Original Article

MOLECULAR METABOLISM

Absence of the kinase *S6k1* mimics the effect of chronic endurance exercise on glucose tolerance and muscle oxidative stress

C. Binsch [1,4,5], T. Jelenik [2,4,5], A. Pfitzer [1], M. Dille [1,4], S. Müller-Lühlhoff [1], S. Hartwig [1,4], S. Karpinski [1], S. Lehr [1,4], D. Kabra [1,4], A. Chadt [1,4], M. Roden [2,3,4], H. Al-Hasani [1,4,*], T.R. Castañeda [1]

ABSTRACT

Objective: Ribosomal protein S6 Kinase-1 (S6K1) has been linked to resistance exercise-mediated improvements in glycemia. We hypothesized that S6K1 may also play a role in regulating glycemic control in response to endurance exercise training.
Methods: *S6k1*-knockout (S6K1KO) and WT mice on a 60 cal% high-fat diet were trained for 4 weeks on treadmills, metabolically phenotyped, and compared to sedentary controls.
Results: WT mice showed improved glucose tolerance after training. In contrast, S6K1KO mice displayed equally high glucose tolerance already in the sedentary state with no further improvement after training. Similarly, training decreased mitochondrial ROS production in skeletal muscle of WT mice, whereas ROS levels were already low in the sedentary S6K1KO mice with no further decrease after training. Nevertheless, trained S6K1KO mice displayed an increased running capacity compared to trained WT mice, as well as substantially reduced triglyceride contents in liver and skeletal muscle. The improvements in glucose handling and running endurance in S6K1KO mice were associated with markedly increased ketogenesis and a higher respiratory exchange ratio.
Conclusions: In high-fat fed mice, loss of S6K1 mimics endurance exercise training by reducing mitochondrial ROS production and upregulating oxidative utilization of ketone bodies. Pharmacological targeting of S6K1 may improve the outcome of exercise-based interventions in obesity and diabetes.

Keywords S6K1; Exercise; Glycemic control; Metabolic phenotyping; Reactive oxidative species

1. INTRODUCTION

Physical exercise training is associated with increased insulin sensitivity, improved glycemic control, and reduced risk for developing type 2 diabetes mellitus. While the beneficial effect of exercise has been attributed to metabolic adaptation in energy substrate preference, mitochondrial density, and skeletal muscle function, the regulatory network responsible for the diabetes-protective effect of exercise is not well understood.

The ribosomal protein S6 kinase beta-1 (S6K1), also known as p70S6 kinase, is part of the mTORC1 (mammalian target of rapamycin complex 1) signaling pathway that regulates cell growth, motility, and survival [1]. Chronic resistance training that implies high-intensity muscle contraction activates the mTORC1 pathway, thereby increasing protein synthesis and muscle mass, as evidenced by the positive correlation between the exercise-induced increase in S6K1 phosphorylation and skeletal muscle hypertrophy [2]. Conversely, muscle atrophy resulting from muscle unloading decreased mTORC1 signaling, respectively [3,4]. Moreover, rapamycin, an inhibitor of mTORC1, blunted protein synthesis and muscle growth in response to increased mechanical loading [5] but did not affect endurance exercise mediated muscle myofibrillar and mitochondrial protein synthesis [6]. On the other hand, endurance training that induces low-intensity high-volume muscle contraction, increases muscle oxidative capacity and glucose uptake [7,8] through the activation of AMPK [9,10]. The disruption of the mTORC1 pathway through the absence of *S6k* results in enhanced lipid utilization in mice consuming a high fat diet (HFD), and protection from HFD-induced insulin resistance mediated by phosphorylation of IRS-1 at Ser-1101 [11–13]. Hence, both increased lipid oxidation and lower inhibitory Ser-1101 phosphorylation of IRS-1

[1]Institute for Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, Düsseldorf, Germany [2]Institute for Clinical Diabetology, German Diabetes Center, Düsseldorf, Germany [3]Division of Endocrinology and Diabetology, Medical Faculty, Heinrich-Heine University, Düsseldorf, Germany [4]German Center for Diabetes Research, München-Neuherberg, Germany

[5] These authors contributed equally.

