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

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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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[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

62 min at a maximum speed of 50 m/min (a 1.9-km total distance) (Fig. 1A).

To investigate fuel utilization during exercise, VO_2 and VCO_2 were monitored simultaneously until the mice reached exhaustion (Fig. 1B and C). No differences in VO_2 and VCO_2 were detected between sedentary HeA and By mice, and VO_2 and VCO_2 levels increased along with exercise intensity until exhaustion was reached in both groups (data not shown). The RQ ratio during exercise, which indicates fuel utilization, increased gradually as the treadmill speed increased, except between 0 and 13 min of the exercise protocol (Fig. 1B). After 13 min of exercise, the RQ ratio in HeA mice was lower than that of By mice. After 45 or 55 min of exercise, the ratio increased drastically in By or HeA mice, respectively. Our results also showed that glucose oxidation became predominant just before exhaustion in both HeA and By mice. These data suggested that glucose oxidation was not impaired in HeA mice, irrespective of GPD1 deficiency in the skeletal muscle. Because malate content was shown to be higher in HeA muscle than in By muscle after exercise [13], we reasoned that the reaction from oxaloacetate to malate, a part of malate–aspartate shuttle, might be enhanced in HeA mice. The malate–aspartate shuttle is thought to be involved in endurance [21], and endurance training was shown to increase levels of malate–aspartate shuttle enzymes in human skeletal muscle [22]. One study suggests that the malate–aspartate shuttle compensates for the absence of glycerol-phosphate shuttle within the islets of GPD2 null mice [23]. The malate–aspartate shuttle might also compensate for the glycerol-phosphate shuttle in HeA mice to oxidize glucose aerobically.

The level of lipid oxidation was higher in HeA mice than in By mice during exercise (Fig. 1B). Even when the data are expressed as the percentage of maximal speed, the calculated level of lipid oxidation during exercise is significantly higher in HeA mice than in By mice (Fig. 1C). These results suggest that the increase in exercise capacity of HeA mice is due to enhancement of exercise-induced lipid oxidation.

3.2. Anaerobic glycolysis during exercise in GPD1 null mice

Intramuscular glycogen is the primary energy source for anaerobic glycolysis during vigorous exercise. Increased reliance on anaerobic glycolysis during exercise produces lactate in skeletal muscle and induces the accumulation of lactate in the blood

