# Differential Mitochondrial Adaptation of the Slow and Fast Skeletal Muscles by Endurance Running Exercise in Streptozotocin-Induced Diabetic Mice

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**Summary**

The skeletal muscle is the main organ responsible for insulin action, and glucose disposal and metabolism. Endurance and/or resistance training raises the number of mitochondria in diabetic muscles. The details of these adaptations, including mitochondrial adaptations of the slow and fast muscles in diabetes, are unclear. This study aimed to determine whether exercise training in streptozotocin (STZ)-induced mice leads to differential adaptations in the slow and fast muscles, and improving glucose clearance. Eight-week-old mice were randomly distributed into normal control (CON), diabetes (DM), and diabetes and exercise (DM+Ex) groups. In the DM and DM+Ex groups, mice received a freshly prepared STZ (100 mg/kg) intraperitoneal injection on two consecutive days. Two weeks after the injection, the mice in the groups ran on a treadmill for 60 min at 20 m/min for a week and subsequently at 25 m/min for 5 weeks (5 days/week). The analyses indicated that running training at low speed (25 m/min) enhanced mitochondrial enzyme activity and expression of lactate and glucose transporters in the plantaris (low-oxidative) muscle that improved whole-body glucose metabolism in STZ-induced diabetic mice. There were no differences in glucose transporter expression levels in the soleus (high-oxidative) muscle. The endurance running exercise at 20-25 m/min was sufficient to induce mitochondrial adaptation in the low-oxidative muscles, but not in the high-oxidative muscles, of diabetic mice. In conclusion, the present study indicated that running training at 25 m/min improved glucose metabolism by increasing the mitochondrial enzyme activity and glucose transporter 4 and monocarboxylate transporter 4 protein contents in the low-oxidative muscles in STZ-induced diabetic mice.

**Key words**

Exercise training • Mitochondria • Skeletal muscles • Streptozotocin

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## Introduction

The skeletal muscle is the main organ responsible for insulin action, and glucose disposal and metabolism. The skeletal muscle weight decreases, and mitochondrial biogenesis and function in the skeletal muscle are impaired in type 1 [1] and type 2 diabetes [2-4]. These negative changes in the skeletal muscles are caused by a reduction in the expression of genes involved in oxidative capacity, nuclear genes encoding mitochondrial proteins, and those involved in mitochondrial biogenesis [5,6]. Aerobic exercise is known to effectively prevent and/or improve insulin resistance and impaired glucose metabolism, as demonstrated by a previous study [7]. Area under the curve measurements following an intravenous glucose tolerance test in rats with type 1 diabetes was found to significantly decreased compared with diabetic control group by resistance (climbing a ladder with incremental loads) or aerobic treadmill exercise training for 6 weeks

[8]. Improved insulin resistance is associated with increased glucose transporter 4 (GLUT4) content in the skeletal muscles [8]. King *et al.* [9] developed an animal model of type I diabetes using streptozotocin (STZ) that helped study the impairment of insulin production and hyperglycemia. High monocarboxylate transporter 1 (MCT1) levels correlate with oxidative metabolism, such as citrate synthase activity, and high MCT4 levels correlate with glycolytic metabolism in the skeletal muscles of rodents [10-12]. Previous research suggested that endurance exercise training for 30 min/day induced an increase in MCT1 levels in the plantaris and soleus muscles, and MCT4 in the plantaris muscles in the STZ-induced diabetic rats than in sedentary diabetic rats [13].

A previous study showed that voluntary running exercise in rats with type 2 diabetes improved oxidative capacity, *via* increased succinate dehydrogenase activity and peroxisome proliferator-activated receptor γ coactivator-1α (Pgc-1α) mRNA levels in the soleus and plantaris muscles [14]. This previous study [14] showed that both the slow (soleus) and fast (plantaris) muscles undergo the same adaptation with running exercise; but another study has indicated the differences in exercise adaptations, such as the level of monocarboxylate transporter, between the slow and fast muscles in diabetic animals [8,13]. However, the differences in adaptation, including glucose metabolism and mitochondrial adaptation of the slow and fast muscles, by endurance running exercise in STZ-induced diabetic mice, have not yet been completely elucidated. The present study aimed to determine whether exercise training in STZ-induced mice leads to different adaptations in the slow and fast muscles and improves glucose clearance.

## Methods

*Animals*

Male ICR (Institute of Cancer Research) mice (8-week-old; Japan SLC, Inc., Tokyo, Japan) were used. Animals were housed individually in standard cages in an air-conditioned animal room at 22 °C under a 12:12 h light/dark cycle (dark period from 7:00 to 19:00). They were fed standard laboratory chow (MF; Oriental Yeast, Tokyo, Japan) and water ad libitum. All experiments were approved by the Animal Experimental Committee of the University of Tokyo (approval number: 2021-12).

*Experimental procedures*

We randomly distributed 8-week-old mice into normal control (CON, n=7), diabetes (DM, n=7), and diabetes and exercise (DM+Ex, n=8) groups. In the DM and DM+Ex groups, mice received a freshly prepared STZ (100 mg/kg, Sigma, St. Louis, MO, USA) intraperitoneal (i.p.) injection to induce diabetes after fasting for 6 h on two consecutive days. Mice in the control group were administered the same dose of saline. Mice in the DM+Ex group were familiarized with running exercise at 15 m/min for 10 min on a treadmill with shock grids (MK-680, Muromachi Kikai Co., Inc., Tokyo, Japan) for 3 days. Two weeks after the injection, the mice in the DM+Ex group were made to run on the treadmill for 60 min at 20 m/min for a week and subsequently at 25 m/min for 5 weeks (5 days/week). The expired gas measurement in sedentary conditions and the oral glucose tolerance test (OGTT) were performed on 14 and 15-week-old mice, respectively. The soleus, plantaris, and tibialis anterior muscles, heart and mesenteric, epididymal and perirenal fats were removed, rapidly frozen in liquid nitrogen, and stored at -80 °C until further analysis.

***Analytical methods***

*Blood analysis*

In the 15-week-old mice, non-fasting blood lactate concentrations and plasma glucose and insulin levels during OGTT were measured. Blood samples were collected *via* the tail veins of mice. Blood lactate concentrations were determined using portable analyzers (Lactate Pro 2; Arkray, Kyoto, Japan). The blood samples collected during the OGTT were then centrifuged (4 °C, 5000× g, 10 min), and the plasma fraction was rapidly frozen in liquid nitrogen and stored at -80 °C until further analysis.

At the end of the experiment (16-week-old), blood samples were collected from the inferior vena cava of mice anesthetized using isoflurane. Blood samples were handled as described above, and the plasma fraction after centrifugation was stored at -80 °C.

