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MyoD and Myogenin mRNA levels after single session of treadmill exercise in rat skeletal muscle

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Abstract

[Purpose] We examined mRNA levels of MyoD and myogenin in rat skeletal muscle after a single session of treadmill running. [Subjects] A total of 26 male Sprague-Dawley rats aged 4 weeks were used in this study. [Methods] Rats were run on a 16° decline for 30 min. Treadmill speeds were 0, 16, 20, 24 and 28 m/min. At 72 h post-exercise, soleus (SOL) and extensor digitorum longus (EDL) muscles were extracted. The expression of MyoD and myogenin mRNA were analyzed by reverse transcription polymerase chain reaction (RT-PCR) and quantitative RT-PCR. [Results] MyoD and myogenin mRNA expression was detected in all groups. Significant differences in myogenin levels were apparent in SOL between 16 m/min and 28 m/min. MyoD levels in SOL at 16, 24, 28 m/min were lower, while myogenin levels at 20, 24 and 28 m/min were higher, when compared with the 0 m/min group, although significant differences were not seen. [Conclusion] These results suggest that a single session of exercise has little effect on proliferation of satellite cells, or myotube production.

Key words: satellite cell; myogenic regulatory factor, treadmill running

INTRODUCTION

Muscle strength exercises are one of the most common techniques in physical therapy, and physical therapist experience has indicated that muscle hypertrophy can improve ability in walking capacity and activities of daily living. Satellite cells, quiescent myogenic precursor cells located between the plasma membrane and basal lamina in adult muscle, are responsible for the hypertrophic capacity of muscle[1)]. Stimuli, which include treadmill running, stretching or tenotomy, induce satellite cell activation and proliferation[2)]. Proliferating satellite cells fuse to produce new myotubes and/or fuse to existing myofibers, contributing to hyperplasia or increased numbers of muscle nuclei[1)].

Conversely, myogenic regulatory factors (MRFs) are responsible for growth in embryogenesis[1)]. MRFs include MyoD, myogenin, MRF4 and Myf5, the expression of which is able to transform any cell into a muscle cell. MRFs are also responsible for activating transcription of protein synthesis. MyoD is expressed when satellite cells are proliferating, and myogenin is expressed when satellite cells differentiate into myotube. MyoD and myogenin are expressed in both embryogenic muscle and adult muscle during overload[3)] and regeneration[4)]. Numerous reports have described exercise-induced satellite cell activation. For example, Darr[5)] reported that running on a treadmill at a decline of 18% for 105 min at 16-24 m/min activates satellite cells. Tsivitse[6)] used a treadmill on a 16% incline for 90 min at 17 m/min, while Armand[7)] subjected mice to a treadmill on a 14° decline for 150 min at 30-40 m/min. However, the conditions in these reports were aimed at producing myotrauma, and are not realistic for physical therapy. Furthermore, few reports have described MyoD and myogenin expression levels after treadmill running, or have attempted to clarify the threshold of satellite cell activation.

In an effort to identify the most efficient exercise frequency, we examined the relationship between exercise intensity and satellite cell activation using MyoD and myogenin levels, as determined by reverse transcription polymerase chain reaction (RT-PCR) and quantitative RT-PCR (qRT-PCR), as markers.

METHODS

A total of 26 male Sprague-Dawley rats (age, 4 weeks; mean (± standard deviation (SD)) body weight, 119±10 g) were used for this study. Rats were run on a treadmill on a 16° decline for 30 min. The downward slope of 16° was in accordance with the Armstrong protocol[8)], which found that this angle was the steepest that could be used before the rats began to slide down the tread surface. While exercise period was set at 30 min in accordance with Smith[9,10)], we found that it was difficult for

untrained rats to run constantly for more than 30 min. Treadmill speeds were set at 0, 16, 20, 24 (n=5/group) and 28 m/min (n=6). These speeds were based on the report by Darr[5] and were demonstrated to induce satellite cell activation. 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 in accordance with the Guidelines for the Care and Use of Laboratory Animals at Kanazawa University.

After exercise, rats were returned to their cages and given ad libitum access to food and water. At 72-h post-exercise, when MyoD and myogenin expression reportedly peaks in rat soleus (SOL) and extensor digitorum longus (EDL) after injection of notexin[4], rats were injected with pentobarbital sodium (4.0-7.5 mg/100 g body weight) and intracardially exsanguinated. The right SOL and EDL muscles were extracted and placed in stabilization reagent (Takara Bio, Shiga, Japan) overnight at 4°C. Muscles were stored at -70°C until processing.