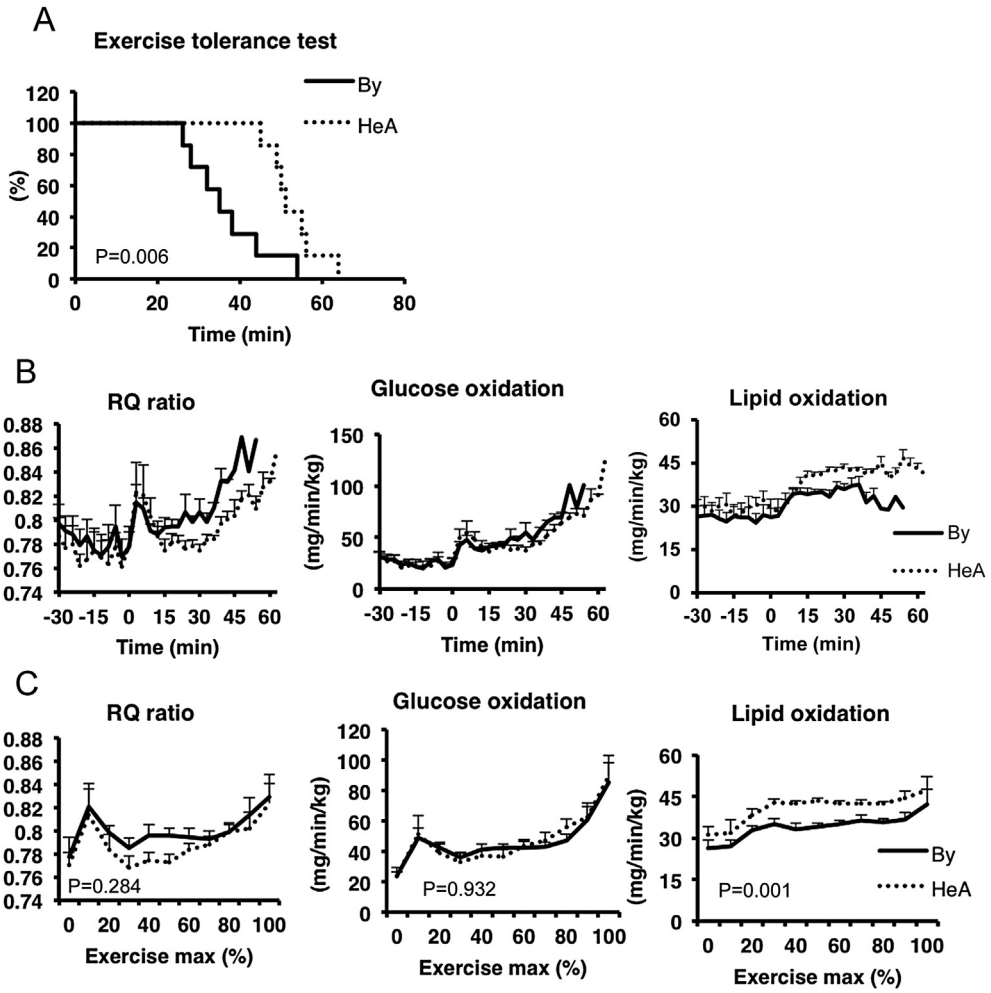

Fig. 1. Exercise tolerance, RQ ratio, and calculated glucose and lipid oxidation rates during exercise. (A) Male BALB/cHeA (HeA, n = 7) and BALB/cBy (By, n = 7) mice (age-matched at 10-weeks-old) were exercised by forced running on a treadmill at 10 m/min. The speed increased was by 2 m/min every 3 min until exhaustion. Exercise tolerance is shown as a Kaplan–Meier survival curve. A significant difference (P = 0.006, log-rank test) was observed between the exercise tolerances of HeA and By mice. (B) The RQ ratio was monitored using an O_2/CO_2 metabolism measuring system for small animals, which was equipped with an air-tight treadmill chamber. Calculated glucose and lipid oxidation rates are also shown. Each value is the mean ± SE. (C) The RQ ratio and calculated glucose and lipid oxidation rates are plotted against relative exercise intensity, as estimated by the percentage of mean speed at exhaustion for each genotype. Statistical significance was calculated as the area under the curve by using the mean value for sedentary animals in each group as the base line. P values compared to By mice are shown.

stream. Changes in intramuscular glycogen concentration and blood lactate levels are predictors of reliance on anaerobic glycolysis, as well as performance in endurance events. To investigate whether aerobic exercise capacity is increased in HeA mice, we measured glycogen concentration in the skeletal muscle and blood lactate concentration when animals were sedentary and 30 min after the exercise tolerance test. When sedentary, glycogen content in the gastrocnemius from HeA mice was 1.32-fold greater than that in By mice (Fig. 2A). Thirty minutes after the exercise tolerance test, skeletal muscle glycogen content decreased by 38% in By mice, whereas glycogen content only decreased by 18% in HeA mice.

Blood lactate concentration was measured 30 min after starting the exercise tolerance test to determine anaerobic glycolysis activity. By mice exhibited a 2-fold increase in blood lactate concentration; however, an increase was not observed in HeA mice (Fig. 2B). However, a 3-fold increase in blood lactate concentration was observed in HeA mice upon exhaustion (data not shown). These results suggested that, in addition to aerobic glycolysis, anaerobic glycolysis contributes to energy production in By mice after 30 min of exercise. These data also suggest that aerobic exercise capacity was increased in HeA mice. These mice may not need to switch to anaerobic energy pathways as early as By mice during high intensity exercise owing to, in part, an increase in exercise-induced lipid oxidation.