Plasma glucose concentrations during the OGTT and at the end of the experiment were determined enzymatically using commercial kits (Glucose CII Test Wako, Cat# 439-90901, Wako, Tokyo, Japan). Plasma insulin concentrations during the OGTT were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Ultra Sensitive Mouse/Rat Insulin ELISA Kit, MIoBS, Yokohama, Japan). Plasma free fatty acid concentrations at the end of the experiment were determined enzymatically using commercial kits (Lab

AssayTM NFFA, Cat# 633-52001, Wako, Tokyo, Japan).

*Expired gas analysis*

The expired gas in sedentary condition was measured once every 5 min for 24 h in 14-week-old mice using $O_2/CO_2$ metabolism measuring system for small animals ($O_2/CO_2$ Analyzer MM204R; Muromachi Kikai, Tokyo, Japan). $VO_2$ and $VCO_2$ were monitored, and the respiratory quotient (RQ) ratio ($VCO_2/VO_2$) was calculated at a 12:12 h light/dark cycle (dark period from 7:00 to 19:00). The mice had access to water and food ad libitum during the experiments. Glucose and lipid oxidation, and energy expenditure were calculated using formulas described previously [15-17].

*Oral glucose tolerance test*

The oral glucose tolerance test (OGTT) was performed in 15-week-old mice after overnight fasting. Blood samples were collected before the OGTT (time 0), followed by the oral administration of glucose solution (2 g/kg body weight). Blood samples were collected 15, 30, 60, and 120 min after glucose administration. For OGTT analysis, total area under the curve (AUC) was calculated using trapezoidal integration.

*Glycogen levels in the skeletal muscles*

The glycogen concentrations in the soleus and plantaris muscles were determined using the phenol-sulfuric acid method as described previously [18,19]. Briefly, the muscles were heated at 98 °C for 5 min with 30 % KOH saturated with $Na_2SO_4$ until they were completely dissolved, followed by addition of 99.5 % ethanol. After placing the samples on ice for 30 min, they were centrifuged at 5000× g for 15 min at 4 °C, and the supernatants were removed. Pellets (glycogen-precipitates) were dissolved in distilled water, phenol and sulfuric acid were added, and the mixture was incubated for 20 min. The absorbance at 490 nm was measured.

*Western blotting*

The frozen skeletal muscle and heart tissues were homogenized in radioimmunoprecipitation assay lysis buffer (20-188, Millipore, MA, USA) containing a protease inhibitor (1183617001, Complete Mini EDTA-free, Roche Life Science, Indianapolis, IN, USA) and phosphatase inhibitor mixture (04906837001, PhosSTOP phosphatase inhibitor cocktail, Roche Life Science). The protein concentrations of the samples were determined using BCA Protein Assay Kit (23,227, Pierce, Rockford, IL, USA). The protein samples (10 μg) were electrophoresed on 12 % sodium dodecyl sulfate-polyacrylamide gels for 40 min at 200 V. The proteins were transferred from the gels to polyvinylidene difluoride membranes. Further, the membranes were blocked with 5 % bovine serum albumin in Tris-buffered saline with 0.1 % Tween 20 (TBS-T) for 60 min at room temperature. The membranes were incubated overnight (4 °C) with the following primary antibodies: anti-glucose transporter 4 (GLUT4, 07-1404, Merck, Tokyo, Japan), anti-peroxisome proliferator-activated receptor γ coactivator 1 alpha (PGC-1α, 516557, Merck, Tokyo, Japan), and anti-oxidative phosphorylation (OXPHOS, ab110413, Abcam, Cambridge, UK). Anti-MCT1 and anti-MCT4 antibodies were raised in rabbits against the C-terminal region of the respective MCT (Qiagen, Japan), as described previously [20-22]. After overnight incubation, they were washed in TBS-T, incubated for 60 min at room temperature with the following secondary antibodies: rabbit anti-goat IgG (H&L) (A106PU, American Qualex, San Clemente, CA, USA) and mouse anti-goat IgG (H&L) (A102PT, American Qualex).

The proteins were detected using Pierce ECL Western blotting Substrate (Thermo Fisher Scientific) and visualized using ChemiDoc XRS (170-8071, Bio-Rad, Hercules, CA, USA). The blots were quantified using the Quantity One software (170-9600, Bio-Rad, Hercules, CA, USA). Consistent loading was verified using Ponceau-S solution (P7170-1L, Sigma-Aldrich) as previously described [23].

*Mitochondrial enzyme activity analysis*

The whole tibialis anterior muscles were homogenized in 100 times (vol/wt) 100 mM potassium phosphate buffer to determine mitochondrial citrate synthase enzyme activity. To disrupt the mitochondrial membrane, the sample homogenates were freeze (liquid nitrogen)-thawed twice. After centrifugation, the supernatant was collected and used for measurement of citrate synthase activity. The citrate synthase activity was measured spectrophotometrically as previously described [24].

*Statistical analysis*

Data are expressed as mean ± standard deviation (SD). Differences between the CON, DM, and DM+Ex groups were evaluated using one-way analysis of variance (ANOVA), followed by the Tukey-Kramer multiple-comparison test. Nonparametric measures were analyzed using the Kruskal-Wallis test, followed by the

Bonferroni *post hoc* test. Differences between repeated measurements of each trial were evaluated using two-way ANOVA (time × group), followed by the Bonferroni *post hoc* test to analyze plasma glucose and insulin concentrations in the OGTT. $P<0.05$ was considered to be statistically significant. The relationship between muscle glycogen content and energy expenditure was assessed by calculating the Pearson's correlation coefficient. Statistical analyses were performed using IBM SPSS Statistics version 27 (Armonk, NY, USA).

## Results

*Food and water consumption and body weight*

Average food and water consumption from 10 to 16 weeks of age was higher in the DM and DM+Ex groups than in CON group (Table 1, $P<0.05$). The body weight at 16 weeks of age in the DM+Ex group was lower than that in CON and DM groups (Table 1, $P<0.05$).

*Muscle, fat and heart weight*

The soleus muscle weight (mg/g BW) at 16 weeks of age was higher in the DM+Ex group than in DM group (Table 1, $P<0.05$). The plantaris muscle weight (mg/g BW) at 16 weeks of age was lower in the DM group than in CON group (Table 1, $P<0.05$). The intraperitoneal fat weight including the mesenteric, epididymal and perirenal fats at 16 weeks of age decreased in the DM and DM+Ex groups than that in CON group (Table 1, $P<0.05$). The heart weight (mg/g BW) at 16 weeks of age decreased in the DM and DM+Ex groups than that in CON group (Table 1, $P<0.05$).

