Biochemical and Biophysical Research Communications xxx (2015) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Glycerol 3-phosphate dehydrogenase 1 deficiency enhances exercise capacity due to increased lipid oxidation during strenuous exercise

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ARTICLE INFO

Article history:
Received 24 December 2014
Available online xxx

Keywords:
Glycerophosphate shuttle
Glycolysis
AMPK
BALB/cHeA mice

ABSTRACT

A large percentage of energy produced during high-intensity exercise depends on the aerobic glycolytic pathway. Maintenance of a cytoplasmic redox balance ([NADH]/[NAD^+] ratio) by the glycerophosphate shuttle involves sustained aerobic glycolysis. Glycerol 3-phosphate dehydrogenase 1 (GPD1) catalyzes an oxidation reaction in the glycerophosphate shuttle. In this study, we examined whether GPD1 deficiency decreases exercise capacity due to impairment of aerobic glycolysis by using the GPD1 null mouse model BALB/cHeA (HeA). Unexpectedly, we found that exercise endurance was significantly higher in HeA mice than in BALBc/By (By) mice used as controls. Furthermore, aerobic glycolysis in HeA mice was not impaired. During exercise, lipid oxidation was significantly higher in HeA mice than in By mice, concomitant with an increase in phosphorylation of AMP-activated protein kinase (AMPK). HeA mice also showed a delay in the onset of muscle glycogen usage and lactate production during exercise. These data suggest that contribution of lipid oxidation as a fuel source for exercise is increased in HeA mice, and GPD1 deficiency enhances exercise capacity by increasing lipid oxidation, probably due to activation of AMPK. We propose that GPD1 deficiency induces an adaptation that enhances lipid availability in the skeletal muscle during exercise.

1. Introduction

During exercise, several metabolic pathways contribute to energy production in skeletal muscle. The main energy sources are glycogen and triglycerides within the muscle tissue, and glucose and free fatty acids in the blood stream. The intensity of exercise affects carbohydrate usage by skeletal muscle, and the source of carbohydrate varies with exercise intensity [1]. During exercise at 25% of maximal oxygen uptake (VO_2 max), carbohydrates contribute little to energy production; however, the use of intramuscular glycogen and blood glucose increases at 65% and 85% VO_2 max. Almost 50% of energy is produced from carbohydrates (blood glucose and intramuscular glycogen) at 65% VO_2 max. During vigorous exercise (85% VO_2 max), approximately 70% of energy is produced from carbohydrates, and intramuscular glycogen becomes the predominant energy source.

Intramuscular glycogen acts as a readily available source of glucose-6-phosphate for glycolysis within skeletal muscle. During exercise, activation of glycogen phosphorylase increases glycogenolysis to generate glucose-6-phosphate [2]. Hexokinase also produces glucose-6-phosphate from circulating glucose taken up by the skeletal muscle during exercise [3]. Under aerobic conditions, ATP is produced from glucose 6-phosphate by glycolysis in the cytosol and oxidative phosphorylation in the mitochondria. Within the cytosol, NADH is produced from NAD^+ by the oxidation of glyceraldehyde-3-phosphate [4]. NADH must then be re-oxidized to NAD^+ in order to continue glycolysis. NADH cannot penetrate the mitochondrial membrane [5]. Within the mitochondria, NADH is presumed to be oxidized by the respiratory chain

Abbreviations: AICAR, 5-aminoimidazole-4-carboxamide-1-b-D-ribofuranoside; AMPK, AMP activated protein kinase; AMP, adenosine monophosphate; ATP, adenosine triphosphate; By, BALB/cBy; COX2, cytochrome c oxidase subunit 2; COX4, cytochrome c oxidase subunit 4; CPT, carnitine palmitoyltransferase; GPD1, glycerol-3-phosphate dehydrogenase 1; GPD2, glycerol-3-phosphate dehydrogenase 2; HeA, BALB/cHeA; NADH, reduced nicotinamide adenine dinucleotide; NAD^+, oxidized nicotinamide adenine dinucleotide; PGC-1 alpha, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; Tg, transgenic; VEGF, vascular endothelial growth factor.

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http://dx.doi.org/10.1016/j.bbrc.2015.01.043
0006-291X/

[6]. Several possible mechanisms such as the glycerophosphate shuttle and the malate shuttle have been considered to permit re-oxidation of NADH in the mitochondria [7,8]. In the glycerophosphate shuttle, cytosolic GPD (GPD1) and mitochondrial GPD (GPD2) are involved in transfer of reducing equivalents from the cytosol into mitochondria [7]. These enzymes were shown to play important roles in nutrient metabolism in various tissues [7,9,10]. GPD2 activity is higher in mitochondria isolated from skeletal muscle than from other tissues, strongly suggesting that skeletal muscle is more dependent on the glycerophosphate shuttle [11,12]. Furthermore, mice lacking GPD1 showed that the lactate/pyruvate ratio in skeletal muscle was low, signifying a low cytosolic NAD^+/NADH ratio [13]. This study also suggested that glycolysis was inhibited at the step catalyzed by GPD1 due to an impaired glycerophosphate shuttle required for the maintenance of a normal NAD^+/NADH ratio. Since the glycerophosphate shuttle is required for maintaining aerobic glycolysis during high-intensity exercise, GPD1 deficiency may decrease exercise capacity or increase the usage of energy substrates other than carbohydrates.

