MyoD, Myogenin and Myosin Heavy Chain mRNA Expression in Rat Skeletal Muscle after a Single Session of Low-Intensity Treadmill Exercise

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Abstract. [Purpose] We examined the time course of changes in the activation of muscle satellite cells and muscle protein synthesis after a single session of low-intensity treadmill exercise. [Subjects] A total of 25 male Wistar rats aged 11 weeks were used. [Methods] Rats were run on a treadmill with a 16° decline for 30 min at 24 m/min, except for the control group (CON). Blood was collected to measure creatine phosphokinase (CPK) and both soleus muscles were removed to analyze histological and muscle gene expression at 24, 48, 72 and 96 h post-exercise. [Results] CPK levels in exercised rats at 24 and 96 h were significantly higher than in CON, and CPK levels at 96 h were higher than those at 48 h. Infiltration of macrophages and gathering of small myofibers were detected in the exercise group, but there were no significant differences in MyoD, myogenin, myosin heavy chain (MHC)-1 and MHC-2a mRNA levels. [Conclusion] These results suggest that muscle satellite cells and muscle protein synthesis are not significantly activated, despite the occurrence of myotrauma, in the acute phase (24 h to 96 h) after a single session of low-intensity exercise.

Key words: Satellite cell, Muscle protein synthesis, Treadmill running

INTRODUCTION

Skeletal muscle hypertrophy occurs as a result of various types of stimulation. Such hypertrophy is based on muscle protein metabolism, and occurs when the synthesis of muscle protein is higher than its disintegration. Satellite cells play a very important role in muscle hypertrophy1). Satellite cells are quiescent myogenic precursor cells located between the plasma membrane and basal lamina in adult muscle, and they are activated by exercise stimulation2,3). Activated satellite cells enter the cell cycle and proliferate, thereafter fusing to produce new myotubes and/or fuse to existing myofibers, contributing to hyperplasia or increased numbers of muscle nuclei1).

Myogenic regulatory factors, including MyoD and myogenin, control the transcription and translation of myosin heavy chain (MHC), which is unique to skeletal muscle4). Moreover, MyoD and myogenin regulate muscle differentiation. MyoD is expressed when satellite cells proliferate and myogenin is expressed when satellite cells differentiate into myotubes.

With regard to the study of differences in the activation of muscle satellite cells and muscle
protein synthesis in hypertrophy, experiments that have prevented hypertrophy through ablation of satellite cells and inhibition of myonuclear addition using gamma irradiation have been reported. Gamma irradiation completely prevented hypertrophy following synergist ablation and voluntary running, while gamma irradiation reduced only half of the muscle hypertrophy in response to either Insulin-like growth factor 1 gene delivery or reloading after hindlimb suspension. These reports indicate that the cellular mechanisms regulating increases in myofiber size differ depending on the hypertrophic stimulus, and it is now thought that muscle growth consists of multiple phases, including accelerated transcriptional and translational responses, followed by satellite cell proliferation during the later stages of hypertrophy.

Although muscle strength exercises are often used in physical therapy, it is difficult to apply the conditions reported to date in hypertrophy models, such as synergist ablation or long-term exercise, to physical therapy treatment. Although many reports have confirmed regeneration after myotrauma, it is necessary for clinical physical therapy to prevent myotrauma as much as possible in muscle strength exercises. It is therefore necessary to understand the activation of muscle satellite cells and muscle protein synthesis after low-intensity exercise, but no such reports are currently available. Therefore, we examined the time course of changes in the activation of muscle satellite cells and muscle protein synthesis in rat skeletal muscle after a single session of low-intensity treadmill exercise.

We hypothesized that muscle protein synthesis would be activated to a greater degree than satellite cells, as we focused our examination on the early phase after a single session of exercise. In this study, we used creatine phosphokinase (CPK) as a marker of myotrauma, MyoD and myogenin mRNA expression as markers of satellite cell activation, and MHC-1 and MHC-2a mRNA expression as markers of muscle protein synthesis.

METHODS

A total of 25 male Wistar rats (age, 11 weeks; mean ± standard deviation (SD) body weight, 378 ± 12 g) were used for this study. All rats were given access to standard laboratory diet and water under a 12-h light/dark cycle. All procedures for animal care and treatment were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals at Kanazawa University. Rats were randomly allocated into one of 5 groups: the control group (CON), and 4 experimental groups (Downhill running). Rats in the experimental groups performed running at 24 m/min on a treadmill with 16° decline for 30 min. The exercise protocol was based on that used in previous studies. After the exercise, rats were returned to their cages and given ad libitum access to food and water. Rats were injected with pentobarbital sodium (4.0 mg/100 g body weight), and blood was collected by intracardial exsanguination at 24 h (P24), 48 h (P48), 72 h (P72) or 96 h (P96) post-exercise. Blood was left for 10 min at room temperature following centrifugation at 2500 rpm for 10 min, then centrifuged blood plasma was stored frozen until use. The right SOL muscles were quick-frozen in liquid nitrogen-cooled isopentane, and stored at –70 °C until use. As a marker of myotrauma after exercise, CPK levels, which are easily analyzed in clinical settings, were measured. Stored blood plasma was thawed at room temperature, and analyzed using the appropriate reagent (Shino-test, Tokyo, Japan) and an automated device (Nihonkohden, Tokyo, Japan).