**Table 1.** Food intake, drinking water, body weight, and tissue weights.

| | CON (n=7) | DM (n=7) | DM+Ex (n=8) |
|---|---|---|---|
| *Food intake (g/day)* | 5.6 ± 0.3 | 12.4 ± 3.6[a] | 11.1 ± 2.1[a] |
| *Water consumption (ml/h)* | 0.4 ± 0.1 | 2.8 ± 0.7[a] | 2.8 ± 0.3[a] |
| *Body weight (g)* | 41.1 ± 2.4 | 38.7 ± 2.9 | 35.2 ± 3.5[a,b] |
| *Soleus muscle (mg/g BW)* | 0.22 ± 0.02 | 0.21 ± 0.02 | 0.24 ± 0.02[b] |
| *Plantaris muscle (mg/g BW)* | 0.50 ± 0.05 | 0.39 ± 0.06[a] | 0.44 ± 0.05 |
| *Intraperitoneal fat (mg/g BW)* | 31.91 ± 5.89 | 5.96 ± 3.34[a] | 5.10 ± 0.85[a] |
| *Heart (mg/g BW)* | 4.54 ± 0.11 | 3.72 ± 0.40[a] | 4.15 ± 0.27[a] |

Values are presented as means ± SD (n=7-8). [a] $P<0.05$ compared with the CON group. [b] $P<0.05$ compared with the DM group. CON, control; DM, diabetes; DM+Ex, diabetes with exercise.

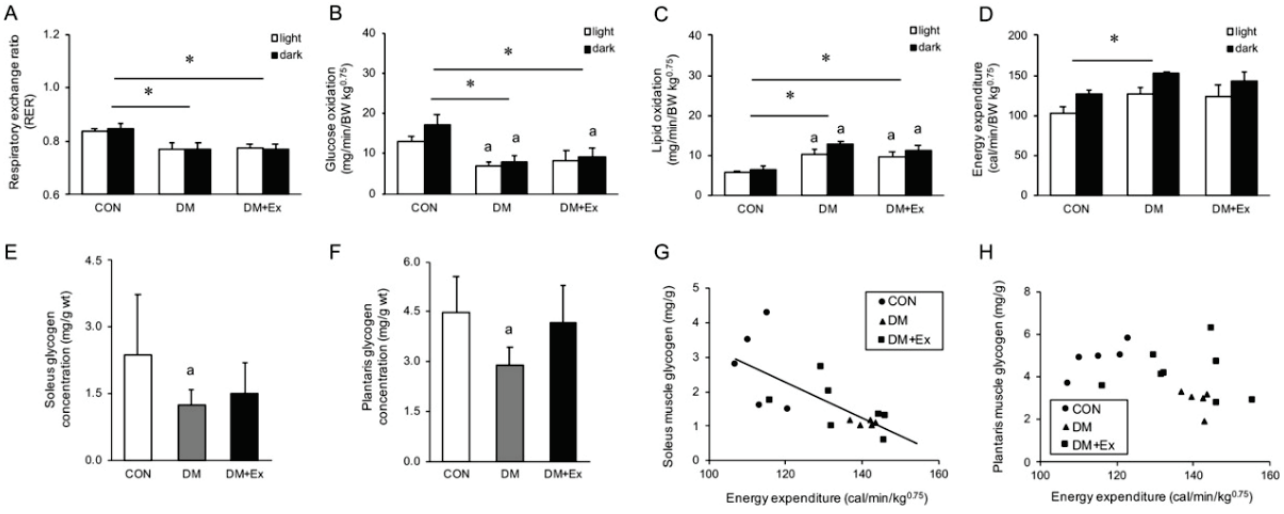


**Fig. 1.** (**A**) Respiratory exchange ratio (RER), (**B**) glucose oxidation, (**C**) lipid oxidation, and (**D**) energy expenditure and glycogen concentration in the (**E**) soleus and (**F**) plantaris muscles. Correlation between energy expenditure and glycogen content in the (**G**) soleus and (**H**) plantaris muscles in mice in all groups. Values are presented as means ± SD (n=7-8). * $P<0.05$ between CON and DM or DM+Ex group, and [a] $P<0.05$ compared with the time matched CON group. CON, control; DM, diabetes; DM+Ex, diabetes with exercise.

*Respiratory exchange ratio, energy expenditure, and glucose and lipid oxidations*

Figure 1A shows that there was no main effect of time (light or dark condition) or an interaction between time and group on respiratory exchange ratio (RER). There was a main effect of group on RER ($P<0.001$). A decreased RER was observed in the DM and DM+Ex groups than that in CON group ($P<0.001$). There were main effects of time and group, and an interaction between time and group on the glucose oxidation levels (Fig. 1B, $P<0.05$). Glucose oxidation levels in the DM and DM+Ex groups were lower than those in the CON group ($P<0.05$). There were main effects of time and group, and an interaction between time and group on the lipid oxidation levels (Fig. 1C, $P<0.001$, $P<0.001$, $P<0.05$, respectively). Lipid oxidation levels in the DM and DM+Ex groups were higher than those in the CON group ($P<0.05$). There were main effects of time and group on energy expenditure (Fig. 1D, $P<0.001$). Energy expenditure was higher in the DM group than in CON group ($P<0.001$). There was no interaction between time and group on energy expenditure.

*Muscle glycogen levels*

Glycogen levels in the soleus and plantaris muscles in the DM group were lower than those in CON group (Fig. 1E, F, $P<0.05$).

*Relation between energy expenditure and glycogen concentration in the soleus and plantaris muscles*

A significant negative correlation was observed between glycogen levels in the soleus muscle and energy expenditure, whereas there was no significant correlation between them in the plantaris muscle (Fig. 1G, H, $r=-0.721$, $P<0.05$ and $r=-0.381$, $P=0.119$, respectively).

*Non-fasting glucose, lactate, triglyceride, and free fatty acid (FFA) levels*

Non-fasting plasma glucose levels at 15 weeks of age were higher in the DM and DM+Ex groups than those in CON group (Fig. 2A, $P<0.05$). Non-fasting blood lactate (sedentary) levels at 15 weeks of age were lower in the DM group than in the CON group (Fig. 2B, $P<0.05$). There were no differences in non-fasting plasma triglyceride levels between the three groups (Fig. 2C). Non-fasting plasma FFA levels were higher in the DM group than in CON group (Fig. 2D, $P<0.05$).

