BBA - Molecular Basis of Disease xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

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

Exercise improves glucose uptake in murine myotubes through the AMPKα2-mediated induction of Sestrins

Tianyi Wang[a], Yanmei Niu[b], Sujuan Liu[c], Hairui Yuan[d], Xiaolei Liu[e], Li Fu[a,*]

[a] Department of Physiology and Pathophysiology, School of Basic Medical Science, Tianjin Medical University, Tianjin 300070, China
[b] Department of Rehabilitation, Tianjin Medical University, Tianjin 300070, China
[c] Department of Anatomy and Embryology, School of Basic Medical Science, Tianjin Medical University, Tianjin 300070, China
[d] Key Laboratory of Hormones and Development (Ministry of Health), Tianjin Key Laboratory of Metabolic Diseases, Tianjin Metabolic Diseases Hospital & Tianjin Institute of Endocrinology, Tianjin Medical University, Tianjin 300070, China
[e] Department of Medicine (Hematology-Oncology), University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA

ARTICLE INFO

Keywords:
Sestrins
AMPKα2
Autophagy
Exercise training
Glucose uptake

ABSTRACT

Exercise training increases insulin sensitivity. Over the past decades, considerable progress has been made in understanding the molecular basis for this important effect of physical exercise. However, the underlying mechanism is still not fully described. Recent studies have revealed that the stress responsive protein family Sestrins (SESNs) may play an important role in improving insulin sensitivity of skeletal muscle under exercise training. In this study, we aim to better understand the relationship between SESNs and AMPK in response to exercise training and the possible mechanism by which SESNs mediate glucose metabolism. We used wild type, AMPKα2$^{+/-}$ and AMPKα2$^{-/-}$ C57BL/6 mice to reveal the pathway by which 6 weeks of exercise training induced SESNs. We explored the mechanism through which SESNs regulated glucose metabolism in vitro by overexpressing or inhibiting SESNs, and inhibiting AMPK or autophagy in myotubes. We found that a 6-week exercise training regime improved oxidative metabolism, activated the insulin signaling pathway and increased the level of SESN2 and SESN3 in an AMPKα2-dependent manner. Overexpression of SESN3 or SESN2 and SESN3 together increased glucose uptake, activated the insulin signaling pathway, and promoted GLUT4 translocation in myotubes. Although inhibition of SESNs had no effect on glucose uptake, SESNs could reverse reduced glucose uptake following autophagy inhibition, and may be downstream effectors of AMPK responses in myotubes. Taken together our data show that SESNs are induced by AMPKα2 after exercise training, and SESNs, specifically SESN3, play a key role in exercise training-mediated glucose metabolism in skeletal muscle.

1. Introduction

A sedentary lifestyle predisposes individuals to many health risks. Numerous studies have shown that exercise training can significantly reduce the risk of obesity, type 2 diabetes, and cardiovascular-related diseases by improving insulin sensitivity in target tissues for insulin, primarily skeletal muscle [1–3]. However, the underlying mechanism by which exercise training increases insulin sensitivity in skeletal muscle is unclear and has therefore become the focus in this field. Recent studies have found that stress-responsive SESNs may serve a key function in exercise training to improve insulin sensitivity in skeletal muscle [4].

SESNs are highly conserved proteins encoded by genes whose expression is upregulated in cells exposed to a variety of environmental stresses such as DNA damage, oxidative stress, and hypoxia [4]. In mammals, the Sestrin family consists of three proteins. Lee et al. reported that concomitant ablation of *Sesn2* and *Sesn3* provokes hepatic mTORC1-S6K activation and insulin resistance in the absence of

