remain to be defined. We aimed to determine whether loss of GCN5 in skeletal muscle enhances mitochondrial density and function, and the adaptive response to endurance exercise training.

Methods: We used Cre-LoxP methodology to generate mice with muscle-specific knockout of GCN5 (mKO) and floxed, wildtype (WT) littermates. We measured whole-body energy expenditure, as well as markers of mitochondrial density, biogenesis, and function in skeletal muscle from sedentary mice, and mice that performed 20 days of voluntary endurance exercise training.

Results: Despite successful knockdown of GCN5 activity in skeletal muscle of mKO mice, whole-body energy expenditure as well as skeletal muscle mitochondrial abundance and maximal respiratory capacity were comparable between mKO and WT mice. Further, there were no genotype differences in endurance exercise-mediated mitochondrial biogenesis or increases in PGC-1α protein content.

Conclusion: These results demonstrate that loss of GCN5 *in vivo* does not promote metabolic remodeling in mouse skeletal muscle.

Keywords Acetylation; GCN5; Mitochondria; SIRT1; Deacetylase; PGC-1α

1. INTRODUCTION

For over 50 years endurance exercise has been known to induce mitochondrial adaptations in skeletal muscle [1—4]. While the mechanisms by which contraction initiates mitochondrial biogenesis remain to be fully elucidated, it is clear that perturbation in allosteric factors such as calcium (Ca^{2+})[1], adenosine monophosphate (AMP), nicotinamide-adenine dinucleotide (NAD^+), and acetyl-CoA are important initiators of the adaptive response [5—8]. Accordingly, considerable research has focused on the transduction pathways that are modulated by these metabolic intermediates and their contribution to exercise-induced mitochondrial adaptations in skeletal muscle.

[1]School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, UK [2]Department of Orthopaedic Surgery, University of California, La Jolla, San Diego, CA, USA [3]Program in Physical Therapy, Washington University School of Medicine, St Louis, MO, USA [4]School of Sport, Stirling University, Stirling, UK [5]Biomedical Sciences Graduate Program, University of California, La Jolla, San Diego, CA, USA

*Corresponding author. MRC-ARUK Centre for Musculoskeletal Ageing Research, School of Sport Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, B15 2TT, UK. Fax: +44 0 121 414 4121. E-mail: a.philp@bham.ac.uk (A. Philp).

**Corresponding author. Department of Orthopaedic Surgery, University of California, San Diego 9500 Gilman Drive MC0863, La Jolla, CA 92093-0863, USA. Fax: +1 858 822 3807. E-mail: sschenk@ucsd.edu (S. Schenk).

Abbreviations: AMP, adenosine monophosphate; Ca^{2+}, calcium; CBP, CREB-binding protein; Cre-MCK, creatine kinase promoter; CHO, carbohydrate; DAC, deacetylase; ETC, electron transport chain; ExT, endurance exercise training; FA, fatty acid; GA, gastrocnemius; GCN5, general control of amino acid synthesis 5; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HKII, hexokinase 2; KAT, acetyltransferase; mKO, muscle knockout; LCAD, long chain acyl CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; Mef2, myocyte enhance factor 2; Myf6, myogenic factor 6; MyoG, myogenin; NAD^+, nicotinamide-adenine dinucleotide; PARPs, poly (ADP-ribose) polymerases; PCAF, p300/CBP-associated factor; PDH, pyruvate dehydrogenase; PGC-1α, peroxisome proliferator activated receptor-γ coactivator-1α; Pln, plantaris; Q, quadriceps; RQ, respiratory quotient; SDH, succinate dehyrogenase; SIRT, sirtuin; TA, tibialis anterior; TCA, tricarboxylic acid; Tfam, mitochondrial-specific transcription factor A; TRI, triceps; WT, wildtype; mHZ, muscle heterozygous

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Reversible lysine acetylation has emerged as an important post-translational modification that links cellular flux to the adaptive responses [9,10]. An underlying reason for this is that NAD^+ is the primary substrate for the sirtuin class of deacetylases (DACs), which remove acetyl groups from lysine residues [11], whilst acetyl-CoA is the substrate for acetyltransferases (KATs), which add acetyl groups to lysine residues [12]. However, despite the high prevalence of lysine acetylation in skeletal muscle [13,14] much remains unknown regarding its contribution to the remodeling of skeletal muscle, both at rest and also in response to endurance exercise [15].

Peroxisome proliferator activated receptor-γ coactivator-1α (PGC-1α) is an important contributor to mitochondrial biogenesis and function in skeletal muscle [2,5,6], with its transcriptional activity being regulated, at least in part, by reversible acetylation [16–18]. Deacetylation by sirtuin 1 (SIRT1) is a potent activator of PGC-1α [16,17,19,20], while general control of amino acid synthesis 5 (GCN5) acetylates and inhibits its transcriptional activity [8,17,18,21]. GCN5 and PGC-1α form a complex in PGC-1α immunoprecipitates from Fao hepatocytes, while GCN5 overexpression in HEK293 cells represses PGC-1α intrinsic transcriptional activity [18]. In relation to skeletal muscle, overexpression of GCN5 in C2C12 myotubes represses PGC-1α-mediated induction of mitochondrial and fatty acid metabolism genes [17]. Moreover, we previously reported that deacetylation of PGC-1α following acute endurance exercise occurs in conjunction with a reduction in both the nuclear presence of GCN5 and its association with PGC-1α [8]. This relationship was maintained in both the presence and absence of SIRT1 deacetylase activity, suggesting that exercise-induced deacetylation of PGC-1α occurs as a result of reduced GCN5-PGC-1α interaction, rather than solely through SIRT1-dependent deacetylation of PGC-1α [8].