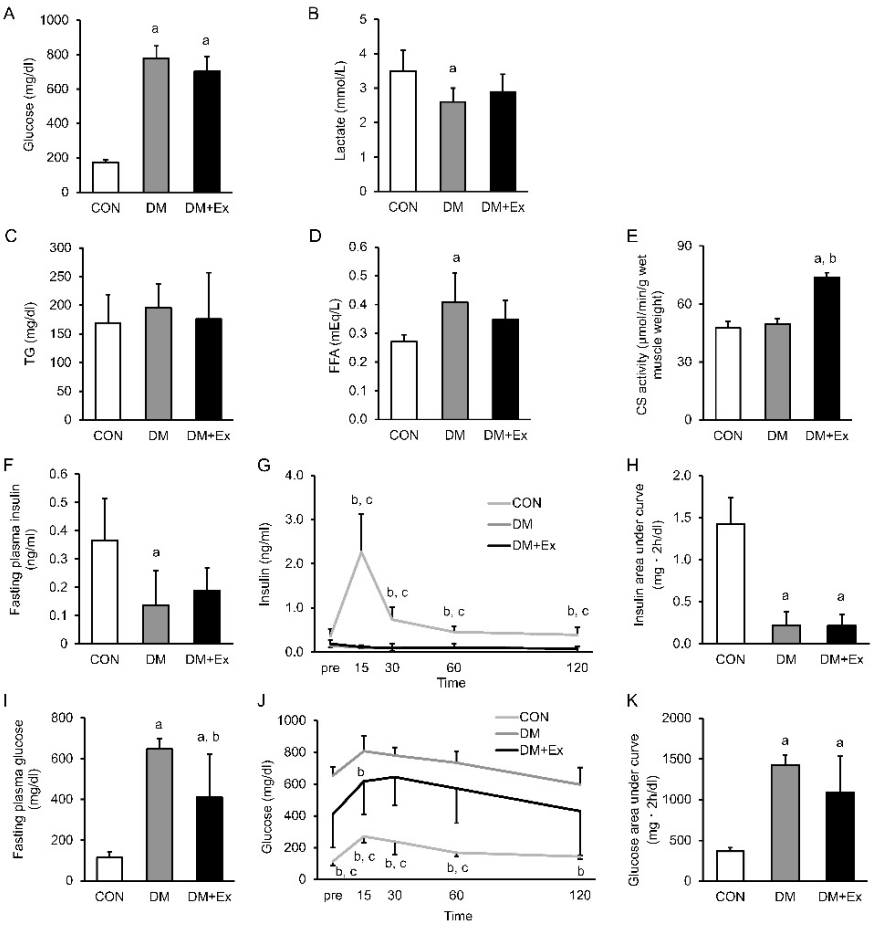


**Fig. 2.** (**A**) Glucose, (**B**) lactate, (**C**) triglyceride and (**D**) free fatty acid levels in the plasma. (**E**) Citrate synthase (CS) activity in the tibialis anterior muscle. (**F**) Fasting plasma insulin, (**G**) insulin levels after the oral administration of glucose solution, (**H**) insulin area under the curve, (**I**) fasting plasma glucose, (**J**) glucose levels after the oral administration of glucose solution, and (**K**) glucose area under the curve. [a] $P<0.05$ compared with the CON group. [b] $P<0.05$ compared with the DM group. [c] $P<0.05$ compared with the DM+Ex group. Values are presented as means ± SD (n=7-8). CON, control; DM, diabetes; DM+Ex, diabetes with exercise.

*Citrate synthase activity*

The citrate synthase activity in the tibialis anterior (TA) muscle was higher in the DM+Ex group than that in CON and DM groups (Fig. 2E, P<0.05).

*OGTT*

OGTT was performed at 15 weeks of age. The fasting plasma insulin levels were lower in the DM group than in the CON group (Fig. 2F, P<0.05). Plasma insulin levels in the CON group were higher at 15, 30, 60, and 120 min after glucose administration than those in DM and DM+Ex groups (Fig. 2G, P<0.05). The insulin AUC was lower in the DM and DM+Ex groups than in the CON group (Fig. 2H, P<0.05). Fasting plasma glucose levels were higher in the DM and DM+Ex groups than in the CON group and were lower in the DM+Ex group than in the DM group (Fig. 2I, P<0.05). Plasma glucose levels in the CON group were lower at 0, 15, 30, and 60 min after glucose administration than those in DM and DM+Ex groups (Fig. 2J, P<0.05). Additionally, plasma glucose levels in the DM group were higher than those in DM+Ex and CON groups at 15 and 120 min after glucose administration, respectively (Fig. 2J, P<0.05). The glucose AUC was higher in the DM and DM+Ex groups than in CON group (Fig. 2K, P<0.05).

*Protein levels in the heart and soleus and plantaris muscles*

There were no differences between each group on the protein loading amount of the heart and soleus and plantaris muscles. The protein levels of GLUT4 in the soleus muscle were lower in the DM group than those in the CON group (Fig. 3A, P<0.05). There were no differences in the levels of MCT1, MCT4, and PGC1α in the soleus muscle between the three groups (Fig. 3B, C, D). The levels of the GLUT4 protein in the plantaris muscle were higher in the DM+Ex group than those in the DM group (Fig. 3E, P<0.05). There were no significant differences in the levels of MCT1 in the plantaris muscle between the three groups (Fig. 3F). The levels of MCT4 and PGC1α in the plantaris muscle in DM+Ex group were higher than those in the CON and DM groups (Fig. 3G and H, P<0.05). The levels of the mitochondrial protein complex I in the DM and DM+Ex groups were lower than those in the CON group in the soleus muscle (Fig. 4A, P<0.05). There were no differences in the levels of mitochondrial proteins (complex II-V) in the soleus muscle between the three groups (Fig. 4A). The levels of complex I, III, and IV of mitochondrial proteins in the plantaris muscle in the DM+Ex group were higher than those in the CON and DM groups (Fig. 4B, P<0.05). There were no significant differences in the levels of complex II and V of the mitochondrial proteins in the plantaris muscle between the three groups (Fig. 4B). In the heart, there were no differences in the levels of GLUT4, MCT1, PGC1α, and mitochondrial proteins (oxphos) between the three groups (Fig. 5).