Abbreviations: 2-NBDG, 2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2- Deoxyglucose; 3-MA, 3-Methyladenine; ACC, Acetyl-CoA Carboxylase; Ad, adenoviruses; AKT, Protein Kinase B, also known as AKT; AMPK, Adenosine 5′-monophosphate (AMP)–activated protein kinase; ATG1, Autophagy-related 1; ATP1A1, ATPase Na^+/K^+ transporting subunit alpha 1; C2C12, Mouse myoblast cell line; COX, Cytochrome c oxidase; DN, Dominant negative; LKB1, liver kinase B1; MOI, Multiplicity of infection; PMSF, Phenylmethanesulfonyl fluoride; S6K, Ribosomal protein S6 kinase; SESN, Sestrin; siRNA, Small interfering RNA; ULK1, Unc-51 like autophagy activating kinase 1

* Corresponding author at: Department of Physiology and Pathophysiology, School of Medicine; Tianjin Medical University, 22 Qixiangtai Road, Tianjin 300070, China.
E-mail address: lifu@tmu.edu.cn (L. Fu).

https://doi.org/10.1016/j.bbadis.2018.07.023
Received 23 April 2018; Received in revised form 12 July 2018; Accepted 18 July 2018

nutritional overload and obesity, which reveals that Sestrin2 (SESN2) and Sestrin3 (SESN3) have an important homeostatic function in the control of mammalian glucose and lipid metabolism. Relatively few studies have measured the effects of exercise training on Sestrins levels, but the results available demonstrated that physical exercise increased the protein content of Sestrins [5,6]. Although the relationship between Sestrins and exercise training has not yet been explored, it is well established that Sestrins are involved in the AMP-activated protein kinase (AMPK) signaling pathway in different tissues or cell lines [6–8]. Sestrins modulate AMPK subunit expression [7] and act as a "scaffold" between AMPK and liver kinase B1 (LKB1). They promote phosphorylation of AMPK-Thr172 by promoting binding between AMPK and LKB1 [8], which indicate that Sestrins are involved in the regulation of the AMPK signaling pathway. Inhibition of Sestrin2 expression by siRNA in the human nasopharyngeal carcinoma cell led to a significant decrease in autophagy [9], suggesting that Sestrins may be involved in its regulation.

AMPK is a crucial cellular energy sensor [10]. Acute and long-term exercise training effectively activates AMPK kinase activity in human and rodent skeletal muscle [11], the extent of which depends on the intensity and duration of exercise and is closely associated with an increase in insulin sensitivity [12]. Importantly, AMPK activation is an exercise- induced regulator of glucose uptake in skeletal muscle, which results in greater systemic insulin sensitivity [13]. However, the downstream signaling pathways through which exercise-induced AMPK regulates glucose metabolism have not yet been elucidated.

Autophagy is an evolutionarily conserved lysosomal catabolic pathway that plays a key role in skeletal muscle metabolism [14]. It has been described as a quality control mechanism that removes long-lived proteins and dysfunctional organelles in various tissues [15]. In one recent study, autophagy was shown to regulate muscular glucose homeostasis and increase insulin sensitivity to exercise [15]. This potential link between exercise and autophagy may be related to the function of AMPK because it interacts directly with and phosphorylates Atg1/ULK1, resulting in the activation of downstream signals of autophagy [16]. Our earlier study demonstrated the role of AMPK in exercise regulation of muscle autophagy and insulin resistance [6]. This research also showed that AMPKα2 regulates the effect of exercise on the activation of muscle autophagy, which is involved in the regulation of glucose metabolism and insulin sensitivity in insulin target cells and tissues.

Because of the physiological relevance between Sestrins and AMPK, we postulated that both Sestrins and AMPK-modulating functions of autophagy could be pertinent to exercise-related metabolic benefits. In this study, we aim to better understand the relationship between Sestrins and AMPK as it relates to the physiological response to exercise and the possible mechanism by which Sestrins mediates glucose metabolism.

2. Material and methods

2.1. Animals

All animal protocols were approved by the Tianjin Medical University Animal Care and Use Committee under the guidelines of the Chinese Academy of Sciences animal welfare and experimental protocol. AMPKα2$^{-/-}$ C57BL/6J mice were kindly provided by Dr. B. Viollet (Institute Cochin INSERM U1016, France) and bred at the Department of Laboratory Animal Science of Tianjin Medical University. Four-week old male wild-type, AMPKα2$^{+/-}$ and AMPKα2$^{-/-}$ C57BL/6J mice were housed in a controlled environment with a reversed 12/12 h light–dark cycle and free access to food and water.