*Corresponding author. Institute for Clinical Biochemistry and Pathobiochemistry, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich-Heine University Düsseldorf, Auf'm Hennekamp 65, Düsseldorf 40225, Germany. Fax: +49 211 3382 430. E-mail: hadi.al-hasani@ddz.uni-duesseldorf.de (H. Al-Hasani).

Abbreviations: HFD, high-fat diet; ROS, reactive oxygen species; TCA, tricarboxylic acid cycle; mTOR, mammalian target of rapamycin

Received July 20, 2017 • Revision received August 22, 2017 • Accepted August 22, 2017 • Available online xxx

http://dx.doi.org/10.1016/j.molmet.2017.08.008

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may improve glucose metabolism and insulin sensitivity, and protect against type 2 diabetes mellitus. Mice deficient in *S6k1* as well as *S6k1*/*S6k2* double knockouts show higher expression of *Ppargc1a* in *plantaris* muscle and higher AMPK activation in hepatocytes and adipose tissue [12,14], which could lead to increased mitochondrial biogenesis. Therefore, it is possible that the absence of *S6k1* ameliorates the decrease in AMPK activation induced by HFD consumption [15], thus facilitating synergistic improvements of glucose homeostasis through changes in mitochondrial activity.

The aim of our present study was to investigate the impact of *S6k1* deletion on chronic endurance exercise, glucose metabolism, and running performance in mice on a HFD and whether the effects relate to lipid utilization and mitochondrial activity in skeletal muscle.

2. MATERIALS AND METHODS

2.1. Animals

Age- and sex-matched *S6k1* knockout (S6K1KO) mice were generated as described [11,12]. Male S6K1KO mice and the corresponding wild type (WT) littermate controls were originally generated in a mixed 129/SveJ × C57Bl/6 line [16]. All mice were housed under a 12:12 h light–dark cycle (lights on at 6 a.m.) with *ad libitum* access to a chow diet (V153x R/M-H, Ssniff, Soest, Germany) and tap water for three months. Subsequently, the mice were placed on a HFD (60% kcal from fat [D12492], Research Diets Inc., New Brunswick, NJ, USA) for one month before and during the four weeks of the exercise training intervention. All experiments were approved by the Ethics Committee of the State Ministry of Agriculture, Nutrition and Forestry (State of North Rhine-Westphalia, Germany).

2.2. Genotyping

Isolation of DNA from mouse tail tips was performed with the InViSorb Genomic DNA Kit II (Invitek, Berlin, Germany). Genotyping of mice was performed by a two-step PCR with primers for the *S6k1* knockout. Isolated genomic DNA served as template for the first PCR (WT outside fwd: 5′- GATGGGGCAGGGCTTAGGAGGC -3′, WT outside Rev: 5′- CGCTGTGTCCCTTCCTCTCCC -3′, Neo outside: 5′- GAGCTTGGCGGCGAATGGGCTG -3′). For the subsequent second PCR, the product of the first PCR was used as template (WT inside forward: 5′- GAAGGAGCTACTGGCTATTGGGG -3′, WT inside reverse: 5′- CTCCCCCCTCTGCCCCCCTC -3′, Neo inside: 5′- GGGCTGACCGCTTCCTCGTGC -3′).

2.3. Exercise training protocol

Four months old male mice consuming HFD for one month were subsequently trained on 4-lane treadmills (TSE systems, Bad Homburg, Germany) for 4 weeks, 1 h per day, 5 days per week followed by a two-day recovery phase. During these 4 weeks, training parameters were gradually increased from low intensities in the first week to higher intensities in the last week (i.e. maximum speed up to 18 m/min, running time up to 45 min and treadmill slope from 0° to 10° in order to simulate uphill running). Glucose tolerance and running performance were analyzed in the last week of the training intervention. Tissues were harvested after 2 h fasting (1 h after the last training bout) for subsequent analyses.