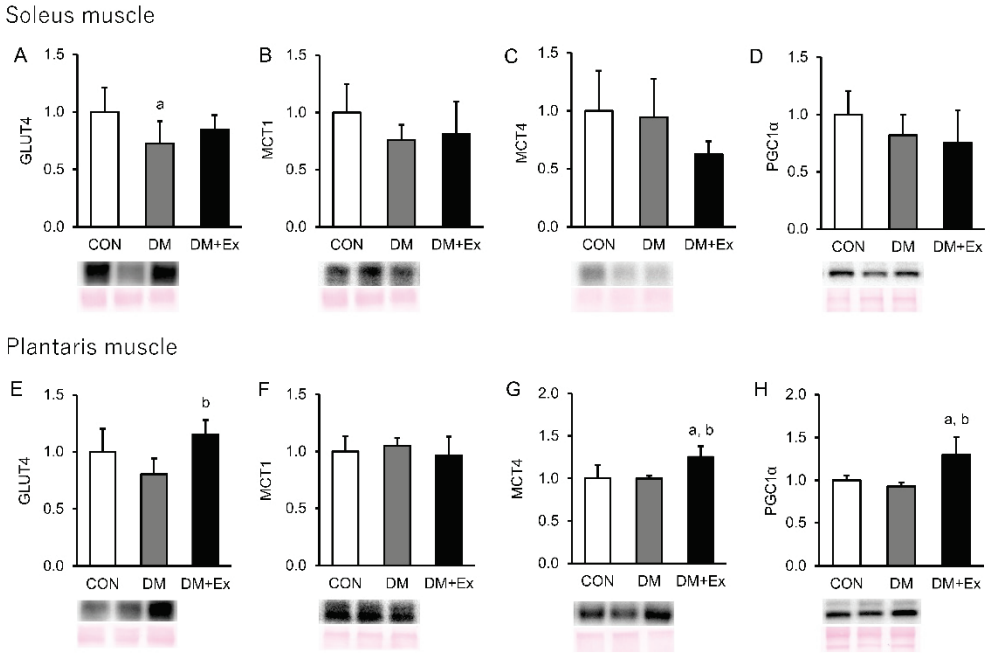


**Fig. 3.** (**A**) GLUT4, (**B**) MCT1, (**C**) MCT4, and (**D**) PGC1α protein levels in the soleus and (**E-H**) plantaris muscles. [a] P<0.05 compared with the CON group. [b] P<0.05 compared with the DM group. Values are presented as means ± SD (n=7-8). GLUT4, glucose transporter 4; MCT, monocarboxylate transporter; PGC1α, peroxisome proliferator-activated receptor γ coactivator-1α; CON, control; DM, diabetes; DM+Ex, diabetes with exercise.

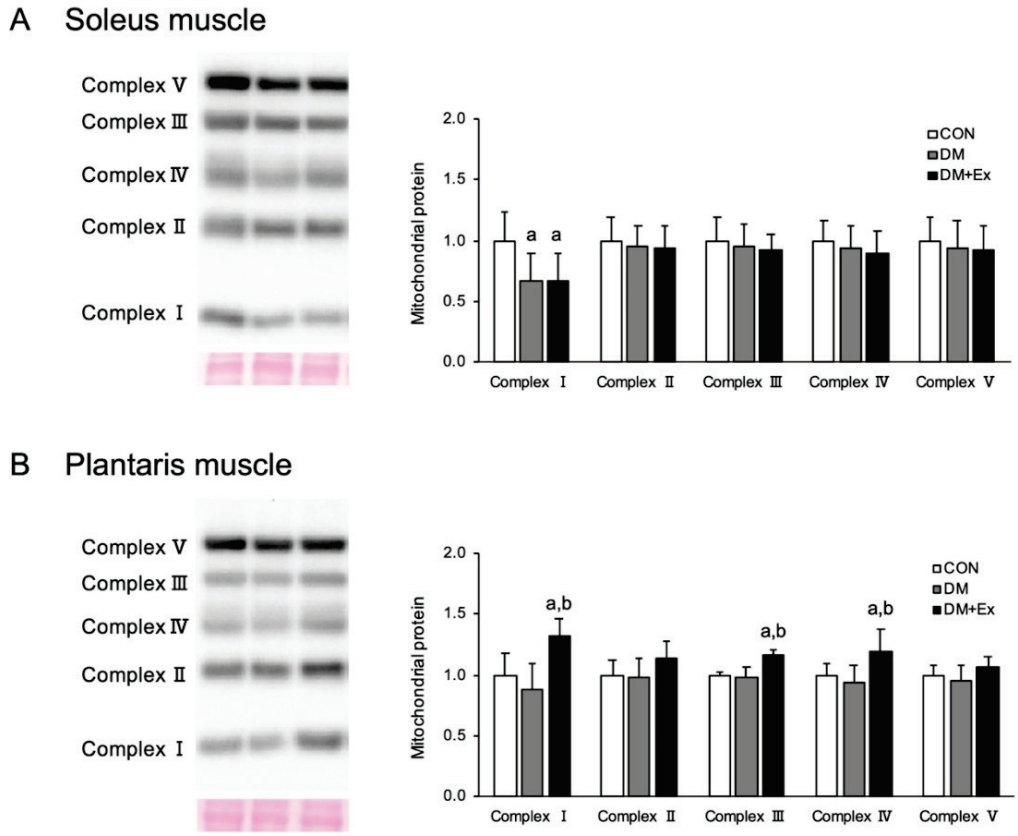


**Fig. 4.** (**A**) Mitochondrial protein levels in the soleus and (**B**) plantaris muscle. [a] P<0.05 compared with the CON group. Values are presented as means ± SD (n=7-8). CON, control; DM, diabetes; DM+Ex, diabetes with exercise.