In order to analyze the influence of exercise on the histology of myofibers, 10-µm frozen sections were cut from the central part of the right SOL muscle using a cryostat (Sakura Finetek, Tokyo, Japan). Sections were then stained with hematoxylin and eosin (HE), and observed and photographed under a microscope (Keyence, Osaka, Japan).

Total RNA was isolated from the central part of 20–30 mg of the left SOL using a spin column (QIAGEN, Tokyo, Japan). DNAaseI was added in order to increase RNA purity. Absorbance at 260 nm (A260) and 280 nm (A280) was measured with a spectrophotometer (TAITEC, Saitama, Japan) to calculate the purity (A260/A280) and density of RNA. Total RNA with a purity of 1.7–2.0 was used for analysis. DNA was synthesized from 1.0 µg of total RNA using a first-strand cDNA synthesis kit (Takara Bio) with random hexamer primers. The reaction conditions followed standard protocols.
A quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used for analysis of mRNA expression. A Lightcycler® (Roche Diagnostic Corporation, Tokyo, Japan) was used for qRT-PCR with SYBR® Premix Ex Taq (Takara bio) and primers (Table 1)\(^{17,19}\). Each primer pair was synthesized by Nihon Gene Research Laboratories (Miyagi, Japan). The PCR conditions were an initial step of 10 s at 95 °C, followed by 5 s of denaturation at 95 °C and 20 s of annealing and extension at 60 °C (for MHC-2a, this step was carried out at 62 °C). The melting curve was then produced by increasing the temperature at + 0.1 °C/s, and it was used to verify amplification product specificity. Relative expression levels of MyoD, myogenin, MHC-1 and MHC-2a were normalized by subtracting the corresponding levels of glyceradehyde-3-phosphate dehydrogenase (GAPDH).

The results of CPK assays and qRT-PCR were analyzed using Smirnov-Grubbs analysis to exclude outliers, followed by Bartlett’s test. One-way analysis of variance (ANOVA) and Scheffe’s post-hoc test or the Kruskal-Wallis test, and Steel-Dwass’s post-hoc test were then performed. All data for qRT-PCR are reported as mean values relative to CON. Values of p<0.05 were considered to be statistically significant.

### RESULTS

The histological results are shown in Fig. 1. Infiltration of macrophages at P24 and P48 and the gathering of small myofibers at P72 and P96 were seen in the exercised groups, but not in CON. CPK levels at P24 and P96 were significantly higher than in CON, and the CPK levels at P96 were higher than at P48 (Table 2). The qRT-PCR results are shown in Table 2. MyoD and MHC-1 mRNA levels showed no changes. Myogenin mRNA levels at P24 were about 1.7-fold those in CON, and MHC-2a mRNA levels at P24 and P96 were about 1.8-fold higher, although there were no significant differences.

### DISCUSSION

In this study, we examined the time course of changes in myotrauma and activation of muscle satellite cells and muscle protein synthesis in rat skeletal muscles after a single session of low-intensity treadmill exercise.

Satellite cells are known to be activated by treadmill exercise\(^3\) (16-24 m/min, −16°, 105 min), and MyoD-positive myofibers increase in number\(^{20}\) (17 m/min, −13.5°, 90 min). In this study (24 m/min, −16°, 30 min), however, MyoD mRNA expression showed no changes, and myogenin mRNA expression at P24 was about 1.7-fold that in CON, although the difference was not significant. Our previous study showed that BrdU-positive cells

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**Table 1. Primers used for qRT-PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession Nos.</th>
<th>Sequence (5’-3’)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MyoD (NM_176079)</td>
<td>ACT ACA GCC GCC ACT CAG AC</td>
<td>ACT GTA GTA GCC GCC GCC GTC GT</td>
</tr>
<tr>
<td>Myogenin (NM_017115)</td>
<td>TGA ATG CAA CTC CCA CAG C</td>
<td>CAG ACA TAT CCT CCA CCG TG</td>
</tr>
<tr>
<td>MHC-1 (NM_017240)</td>
<td>TTG CTC TAC CCA ACC CTA AGG ATG</td>
<td>TTG TGT TTC TGC CTG AAG GTG C</td>
</tr>
<tr>
<td>MHC-2a (L13606)</td>
<td>CTC AGG CTT CAA GAT TTG GTG G</td>
<td>TTG TGC TCT CTC TCT GTC ATT C</td>
</tr>
<tr>
<td>GAPDH (AF106860)</td>
<td>AAC GGG AAA CCC ATC ACC A</td>
<td>CGG AGA TGA TGA CCC TTT TG</td>
</tr>
</tbody>
</table>

* Upper = forward primer; lower = reverse primer.
were significantly elevated using the same exercise protocol in 4-wk-old rats. The difference between the present and previous studies was apparently due to age, as the number of satellite cells decreased in SOL, and the number of satellite cell activated by exercise is known to decrease with aging. It is also possible that the mRNA levels increased more rapidly than BrdU expression, in first 24 hours after exercise; thus, we need to further investigate the changes in the period immediately after exercise.