2.4. Running performance test

In the 4th week of training, mice performed a running performance test on the treadmill until exhaustion. At a 10° slope, the mice underwent a 15 min adaptation and warm-up phase and the subsequent measurement phase, which spanned over a time course of max. 2 h and gradually increased speed up to 18 m/min. Mice were motivated by gentle tail tapping. Exhaustion was considered when an animal did not respond to motivation stimuli and broke a light barrier at the rear of the system three times within 15 s. Mice refusing to exercise were also considered exhausted.

2.5. Measurements of body weight and body composition

Body weight was measured with an electronic scale (Sartorius, Göttingen, Germany) and body composition (fat and lean mass) was analyzed using nuclear magnetic resonance technology (Whole Body Composition Analyzer; Echo MRI, TX, USA) at the beginning and the end of the training intervention.

2.6. Intraperitoneal glucose tolerance test (i.p. GTT)

Mice were fasted for 6 h, and glucose was injected intraperitoneally with 2 g glucose per kg body weight (BW) (Glucose 20%, B. Braun Melsungen AG, Melsungen, Germany). Tail-blood glucose levels (mg/dl) were measured with a glucometer and standard glucose strips (Bayer Vital GmbH, Leverkusen, Germany) before (0 min) and at 15, 30, 45, 60, and 120 min after injection.

2.7. Indirect calorimetry

In the 3rd week of the exercise protocol, the animals were placed in a customized calorimetric cage system (TSE PhenoMaster, TSE systems, Bad Homburg, Germany) for the assessment of spontaneous physical activity (SPA), respiratory exchange ratio (RER), and energy expenditure (EE) as described [17]. Measurements were taken on line for 3 consecutive days, every 30 min starting 1 h after the exercise intervention. During this time, the animals were not removed from the cages or further trained, since the measurements were taken in the protocols recovery phase. SPA measurements were taken with a non-invasive infrared based light beam system and the RER and EE measurements through O_2 consumption and CO_2 production quantification at a cage in-flow rate of 0.4 l/min and a sampling flow rate of 0.38 l/min. Total food intake (FI) was quantified manually in parallel. Whole-body carbohydrate and fat oxidation rates (g/min) were calculated using the following equations: carbohydrate oxidation rate $= 4.585 \times VCO_2$ (l/min) $- 3.226 \times VO_2$ (l/min); fat oxidation rate $= 1.695 \times VO_2$ (l/min) $- 1.701 \times VCO_2$ (l/min) [18].

2.8. Plasma analysis

Mice were euthanized by decapitation at the end of the study; blood was collected and added to an anticoagulant cocktail (for 50 ml: 25 ml 0.5 M EDTA, 92 mg aprotinin dissolved in 21 ml saline [0.15 M], 4 ml heparin [10,000 U/ml], 21.6 mg diprotin A) and subsequently centrifuged (15 min, 3000×*g*, 4 °C). Cholesterol and non-esterified fatty acid (NEFA) levels were measured using enzymatic assay kits (Biolatest, Erba Lachema, Karasek, Czech Republic and Autokit NEFA HR(2), Wako, Neuss, Germany), triglyceride levels were determined using Triacylglycerols Liquid 1000 (Erba Lachema, Karásek, Brno, Czech Republic), plasma glucose was measured using LabAssay Glucose (Wako, Neuss, Germany), lactate was measured using Lactate Assay Kit (Sigma-Aldrichm St. Louis, MO, USA), and ketone bodies were assessed with Autokit Total Ketone Bodies, Autokit 3-hydroxybutyrate and ketone body calibrator 300 as standard (all: Wako, Neuss, Germany). The samples were run in technical duplicates and within the same assay according to the manufacturer's instructions.

Plasma corticosterone levels were measured using a [^{125}I] radioimmunoassay kit from MP Biomedicals (Orangeburg, NY, USA). Two multiplex bead based immunoassays (Bio-Plex Pro™ Mouse Diabetes 8-plex, Diabetes adiponectin, Biorad, Hercules, CA, USA) were used to