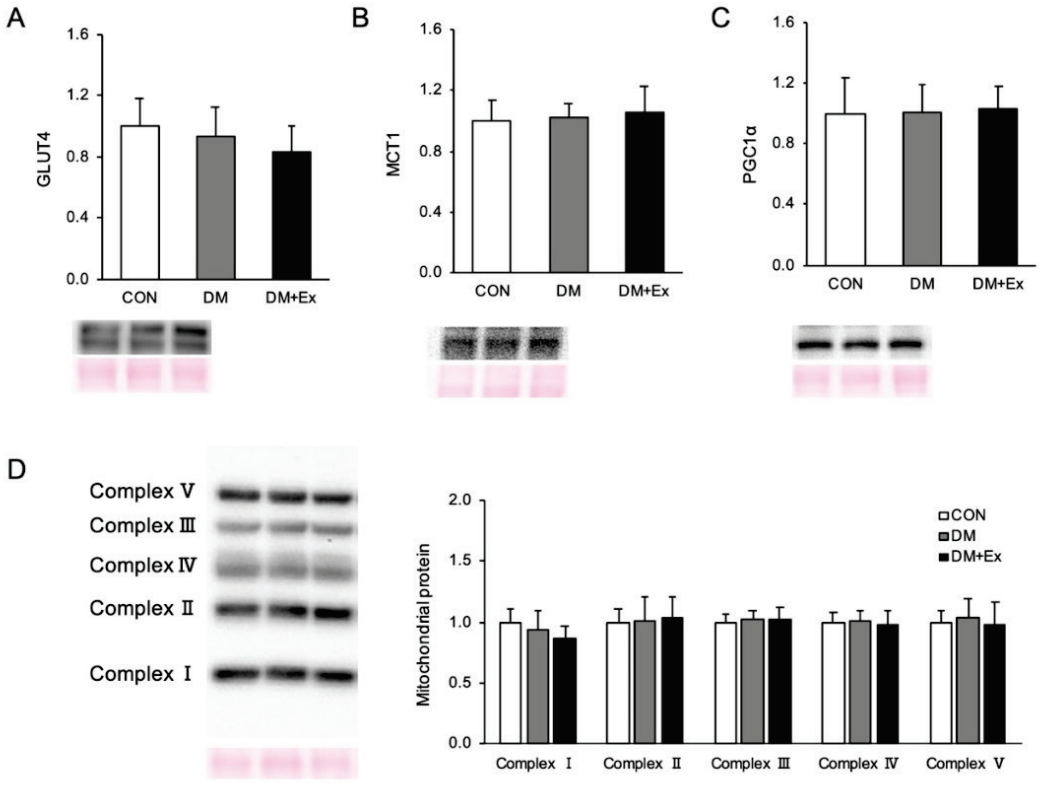


**Fig. 5.** (**A**) GLUT4, (**B**) MCT1, (**C**) PGC1α, and (**D**) mitochondrial protein levels in the heart. Values are presented as means ± SD (n=7-8). GLUT4, glucose transporter 4; MCT1, monocarboxylate transporter 1; PGC1α, peroxisome proliferator-activated receptor γ coactivator-1α; CON, control; DM, diabetes; DM+Ex, diabetes with exercise.

## Discussion

We investigated the effects of exercise training on glucose metabolism in the skeletal muscles of STZ-induced diabetic mice. The analyses suggested that running training at low speed (25 m/min) enhanced mitochondrial enzyme activity and the expression of lactate and glucose transporters in the plantaris muscle that improved whole-body glucose metabolism in STZ-induced diabetic mice.

The diabetic mice showed a significant increase in blood glucose levels, along with an increase in the consumption of food and drinking water. Consistent with previous studies [1,25,26], the body, muscle and fat weights decreased in the diabetic mice compared with the non-diabetic mice. The body weights of the STZ (150 mg/kg)-induced diabetic mice decreased compared with those of non-diabetic mice, although the food intake of the STZ groups was markedly higher than that of non-diabetic group [25]. The lower glucose uptake of muscle and adipose tissues induced by the insulin defect likely resulted in the loss of whole body, muscle and fat weights in spite of higher food intake. Exercise training inhibited weight loss in the plantaris muscle and increased the soleus muscle weight in diabetic mice. The ratio of $CO_2$ production and $O_2$ consumption (RER) and glucose oxidation decreased, and lipid oxidation increased in diabetic mice, including the DM+Ex mice, than that in the control mice. Energy expenditure increased in the diabetic mice, except in DM+Ex mice, than that in the control mice. Consistent with results of the present study, a lower RER was previously observed in a rodent model of type 2 diabetes and obesity [27]. Decreased RER in the diabetic mice suggests lipids, rather than glucose, as the main energy substance. These results are consistent with the increased FFA levels observed in diabetic mice. A previous study showed that lower RER was induced by high-intensity interval training [28] or moderate-intensity physical activity combined with caloric restriction in humans [29]. Additionally, we observed a significantly negative correlation between energy expenditure and glycogen content in the soleus muscle. This finding is consistent with a previous study that investigated the effects of glucose supplementation with casein peptide on the post-exercise recovery period [30]. These results suggest that whole-body energy expenditure is associated with the soleus muscle glycogen content. Increased energy expenditure activates 5'-AMP-activated protein kinase (AMPK) that acts as an intracellular energy sensor. Activated AMPK with 5-aminoimidazole-4-carboxamide-1-β-D-ribonucleoside suppresses glycogen synthase activity [31]. Thus, exercise-suppressed excessive energy expenditure in DM mice may be partially linked to increased glycogen synthesis in the soleus muscle. Glycogen content in diabetic mice with exercise was 22 % and 44 % higher in the soleus and plantaris muscle, respectively, than that in diabetic mice without exercise. In the plantaris muscle, we observed more effective improvement of glycogen content than that in the soleus muscle, which could be related with increased GLUT4 expression triggered by exercise in the plantaris muscle in diabetic mice. In diabetic mice, exercise resulted in increased GLUT4 levels in the plantaris muscle, but not in the soleus muscle. The exercise on a treadmill at 25 m/min for 40-60 min did not increase GLUT4 protein level in the skeletal muscles, including both fast and slow muscles, in normal mice [20,32]. This indicates that the adaptation of the GLUT4 level in the muscle by exercise might be likely to occur in diabetic mice than in normal mice.

A previous study reported that citrate synthase activity, a biomarker of mitochondrial oxidative capacity, is strongly associated with mitochondrial content in the skeletal muscles [33]. The results of the present study, such as increased levels of PGC1α and mitochondrial proteins, and maximal citrate synthase activity, suggest that endurance training enhances mitochondrial content in the fast (plantaris and TA) muscles, but not in the slow (soleus and heart) muscles. In chronic stimulation-induced contractile activity, mitochondrial adaptations, including enhanced enzymatic activity, occur more easily in the low-oxidative muscles, such as the plantaris and TA muscles, than in high-oxidative muscles, such as the soleus and heart muscles [34]. Moreover, previous studies have reported that endurance running training using a treadmill at 20-25 m/min enhances mitochondrial adaptation only in the fast-twitch fiber dominant muscles in normal mice [35,36]. The present study showed that endurance running exercise at 20-25 m/min is sufficient to cause mitochondrial adaptation in the low-oxidative muscles, but not in high-oxidative muscles in diabetic mice.

A previous study showed that the lower levels of MCT1 and MCT4 in the diabetic rats induced by high fat diet and STZ were observed in the extensor digitorum longus and soleus muscles compared with non-diabetic rats [37]. Another study identified significant reduction in the levels of MCT1 and MCT4 only in the plantaris muscles, but not in the soleus muscle and heart, in