3.3. Expression of mitochondrial genes in the skeletal muscle

The ability to oxidize lipids increases in subjects who have improved their heart and lung capacities and muscular strength through exercise training. Therefore, the amount of lipid oxidized during exercise differs in trained and untrained people [24]. Increases in mitochondrial biogenesis and angiogenesis within skeletal muscle tissue also contribute to improved lipid oxidation. In fact, several studies have shown that endurance capacity and lipid oxidation during exercise was significantly increased in mouse models with higher mitochondrial counts in the skeletal muscle [15,25,26]. To determine whether mitochondrial biogenesis and angiogenesis in skeletal muscle were increased in HeA mice, we analyzed the expression of mRNAs coding muscle-specific transcription factors, as well as genes related to mitochondrial biogenesis and angiogenesis, by qRT-PCR. Expression of these genes, such as peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 alpha), cytochrome c oxidase (COX) II and COX IV, was not significantly different at the mRNA level between By mice and HeA mice (data not shown). Expression of vascular endothelial growth factor B (VEGFB), a potent modulator of angiogenesis, also showed no significant difference in mRNA levels among these mice (data not shown). These results suggest that mitochondrial biogenesis and angiogenesis within the skeletal muscle of HeA mice is comparable to that in By mice.

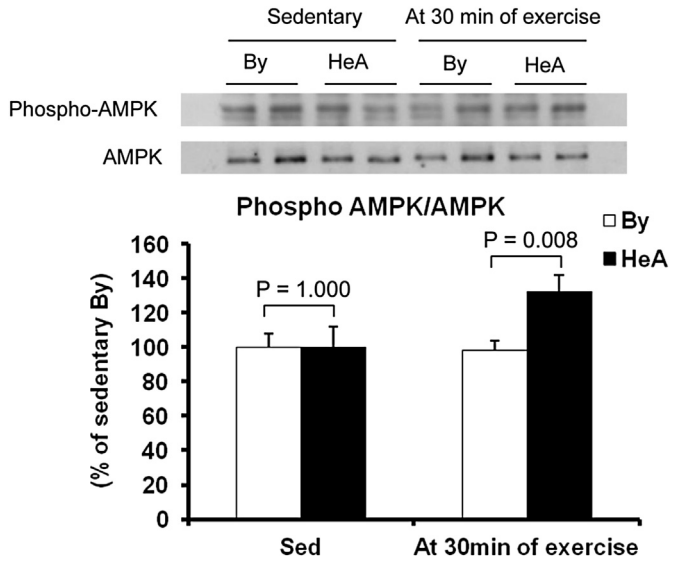

Fig. 3. Phosphorylation of AMPK before and after exercise. Phospho-AMPK/total AMPK in the sedentary (Sed, n = 5 per group) and exercised (n = 6 per group) gastrocnemius of male BALB/cBy (By) and BALB/cHeA (HeA) mice was measured by western blotting. Representative blots are shown. Values are expressed as a percentage of the value for sedentary By. P values compared to By mice are shown in the figure.

3.4. Exercise-induced phosphorylation of AMPK

AMPK activity in the skeletal muscle is required for maintaining exercise capacity. Our previous studies have reported that transgenic mice expressing a dominant-negative (DN) mutant of α1-AMPK (α1-AMPK-DN) in skeletal muscle could not perform high-intensity (30 m/min) treadmill exercise, although their response to low-intensity (10 m/min) treadmill exercise was not compromised [27]. As shown in Fig. 1, By mice could not continue relatively high-intensity exercise (over 30 m/min). In addition, AMP/ATP in the muscle after vigorous exercise was shown to be higher in HeA mice than in By mice [13]. These findings suggested that HeA mice