Total RNA was isolated from the central part of 20 mg of muscle extracts using a spin column (QIAGEN, Tokyo, Japan). DNAaseI was included in the process to increase RNA purity. Absorbance at 260 nm (A_{260}) and 280 nm (A_{280}) was measured to calculate the purity (A_{260}/A_{280}) and density of RNA using a spectrophotometer (TAITEC, Saitama, Japan). 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. Reaction conditions followed standard protocols.

RT-PCR and qRT-PCR were used for the analysis of mRNA expression. For RT-PCR, cDNA at a 100-fold dilution were used as a sample. Each primer pair (Table 1) was synthesized by Nihon Gene Research Laboratories (Miyagi, Japan). Taq polymerase (Takara Bio) and amplification parameters were: denaturation for 10 s at 95°C; annealing for 20 s at 60°C; and extension for 30 s at 72°C. PCR products were analyzed on 3.0% agarose gel and were visualized by staining with Gelstar (Takara Bio).

A Lightcycler® (Roche Diagnostic Corporation, Tokyo, Japan) was used for qRT-PCR using a SYBR® Premix Ex Taq (Takara bio) and the same primers as in RT-PCR (Table 1). Conditions were: 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. The melting curve was then prepared by increasing the temperature at +0.1°C/s, and was used to verify amplification product specificity. Relative expression levels of MyoD or myogenin were normalized by subtracting the corresponding levels of glyceradehyde-3-phosphate dehydrogenase (GAPDH).

Results of qRT-PCR were determined using Smirnov-Grubbs analysis to exclude outliers, following Bartlett's test. One-way analysis of variance (ANOVA) and Scheffe's post-hoc test or Kruskal-Wallis test and Steel-Dwass's post-hoc test were then performed. All data are reported as mean relative values when compared to 0 m/min. Values of $P<0.05$ were considered statistically

significant.

RESULTS

RT-PCR confirmed both MyoD and myogenin mRNA expression in all groups in SOL and EDL (Fig. 1). Results for qRT-PCR are shown in Table 2. Kruskal-Wallis test was performed on MyoD data, and one-way ANOVA was performed on myogenin data. For MyoD mRNA expression, although no significant differences were seen between any groups for SOL or EDL, the levels in SOL for the 16, 24, 28 m/min groups were lower when compared with the 0 m/min group. For myogenin, a significant difference was only seen in SOL between 16 and 28 m/min groups. Myogenin levels at 20, 24 and 28 m/min were higher when compared with the 0 m/min group, although no significant differences were seen.

DISCUSSION

We examined the intensity of exercise that activated satellite cells by measuring mRNA levels of MyoD and myogenin. MyoD is a marker of satellite cell activation and proliferation, while myogenin is a marker of myotube production.

No significant differences in MyoD mRNA expression were seen for SOL or EDL, thus suggesting that this intensity of exercise scarcely activates satellite cells. This is supported by the observation that treadmill exercise at a decline of 16° at 15 m/min for 30 min, did not affect the number of BrdU-labeled myonuclei[10].

A significant difference in myogenin mRNA expression was only seen between the 16 and 28 m/min groups in SOL, and expression levels in the 20, 24 and 28 m/min groups were higher when compared with the 0 m/min group. A treadmill speed of 20 m/min is near the lactate threshold (LT)[11], and this intensities at or above the LT may activate myotube production. Conversely, no significant differences in EDL were identified. Thus, eccentric exercises, such as downhill running, induce muscle injury in SOL[8].

As noted above, a significant difference was seen in myogenin expression, but not in MyoD expression. MyoD and myogenin mRNA expression differ with fiber type[12], and changes in MyoD (about 3-fold) and myogenin (>40-fold) mRNA expression during regeneration of denervated myotrauma[13] may be involved. However, few significant differences were seen in MyoD and myogenin mRNA expression after treadmill exercise. Increased expression of MRFs in representative hypertrophy and regeneration models after functional overload[3] and notexin injection[4] have been clearly identified, and similar findings have been confirmed in humans[14]. In addition, treadmill running activates satellite cells and increased expression of MRFs in rats[6] and

mice[7]. However, the conditions to achieve those results clearly differ from those of muscle strength exercises in physical therapy, and thus a relatively low intensity of exercise, when compared with these reports, was adopted in this study. The present results using this low-intensity treadmill exercise suggest that a single session of exercise has little effect on activating or initiating proliferation of satellite cells, and scarcely activates production of myotubes.