STZ-induced diabetic rats than those in non-diabetic rats [13]. In the present study, there were no significant changes in the levels of MCTs in the skeletal muscles and heart in STZ-induced diabetic mice. The differences in the results of MCTs between the previous and present studies may reflect the metabolic differences between animal species under diabetic conditions, and thus, further studies are needed to clarify them. The running exercise training at 30 m/min increased MCT1 levels in the soleus and plantaris muscles, and increased MCT4 levels in the plantaris muscles in diabetic rats than those in sedentary diabetic rats [13]. The present study showed that endurance exercise did not induce changes in MCT1 expression in the soleus and plantaris muscles in diabetic mice. These results suggest that endurance running exercise at 25 m/min is insufficient to alter MCT1 expression. In the present study, decreased lactate concentrations in sedentary conditions appeared to be unrelated to levels of MCT1 in diabetic mice, although they tended to be related to decreased muscle glycogen concentrations. Consistent with results of a previous study [13], the present study showed that endurance exercise induced an increase in levels of MCT4 in the plantaris muscle, but not in the soleus muscle, in diabetic mice. In patients with type 1 diabetes, lactate accumulation in the vastus lateralis muscle, a fast muscle, with high intensity exercise was higher than that in patients without diabetes [38]. This suggests that type 1 diabetes may be associated with increases lactate production or decreased lactate clearance compared to individuals without diabetes. In the present study, it is possible that endurance running exercise at 25 m/min induced higher lactate concentration in the skeletal muscle and plasma in diabetic mice than that in non-diabetic mice. MCT4 is more abundant in the fast muscles (i.e. plantaris muscle) than in the slow muscles (i.e. soleus muscle) and mediates the efflux of lactate from skeletal muscle [39]. The high lactate concentration observed after endurance exercise may be attributed to the high MCT4 levels in the plantaris muscle in diabetic mice.

In the diabetic mice induced by STZ, oxidative enzyme potential diminished and fiber size reduced in the fast-twitch fibers, although slow-twitch fibers were least affected compared to normal mice [40]. This study revealed that the adaptations, such as GLUT4 and MCT4 level, and protein levels related with mitochondrial contents, by exercise occur more readily in the fast muscles than in the slow muscles. Endurance running exercise at 20-25 m/min is effective in improving hyperglycemia, increasing GLUT4 and MCT4 levels and the proteins related to mitochondrial contents in the fast muscle, but not in the slow muscle.

## Conclusions

In summary, the study findings suggested that running training at 25 m/min improved glucose metabolism by increasing the number of mitochondria and GLUT4 and MCT4 expressions in the low-oxidative muscles in STZ-induced diabetic mice. The results of this study including mitochondrial adaptation, such as increased levels of PGC1α and mitochondrial proteins, and maximal citrate synthase activity, suggest that endurance training improves mitochondrial content in the low-oxidative muscles, but not in the high-oxidative muscles in diabetes. These results suggest glucose clearance in diabetes improved by endurance training *via* to especially low-oxidative muscle adaptation.

## Conflict of Interest

There is no conflict of interest.

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## References

1. Larsen S, Scheede-Bergdahl C, Whitesell T, Boushel R, Bergdahl A. Increased intrinsic mitochondrial respiratory capacity in skeletal muscle from rats with streptozotocin-induced hyperglycemia. Physiol Rep 2015;3:e12467. https://doi.org/10.14814/phy2.12467
2. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes 2002;51:2944-2950. https://doi.org/10.2337/diabetes.51.10.2944
3. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med 2004;350:664-671. https://doi.org/10.1056/NEJMoa031314

4. Yasuda K, Nishikawa W, Iwanaka N, Nakamura E, Seino Y, Tsuda K, Ishihara A. Abnormality in fibre type distribution of soleus and plantaris muscles in non-obese diabetic Goto-Kakizaki rats. Clin Exp Pharmacol Physiol 2002;29:1001-1008. https://doi.org/10.1046/j.1440-1681.2002.03757.x
5. Sparks LM, Xie H, Koza RA, Mynatt R, Hulver MW, Bray GA, Smith SR. A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. Diabetes 2005;54:1926-1933. https://doi.org/10.2337/diabetes.54.7.1926
6. Yokota T, Kinugawa S, Hirabayashi K, Matsushima S, Inoue N, Ohta Y, Hamaguchi S, ET AL. Oxidative stress in skeletal muscle impairs mitochondrial respiration and limits exercise capacity in type 2 diabetic mice. Am J Physiol Heart Circ Physiol 2009;297:H1069-H1077. https://doi.org/10.1152/ajpheart.00267.2009
7. Colberg SR, Sigal RJ, Yardley JE, Riddell MC, Dunstan DW, Dempsey PC, Horton ES, ET AL. Physical activity/exercise and diabetes: A position statement of the American Diabetes Association. Diabetes Care 2016;39:2065-2079. https://doi.org/10.2337/dc16-1728
8. Hall KE, McDonald MW, Grisé KN, Campos OA, Noble EG, Melling CW. The role of resistance and aerobic exercise training on insulin sensitivity measures in STZ-induced Type 1 diabetic rodents. Metabolism 2013;62:1485-1494. https://doi.org/10.1016/j.metabol.2013.05.012
9. King AJ. The use of animal models in diabetes research. Br J Pharmacol 2012;166:877-894. https://doi.org/10.1111/j.1476-5381.2012.01911.x
10. Bonen A, Miskovic D, Tonouchi M, Lemieux K, Wilson MC, Marette A, Halestrap AP. Abundance and subcellular distribution of MCT1 and MCT4 in heart and fast-twitch skeletal muscles. Am J Physiol Endocrinol Metab 2000;278:E1067-E1077. https://doi.org/10.1152/ajpendo.2000.278.6.E1067
11. Juel C, Halestrap AP. Lactate transport in skeletal muscle-role and regulation of the monocarboxylate transporter. J Physiol 1999;517:633-642. https://doi.org/10.1111/j.1469-7793.1999.0633s.x
12. McCullagh KJ, Poole RC, Halestrap AP, O'Brien M, Bonen A. Role of the lactate transporter (MCT1) in skeletal muscles. Am J Physiol 1996;271:E143-E150. https://doi.org/10.1152/ajpendo.1996.271.1.E143
13. Enoki T, Yoshida Y, Hatta H, Bonen A. Exercise training alleviates MCT1 and MCT4 reductions in heart and skeletal muscles of STZ-induced diabetic rats. J Appl Physiol 2003;94:2433-2438. https://doi.org/10.1152/japplphysiol.01155.2002
14. Nakamoto I, Ishihara A. Effects of voluntary running exercise on skeletal muscle properties in nonobese rats with type 2 diabetes. Physiol Res 2020;69:73-84. https://doi.org/10.33549/physiolres.934178
15. Ferrannini E. The theoretical bases of indirect calorimetry: a review. Metabolism 1988;37:287-301. https://doi.org/10.1016/0026-0495(88)90110-2
16. Tadaishi M, Miura S, Kai Y, Kano Y, Oishi Y, Ezaki O. Skeletal muscle-specific expression of PGC-1alpha-b, an exercise-responsive isoform, increases exercise capacity and peak oxygen uptake. PLoS One 2011;6:e28290. https://doi.org/10.1371/journal.pone.0028290
17. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 1949;109:1-9. https://doi.org/10.1113/jphysiol.1949.sp004363
18. Lo S, Russell JC, Taylor AW. Determination of glycogen in small tissue samples. J Appl Physiol 1970;28:234-236. https://doi.org/10.1152/jappl.1970.28.2.234
19. Takahashi Y, Matsunaga Y, Banjo M, Takahashi K, Sato Y, Seike K, Nakano S, ET AL. Effects of nutrient intake timing on post-exercise glycogen accumulation and its related signaling pathways in mouse skeletal muscle. Nutrients 2019;11:2555. https://doi.org/10.3390/nu11112555
20. Hoshino D, Hanawa T, Takahashi Y, Masuda H, Kato M, Hatta H. Chronic post-exercise lactate administration with endurance training increases glycogen concentration and monocarboxylate transporter 1 protein in mouse white muscle. J Nutr Sci Vitaminol (Tokyo) 2014;60:413-419. https://doi.org/10.3177/jnsv.60.413
21. Kitaoka Y, Takahashi Y, Machida M, Takeda K, Takemasa T, Hatta H. Effect of AMPK activation on monocarboxylate transporter (MCT)1 and MCT4 in denervated muscle. J Physiol Sci 2014;64:59-64. https://doi.org/10.1007/s12576-013-0290-7
22. Takahashi K, Kitaoka Y, Matsunaga Y, Hatta H. Effect of post-exercise lactate administration on glycogen repletion and signaling activation in different types of mouse skeletal muscle. Curr Res Physiol 2020;3:34-43. https://doi.org/10.1016/j.crphys.2020.07.002