Smith reported that after a single session of exercise (15 m/min, –16°, 30 min) BrdU-positive cells did not increase, but after two sessions of exercise, the cells increased significantly. Thus, significant activation of satellite cells is expected with increasing exercise volume, either through increasing session length (from 30 min to 90 or 105 min) or in the number of sessions (from one to two).

It is thought to be important for satellite cell activation to adjust both exercise intensity and duration. However, in this study, which showed no significant changes in MyoD and myogenin mRNA expression, and in a previous report, in which no changes in BrdU-positive cells were observed, there were significant increases in CPK and histological myotrauma. Thus, the occurrence of myotrauma is not necessarily related to the activation of satellite cells. On the other hand, as differences were seen in MyoD and myogenin mRNA levels, it is possible that their expression differs with fiber type, as greater increases are seen in myogenin expression than in MyoD expression after synergist ablation and during regeneration of denervated myotrauma. Our previous study also showed greater changes in myogenin expression than in MyoD expression.

The ratios of MHC-1 and MHC-2a are increased by various stimuli, including stretch and mechanical overload, and repeated exercise in rats, while MHC-1, MHC-2a and MHC-2x mRNA expression were found to increase after a single session of heavy resistance exercise in humans. In this study, MHC-1 mRNA levels showed no changes, and MHC-2a mRNA levels at P24 and P96 were about 1.8-fold those in CON, although the differences were not significant. Demirel showed that the ratios of MHC-1 and MHC-2a were not changed by 30-min treadmill exercise, but changed significantly (MHC-1 ratio increased and MHC-2a ratio decreased) with 60-min or 90-min treadmill exercise for 10 weeks. These results show that the influence of short-term exercise on MHC is small in both the later phases and the acute phase. Thus, it is important for the activation of MHC mRNA expression to use exercise of high intensity or moderate duration, similar to the case of satellite cells.

We hypothesized that muscle protein synthesis is activated more strongly than satellite cells, as muscle growth consists of multiple phases, including accelerated transcriptional and translational responses, followed by satellite cell proliferation during the later stages of hypertrophy. However, in this study, no significant changes were seen in MyoD, myogenin, MHC-1 or MHC-2a. Thus, it is probably necessary to increase the exercise workload in order to activate satellite cells and muscle protein synthesis. Although the differences were not significant, myogenin mRNA levels at P24 were about 1.7-fold those in CON, while MHC-2a mRNA levels at P24 and P96 were about 1.8-fold higher. It may therefore be possible to activate the satellite cells and muscle protein synthesis by adjusting the duration and frequency of low-intensity exercise, but consideration is needed as myotrauma may still occur, even though no significant activation of satellite cells and muscle protein synthesis was seen in the present study.

It is necessary for clinical muscle strength exercises in physical therapy to minimize

| Table 2. CPK levels in blood plasma and mRNA expression of markers after treadmill exercise |
|----------------|----------------|----------------|----------------|----------------|
|                | CON   | P24   | P48   | P72   | P96   |
| CPK (IU/L)     | 312 ± 38 | 531 ± 173a | 317 ± 55 | 422 ± 43 | 585 ± 74ab |
| MyoD           | 1.00 ± 0.51 | 1.18 ± 0.49 | 0.48 ± 0.13 | 1.38 ± 0.73 | 0.76 ± 0.47 |
| Myogenin       | 1.00 ± 0.26 | 1.73 ± 0.79 | 1.25 ± 0.48 | 0.86 ± 0.04 | 1.12 ± 0.64 |
| MHC-1          | 1.00 ± 0.21 | 1.20 ± 0.60 | 0.85 ± 0.30 | 0.49 ± 0.17 | 0.48 ± 0.18 |
| MHC-2a         | 1.00 ± 0.21 | 1.83 ± 1.01 | 1.72 ± 0.67 | 1.18 ± 0.24 | 1.79 ± 1.27 |

Values are means ± SD. Levels of mRNA are expressed as relative levels vs. GAPDH levels.
a : vs. CON (p < 0.05), b : vs. P48 (p < 0.05).
myotrauma, and further studies will be needed in order to investigate exercise protocols with regard to intensity, duration, workload, warm-up period and rest in order to apply the findings to physical therapy.

REFERENCES

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