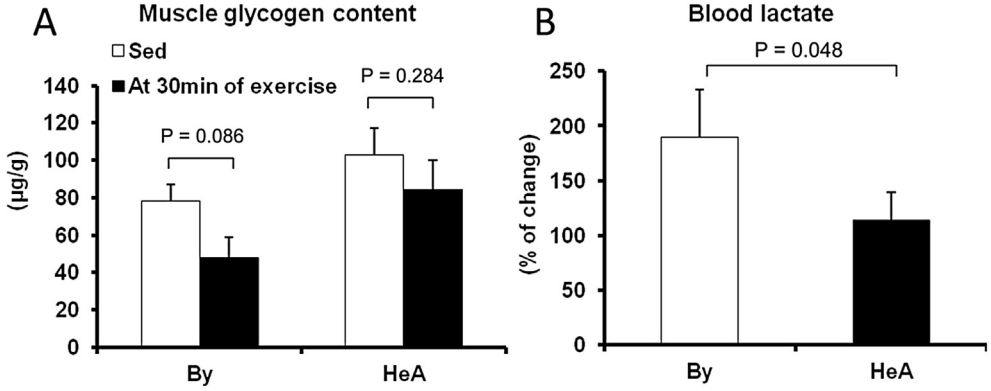

Fig. 2. Skeletal muscle glycogen content and blood lactate concentration before and after exercise. Male BALB/cBy (By) and BALB/cHeA (HeA) mice (each 10–12 weeks old) were exercised by forced running on a treadmill at 10 m/min. The speed was increased 2 m/min every 3 min up to 30 min. (A) Glycogen content in the skeletal muscle (gastrocnemius) was measured when mice were sedentary (Sed, n = 6–7 per group) and after 30 min of exercise (n = 6–7). P values compared to Sed group are shown. (B) Blood lactate concentration was measured before and at 30 min of exercise (n = 6–7 per group). Percentage change after 30 min of exercise as compared to Sed. P value compared to By is shown.

could perform high-intensity exercise because AMPK activity in the skeletal muscle is higher in HeA mice than in By controls. AMPK is activated by phosphorylation of Thr172 [28]. To examine AMPK activation, we measured exercise-induced changes in phosphorylated AMPK levels in HeA and By muscle tissue. Phosphorylation of AMPK was measured in sedentary animals and at 30 min after the start of the exercise tolerance test. Western blot analyses showed no significant difference between sedentary HeA and By mice (Fig. 3). In contrast, phosphorylation of AMPK was significantly increased in HeA mice at 30 min of exercise relative to control mice. Because AMP/ATP was increased in HeA mice during exercise [13], AMPK may be more readily activated in GPD1-deficient mice. The role of AMPK in contraction-induced fatty acid oxidation was estimated with the use of an activator of AMPK, 5-aminoimidazole-4-carboxamide-1-b-D-ribofuranoside (AICAR). AICAR leads to an increase in uptake of fatty acids into mitochondria via carnitine palmitoyltransferase (CPT) 1, thereby stimulating fatty acid oxidation [29]. Increased phosphorylation of AMPK in HeA muscle may increase fatty acid oxidation by activation of CPT1 in the muscle during exercise. Increased fatty acid oxidation during exercise modulates exercise capacity. For example, we previously demonstrated that transgenic overexpression of PGC-1α in mouse skeletal muscle (PGC-1α-Tg mice) increased fatty acid oxidation during exercise, contributing to improved exercise capacity [15]. In addition, it was likely that AMPK activation was involved in improved exercise capacity of the PGC-1α-Tg mice, because AMP/ATP was also increased in the muscle tissue of these mice [30].

In conclusion, we found that GPD1 deficiency increased fatty acid oxidation during exercise, leading to improved exercise capacity. Furthermore, it is likely that AMPK activation is involved in increased fatty acid oxidation. Although the glycerophosphate shuttle was impaired by GPD1 deficiency in HeA mice, aerobic glycolysis was not inhibited during vigorous exercise. We propose that GPD1 deficiency induces an adaptation that enhances lipid availability in the skeletal muscle during exercise. However, the HeA mice used in this study systemically lose GPD1 activity. Because other factors such as cardiac and inspiratory muscle function are also determinants of endurance capacity, we cannot ignore the possibility that tissues other than skeletal muscle contribute to the GPD1-defcient phenotype.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

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Transparency document

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