In this study, we used a GPD1 null model, BALB/cHeA (HeA) mice, to examine whether GPD1 deficiency and the resultant inhibition of the glycerophosphate shuttle decreases exercise capacity or increases usage of alternative, non-carbohydrate energy substrates.

2. Materials and methods

2.1. Experimental animals

BALB/cBy (By) mice were obtained from Japan CLEA (Tokyo, Japan). The origins of HeA mice and their breeding conditions have been previously described [9,14]. The mice were fed a normal laboratory diet (MF, Oriental Yeast, Tokyo, Japan) for 1 week to stabilize their metabolic conditions, and they were maintained on a 12-h light–dark cycle at constant temperature (22 °C). Male mice, 10–12 weeks of age, were used in each experiment. The mice were cared for in accordance with the NIH Guide for the Care and Use of Laboratory Animals and our institutional guidelines. All animal experiments were conducted with the approval of the Institutional Animal Care and Use Committee of the University of Shizuoka (No. 135036).

2.2. Exercise protocol

Exercise capacity was determined by an exercise tolerance test as described previously [15]. To determine O_2 consumption and carbon dioxide (CO_2) production, mice were placed in an air-tight treadmill at a speed of at 0 m/min (Muromachi Kikai, Tokyo, Japan) for 60 min. The mice were then challenged at 10 m/min. The speed was increased at 2 m/min every 3 min until exhaustion. Exhaustion was defined as the point at which the mouse remained on the shocker plate for more than 10–15 s. To measure muscle glycogen after exercise, samples were taken at 30 min of the exercise protocol.

2.3. Measurement of O_2 consumption and carbon dioxide CO_2 production

Open-circuit indirect calorimetry was performed with an O_2/CO_2 metabolism measuring system ARCO-2000 for small animals (ARCO SYSTEM Inc., Chiba, Japan). The system monitored VO_2 and VCO_2 at 1-min intervals and calculated the respiratory quotient (RQ) ratio (VCO_2/VO_2). For measurement of VO_2 and VCO_2 during exercise, mice were allowed to acclimatize to the air-tight treadmill chamber (Muromachi Kikai) for 30 min, the point at which VO_2 and VCO_2 were stable. Measurements were continued for another 30 min, while the mice were maintained in a sedentary state. The mice were then exercised as described above. The rates of glucose and lipid oxidation were calculated using Frayn's equations [16]. The energy production rate was calculated using Lusk's equation [17].

2.4. Quantitative real-time RT-PCR

RNA preparation and quantitative real-time RT-PCR (qRT-PCR) were performed as described previously [18]. The mouse-specific primer pairs used were as those described previously [19].

2.5. Western blot

Frozen skeletal muscle was powdered under liquid nitrogen. Samples were then homogenized in RIPA Lysis Buffer (Merck Millipore, Temecula, CA, USA) containing “PhosSTOP” phosphatase inhibitor cocktail (Roche, Mannheim, Germany) and the “Complete Mini, EDTA-free” protease inhibitor cocktail (Roche, Mannheim, Germany). After three freeze/thaw cycles, the supernatant was separated by centrifugation at 20,400 *g* for 15 min at 4 °C. Sixteen micrograms of the protein from the supernatant was applied to an SDS-PAGE. The protein and phospho protein of AMP-activated protein kinase (AMPK) levels in the gastrocnemius muscle were measured by western blot with the following antibodies: anti-AMPK (Cat. #2532; Cell Signaling Technology, Beverly, MA, USA) and anti-phospho-AMPK (Thr^{172}; Cat. #2531; Cell Signaling Technology). Spots were detected by means of a chemiluminescence reagent ECL Prime Western Blotting Reagent (GE Healthcare, Buckinghamshire, UK) and a luminescent image analyzer LAS-3000(Fujifilm, Tokyo, JAPAN). The band intensity was quantified by densitometry, using Image J software version 1.48.

2.6. Glycogen and blood lactate assays

Glycogen content in the gastrocnemius was measured in glycosyl units after acid hydrolysis as previously described [20]. Blood lactate level was measured by Lactate Pro (Arkray, Kyoto, Japan).

2.7. Statistical analysis

Data were analyzed by one-way ANOVA. In case of significant differences, each group was compared to the other groups by a Student's *t*-test (JMP 5.1.2; SAS, Cary, NC, USA). In the exercise tolerance test, a Kaplan–Meier survival curve was obtained, and a comparison of groups was performed using the log-rank test (JMP 5.1.2). Values are shown as the mean ± SE.

3. Results and discussion

3.1. Exercise capacity of GPD1 null mice was increased relative to controls

GPD1 deficiency caused abnormalities in the glycolytic pathway [13]. Therefore, we hypothesized that GPD1 deficiency, and subsequent inhibition of the glycolytic pathway, may decrease exercise capacity. To examine this possibility, GPD1 null (HeA) and control (By) mice were challenged by running on a treadmill at 10 m/min, with the speed increased by 2 m/min every 3 min until exhaustion. Unexpectedly, HeA mice ran for a significantly longer duration and with a higher exercise-intensity than By mice ($P = 0.006$). By mice ran for a duration of 54 min at a maximum speed of 44 m/min (a 1.5-km total distance), whereas HeA mice ran for a duration of

Please cite this article in press as: T. Sato, et al., Glycerol 3-phosphate dehydrogenase 1 deficiency enhances exercise capacity due to increased lipid oxidation during strenuous exercise, Biochemical and Biophysical Research Communications (2015), http://dx.doi.org/10.1016/j.bbrc.2015.01.043