However, these results do not suggest that hypertrophy is not induced, as the role of satellite cells in skeletal muscle hypertrophy remains unclear[15,16]. Therefore, the effects of exercise intensity, age and timing of muscle extraction must be examined further.

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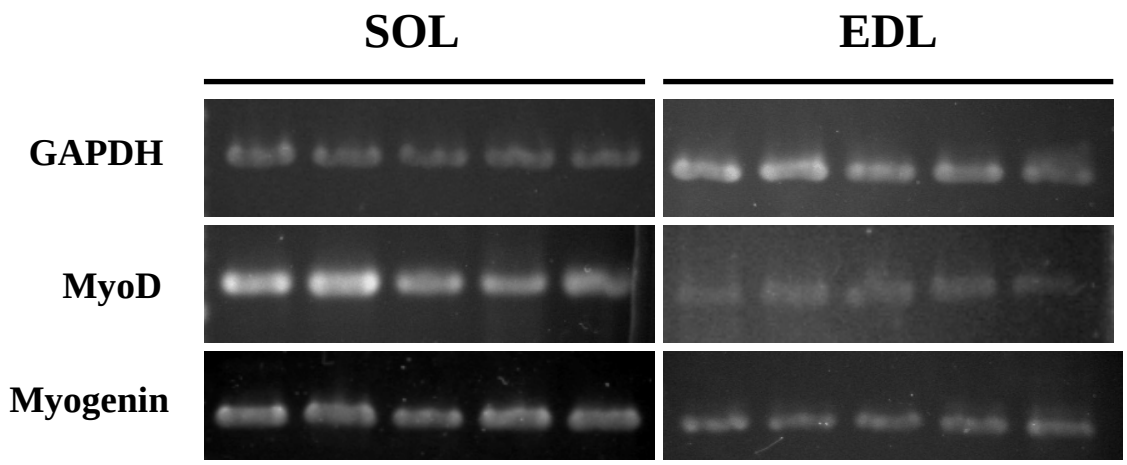
LEGENDS TO FIGURES

Figure 1: Expression of MyoD and myogenin mRNA were analyzed by RT-PCR in SOL and EDL. GAPDH mRNA expression was used as a control. These 3 products were detected in all groups in SOL and EDL. Groups of 0, 16, 20, 24, 28 m/min are expressed sequentially from the left by the right in each SOL and EDL.

FIGURES

Figure 1



TABLES

Table 1: Primers used for RT-PCR and qRT-PCR

Gene	Sequence (5'-3')*	Position (5')
MyoD	ACT ACA GCG GCG ACT CAG AC	782
	ACT GTA GTA GGC GGC GTC GT	884
Myogenin	TGA ATG CAA CTC CCA CAG C	514
	CAG ACA TAT CCT CCA CCG TG	658
PCNA	GCA GAT GTA CCC CTT GTT GT	753
	CAG AAA AGA CCT CAG AAC ACG	953
GAPDH	AAC GGG AAA CCC ATC ACC A	1051
	CGG AGA TGA TGA CCC TTT TG	1191

* Upper = forward primer; lower = reverse primer.

Table 2: MyoD and myogenin mRNA levels in rat SOL and EDL after treadmill exercise

Treadmill	MyoD		Myogenin	
speed	SOL	EDL	SOL	EDL
0 m/min	100.0±23.4	100.0±13.6	100.0±18.8	100.0±16.9
16 m/min	56.6±12.6	87.4±26.3	71.5±14.0[a]	81.3±16.9
20 m/min	93.5±36.5	107.0±34.6	128.8±59.5	102.0±24.0
24 m/min	72.5±7.1	92.2±24.6	124.4±22.4	101.2±21.1
28 m/min	57.8±14.9	104.2±22.3	129.8±34.1[a]	107.6±32.0

Values represent mean ±SD, expressed as relative expression levels normalized by dividing by the GAPDH level. [a]16 m/min< 28 m/min ($p<0.05$).