Chronic exercise study was performed using the treadmill protocol [17]. Thirteen-week-old male mice were divided into control (n = 20) and exercise (n = 20) groups. The mice from the exercise group were exercised on a motor-driven rodent treadmill for 5 days a week for a total of 6 weeks. The mice initially ran at the intensity of 50% VO_2max for 30 min/day during the first week; thereafter, the running intensity and time were increased to 75% VO_2max (12 m/min) for 60 min/day. Thirteen-week old male wild type, AMPKα2$^{+/-}$ and AMPKα2$^{-/-}$ C57BL/6 mice were trained by the same protocol. After 48 h of the last bout of exercise, the mice were fasted for 14 h then anesthetized with isoflurane and sacrificed, skinned and a portion of the quadriceps muscle was dissected.

2.2. Plasmid constructs and adenoviruses

All cloning was performed using the Gateway cloning system (Invitrogen, Carlsbad, CA, USA). SESN3 was subcloned into the pCAG-Flag-DEST expression vector (gifted by Dr. Qi Zhang, University of Pennsylvania). pENTR-AMPKα2 and pENTR-SESN2 were generated using a pENTR Directional TOPO cloning kit. Dominant-negative (K45R) mutation in pENTR-AMPKα2 was generated using a QuickChange™ Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA, USA). Thereafter, SESN2 and AMPKα2 K45R were subcloned into the pAd/CMV/V5-DEST adenoviral expression system. Adenoviruses packaging was performed by the ViraPower Adenoviral expression system (Invitrogen).

2.3. Cell culture and treatments

C2C12 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and maintained at 37 °C in a humidified atmosphere with 5% CO_2 [18]. Cells were seeded and differentiated in DMEM with 2% horse serum for 4 days. For Flag-SESN3 transfection, C2C12 cells were transfected using lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions. For SESN2/3 knockdown experiments, C2C12 cells was transfected with negative control (NC) or siRNA against SESN2 or SESN3 (GenePharma, Shanghai, China) using lipofectamine RNAiMAX (Invitrogen). For adenoviruses treatment, C2C12 myotubes were infected with Ad-SESN2 or Ad-AMPKα2-K45R (DN-AMPK) using a multiplicity of infection (MOI) of 100 and harvested 48 h post-infection on day 6 of differentiation. For 3MA stimulation, myotubes were incubated with 5 mmol/L 3-MA (Sigma-Aldrich) in ethanol vehicle for 18 h in DMEM with 1% fatty acid-free BSA [6]. For insulin-stimulated conditions, 100 nM insulin was added to appropriate wells during the last 30 min of incubations. Glucose uptake was measured using a nonradioactive fluorescent glucose (2-NBDG) method [19]. All experiments were run in triplicate.

2.4. Extraction of outer membrane proteins

Triton X-114 phase-partitioning was used to separate outer membrane proteins following the method [20]. Briefly, myotubes were dissolved in Radio-Immunoprecipitation Assay (RIPA) buffer containing 1 mmol/L PMSF (protease inhibitor, Sigma) and Triton X-114 (1%). This mixture was incubated on ice for 30 min with constant shaking and centrifuged at 10,000*g* for 15 min to pellet the insoluble materials. The resulting supernatant fluid was incubated at 37 °C for 15 min to induce the separation of the detergent phase containing hydrophobic outer membrane proteins from the aqueous phase proteins. The upper aqueous phase was removed and the detergent phase was reconstituted to the original volume with sterile PBS. Extraction of Triton X-114 phase was repeated three times. The proteins were precipitated from the detergent phase by addition of 9 × volume of absolute ethanol, and incubated overnight at −20 °C. The precipitated proteins were harvested by centrifugation at 11,000*g* for 40 min and re-suspended in the sample buffer. The concentration of proteins was determined by Bradford assay (Bio-Rad, Richmond, CA, USA).