Taken together, these data implicate GCN5 as an important negative regulator of PGC-1α transcriptional activity in skeletal muscle and, by extension, mitochondrial biogenesis [8,17,18,21]. However, no studies to date have directly investigated the contribution of GCN5 to skeletal muscle metabolism and mitochondrial function *in vivo*. Accordingly, we used CreLoxP methodology to generate mice with muscle-specific knockout of GCN5 (mKO). We hypothesized that mKO mice would display increased mitochondrial biogenesis in skeletal muscle as compared to their floxed/wildtype (WT) littermates and that mitochondrial adaptations to endurance exercise training (ExT) would be enhanced in mKO mice.

2. MATERIALS AND METHODS

2.1. Generation of mKO mouse

To generate mKO mice, mice harboring LoxP sites flanking exons 3–19 of the GCN5 gene (Figure 1A) [22] (referred to as $GCN5^{flox/flox}$, and kindly provided by Dr. Sharon Dent, The University of Texas MD Anderson Cancer Center, Smithville TX, USA) were crossed with mice expressing Cre recombinase under the control of the muscle creatine kinase promoter (Cre-MCK); after Cre-mediated recombination, exons 3–19 are removed [22]. Our breeding strategy was to breed $GCN5^{flox/flox}$ mice with $GCN5^{flox/+}$ ('+' refers to a WT allele) mice, with one breeder being Cre-MCK positive, and the other Cre-MCK negative. This provided littermates that were Cre-MCK positive or negative and $GCN5^{flox/flox}$ or $GCN5^{flox/+}$; mice that were $GCN5^{flox/+}$ and Cre-MCK positive are heterozygous for loss of GCN5, and are referred to as mHZ. Mice negative for Cre-MCK are referred to as wildtype (WT) and were used as controls for all experiments. Mice were housed on a 12:12 h light–dark cycle, and all experiments were conducted in 13-wk-old littermates. All experiments were approved by and conducted in accordance with the Animal Care Program at the University of California, San Diego.

2.2. Tissue collection

Tissues were excised from fasted (4 h) and anesthetized mice. Skeletal muscles (gastrocnemius [GA], quadriceps [Q], triceps [TRI], tibialis anterior [TA], plantaris [Pln]), heart, liver, and epididymal adipose tissue (AT) were rinsed in sterile saline, blotted dry, weighed, and frozen in liquid nitrogen. The TA that was used for sectioning was pinned on cork and frozen in liquid nitrogen-cooled isopentane. All tissues were stored at −80 °C for subsequent analysis.

2.3. RNA extraction and cDNA synthesis for the quantitation of GCN5 gene expression

RNA was extracted from the Q muscle of WT, mHZ and mKO mice using the standard TRIzol method, and cDNA synthesized, as previously described [8]. The expression of GCN5 in each sample was normalized to values of the reference control glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the comparative ($2^{-\Delta\Delta CT}$) method and expressed relative to WT values. Primer sequences were: GCN5 forward, 5′-CAG GTC AAG GGC TAT GGC AC-3′ and reverse, 5′-GAT AGC GGC TCT TGG GCA C-3′, GAPDH forward 5′-TGGAAAGCTGTGGCGTGAT-3′ and reverse, 5′-TGCTTCACCACCTTCTTGAT-3'.

2.4. Skeletal muscle nuclear isolation

Isolated nuclear fractions were prepared from GA muscle using a commercially available kit (78835: NE-PER; Thermo Fisher Scientific, Waltham, MA, USA) with the addition of 4.8% cOmplete Mini protease inhibitor mixture (Sigma–Aldrich, St. Louis, MO, USA), 1 μM trichostatin A, 10 mM nicotinamide, 1 mM 1,4-Dithiothreitol, 1% Phosphatase Inhibitor Cocktail 2 (Sigma–Aldrich), 1% Phosphatase Inhibitor Cocktail 3 (Sigma–Aldrich).

2.5. GCN5 acetylatransferase activity

GCN5 specific acetyltransferase activity was determined in nuclear fractions from the GA using an immunoprecipitation HAT assay kit (17–284, Merck Millipore, Billerica, MA, USA). For this, the primary antibody in the kit was replaced with a GCN5 primary antibody (607201; Biolegend, San Diego, CA, USA) and the peptide substrate was replaced with Histone HS Peptide (12–403; Merck Millipore).

2.6. Targeted array

Total RNA was isolated from the TRI muscle using a combination of TRIzol (Thermo Fisher Scientific) and Promega ReliaPrep RNA Tissue Miniprep System (Promega, Madison, WI, USA) according to manufacturer instructions. Isolated RNA was reverse transcribed using the RT2 First Strand Kit (SABiosciences-Qiagen, Frederick, MD, USA). A 384-well custom-designed PCR array containing pre-optimized and pre-validated primers was developed in collaboration with SABiosciences. Genes representing the functional groups: electron transport chain (ETC), metabolism, mitochondrial remodeling, mitochondrial protein transport, transcription, mitochondrial transcription, DACs, KATs, angiogenesis, poly ADP ribose polymerases (PARPs), and myogenesis. The arrays also contained a replicate positive PCR control, reaction lacking reverse transcriptase, a mouse DNA positive control and a panel of housekeeping genes including GAPDH, Actb, Hsp90ab1, B2m, Tbp. PCR was performed on the Bio Rad CFX384 thermo cycler following the manufacturer's protocol. Expression of each gene was normalized to the respective average value of the panel of housekeeping genes using the comparative ($2^{-\Delta\Delta CT}$) method and expressed relative to WT.