23. Kitaoka Y, Tamura Y, Takahashi K, Terada K, Takemasa T, Hatta H. Effects of Nrf2 deficiency on mitochondrial oxidative stress in aged skeletal muscle. Physiol Rep 2019;7:e13998. https://doi.org/10.14814/phy2.13998
24. Srere PA. Citrate synthase. Methods Enzymol 1969;13:3-11. https://doi.org/10.1016/0076-6879(69)13005-0
25. Ito M, Kondo Y, Nakatani A, Hayashi K, Naruse A. Characterization of low dose streptozotocin-induced progressive diabetes in mice. Environ Toxicol Pharmacol 2001;9:71-78. https://doi.org/10.1016/S1382-6689(00)00064-8
26. Tandon S, Ayis S, Hopkins D, Harding S, Stadler M. The impact of pharmacological and lifestyle interventions on body weight in people with type 1 diabetes: A systematic review and meta-analysis. Diabetes Obes Metab 2021;23:350-362. https://doi.org/10.1111/dom.14221
27. Choi HM, Kim HR, Kim EK, Byun YS, Won YS, Yoon WK, Kim HC, ET AL. An age-dependent alteration of the respiratory exchange ratio in the db/db mouse. Lab Anim Res 2015;31:1-6. https://doi.org/10.5625/lar.2015.31.1.1
28. Hirsch KR, Greenwalt CE, Cabre HE, Gould LM, Brewer GJ, Blue MNM, Ferrando AA, ET AL. Metabolic effects of high-intensity interval training and essential amino acids. Eur J Appl Physiol 2021;121:3297-3311. https://doi.org/10.1007/s00421-021-04792-4
29. Goodpaster BH, Katsiaras A, Kelley DE. Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. Diabetes 2003;52:2191-2197. https://doi.org/10.2337/diabetes.52.9.2191
30. Matsunaga Y, Sakata Y, Yago T, Nakamura H, Shimizu T, Takeda Y. Effects of glucose with casein peptide supplementation on post-exercise muscle glycogen resynthesis in C57BL/6J mice. Nutrients 2018;10:753. https://doi.org/10.3390/nu10060753
31. Miyamoto L, Toyoda T, Hayashi T, Yonemitsu S, Nakano M, Tanaka S, Ebihara K, ET AL. Effect of acute activation of 5′-AMP-activated protein kinase on glycogen regulation in isolated rat skeletal muscle. J Appl Physiol 2007;102:1007-1013. https://doi.org/10.1152/japplphysiol.01034.2006
32. Takahashi Y, Matsunaga Y, Yoshida H, Shinya T, Sakaguchi R, Hatta H. High carbohydrate diet increased glucose transporter protein levels in jejunum but did not lead to enhanced post-exercise skeletal muscle glycogen recovery. Nutrients 2021;13:2140. https://doi.org/10.3390/nu13072140
33. Larsen S, Nielsen J, Hansen CN, Nielsen LB, Wibrand F, Stride N, Schroder HD, ET AL. Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. J Physiol 2012;590:3349-3360. https://doi.org/10.1113/jphysiol.2012.230185
34. Ljubicic V, Hood DA. Specific attenuation of protein kinase phosphorylation in muscle with a high mitochondrial content. Am J Physiol Endocrinol Metab 2009;297:E749-E758. https://doi.org/10.1152/ajpendo.00130.2009
35. Matsunaga Y, Tamura Y, Takahashi Y, Masuda H, Hoshino D, Kitaoka Y, Saito N, ET AL. Pre-exercise casein peptide supplementation enhances endurance training-induced mitochondrial enzyme activity in slow twitch muscle, but not fast twitch muscle of high fat diet-fed mice. J Phys Fit Sports Med 2015;4:377-384. https://doi.org/10.7600/jpfsm.4.377
36. Takahashi Y, Hijikata K, Seike K, Nakano S, Banjo M, Sato Y, Takahashi K, ET AL. Effects of royal jelly administration on endurance training-induced mitochondrial adaptations in skeletal muscle. Nutrients 2018;10:1735. https://doi.org/10.3390/nu10111735
37. Nikooie R, Rajabi H, Gharakhanlu R, Atabi F, Omidfar K, Aveseh M, Larijani B. Exercise-induced changes of MCT1 in cardiac and skeletal muscles of diabetic rats induced by high-fat diet and STZ. J Physiol Biochem 2013;69:865-877. https://doi.org/10.1007/s13105-013-0263-6
38. Harmer AR, Chisholm DJ, McKenna MJ, Hunter SK, Ruell PA, Naylor JM, Maxwell LJ, ET AL. Sprint training increases muscle oxidative metabolism during high-intensity exercise in patients with type 1 diabetes. Diabetes Care 2008;31:2097-2102. https://doi.org/10.2337/dc08-0329
39. Bonen A. The expression of lactate transporters (MCT1 and MCT4) in heart and muscle. Eur J Appl Physiol 2001;86:6-11. https://doi.org/10.1007/s004210100516
40. Armstrong RB, Gollnick PD, Ianuzzo CD. Histochemical properties of skeletal muscle fibers in streptozotocin-diabetic rats. Cell Tissue Res 1975;162:387-394. https://doi.org/10.1007/BF00220185