Induction of Muscle Hypertrophy in Rats through Low Intensity Eccentric Contraction

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Abstract. [Purpose] The purpose of this study was to examine whether a low intensity exercise using an eccentric contraction would result in skeletal muscle hypertrophy in rats. [Subjects and Methods] Eighteen female Wistar rats were used in this study. The rats were randomly divided into three groups. The control group performed no exercise. The level group ran on a treadmill on a 0° incline. The downhill group ran on a treadmill on a −16° incline. The two exercise groups ran on a treadmill at 16 m/min for 90 minutes, once every three days for a total of twenty sessions. [Results] The muscle wet weights, the relative weight ratios, and the muscle fiber cross-section minor axes of the downhill group were significantly larger than those of the control and level groups. There were no differences in the muscle wet weights, the relative weight ratios, and the muscle fiber cross-section minor axes between the control group and the level group. [Conclusion] The stimulation from the low intensity eccentric contraction may have produced enough mechanical stress to induce muscle hypertrophy without the over-stressing that might have produced muscle fiber damage. These results indicate that this technique may be an effective method of inducing hypertrophy in skeletal muscle.

Key words: Eccentric contraction, Low intensity exercise, Muscle hypertrophy

INTRODUCTION

Muscle weakness and atrophy often affect activities of daily living, disease prevention and disease outcome1). To obtain hypertrophy of skeletal muscle, it has been reported that it is necessary to use a high intensity exercise2). However, high intensity exercise exposes the elderly to high risk3), because it imposes a high burden on articular and cardiovascular functions. Accordingly, the elderly often perform low intensity exercise for safety reasons even though it is not expected to induce hypertrophy of the skeletal muscle. Therefore, we consider that it is necessary to develop a safe and effective low intensity training program for the elderly.

Skeletal muscle contraction is classified into three types4) according to the relationship between the muscle torque and the load torque. The three types are isometric contraction, concentric contraction, and eccentric contraction. In an isometric contraction, the muscle torque is equal to the load torque, and the muscle length does not change. In a concentric contraction, the muscle torque is greater than the load torque, and the muscle length shortens. In an eccentric contraction, the load torque is greater than the muscle torque, and the muscle length lengthens.

An eccentric contraction has been shown to produce greater muscle hypertrophy than a concentric contraction after resistance training5). In addition to greater hypertrophy, eccentric contraction training tends to exert a lower stress on the cardiovascular system6). When eccentric contractions are used, lower-intensity training can be better used than when concentric contractions are used to produce muscle hypertrophy.

Armstrong, et al.7) studied eccentric contractions in downhill running performed by rats. They used downward movement patterns to stimulate the antigravity muscles through eccentric contractions. Downhill running is widely used as a method of eccentric contraction training for rat skeletal muscles7–9).

The purpose of this study was to examine whether a low intensity exercise using an eccentric contraction would result in skeletal muscle hypertrophy in rats. In this experiment, we used level and downhill running speeds of 16 m/min. This speed corresponds to about 50 to 60% of the lactate threshold in rats10).
SUBJECTS AND METHODS

Eighteen female Wistar rats (10 weeks of age, 223.9 ± 2.2 g) were used in this study. The animals were housed in a temperature-controlled room at 23 °C on a 12-hour light-dark cycle, and were given free access to standard rat food and water. The protocols of this study were approved by the Animal Experiment Committee of the Prefectural University of Hiroshima (no.12MA009).

The rats were randomly divided into three groups of six rats each: the control group, the level group, and the downhill group. The level group ran on the treadmill on a 0° incline. The downhill group ran on the treadmill on a −16° incline. The two exercise groups ran on the treadmill at 16 m/min for 90 minutes, once every three days for a total of twenty sessions. A treadmill made for rats and mice (Exer-3/6, Columbus Co., Ltd.) was used. In general, the resistance training period required for muscle hypertrophy is about eight weeks11–13). The control group performed no exercise for 60 days. The experimental groups performed treadmill exercise once every three days for 60 days.

On the 60th day after the start of the experiment, all the rats were sacrificed by excessive inhalation of diethyl ether. The rats were weighed, then the left soleus muscle was excised. The wet weight of the soleus was measured with a precise balance scale. The relative weight ratio of the muscle was calculated as the ratio of the wet weight of the soleus muscle divided by the body weight at the end of the experiment. The excised soleus muscles were quick-frozen and cooled by dry ice in acetone. The frozen muscles were sliced into 10-µm-thick sections using a cryostat microtome and stained with hematoxylin and eosin (HE staining). For histological analysis, all sections were observed under a light microscope to exclude abnormal findings. In addition, all the sections were photographed using a digital camera for microscopy. The section images were transferred to a computer, and the cross-section minor axes of the muscles were measured according to the method reported by Brooke et al14).

The body weight, the muscle wet weight, the relative weight ratio of the muscle, and the cross-section minor axes of the muscle were analyzed using one-way analysis of variance (ANOVA). When a significant difference was present, a multiple comparison test was performed using Tukey’s test. The analyses were performed using the SPSS version 20.0 (SPSS Inc.). Significance was accepted at p<0.05. Values are presented as mean ± SEM.

RESULTS

Body weight at the beginning of the experiment was 223.8±1.9 g in the control group, 223.8±2.9 g in the level group, and 224.2±2.1 g in the downhill group. Body weight at the end of the experiment was 321.5±8.0 g in the control group, 333.8±9.0 g in the level group, and 327.3±3.3 g in the downhill group. There were no differences among the body weights of the three groups at the beginning and at the end of the experiment.

The results for the muscle wet weight, relative weight ratio, and fiber cross-section minor axes are shown in Table 1. The muscle wet weights and the relative weight ratios of the downhill group were significantly larger than those of the control and level groups (p<0.01). There were no differences in the muscle wet weights and the relative weight ratios between the control group and level group. The muscle fiber cross-section minor axes of the downhill group were significantly longer than those of the control and level groups (p<0.01). There was no difference in the muscle fiber cross-section minor axes between the control group and level group. Histologically, no abnormal findings, such as fiber necrosis and inflammatory cell infiltration, were noted in the muscles of any of the groups (Fig. 1).

DISCUSSION

As shown in Table 1, the muscle wet weights and relative weight ratios were not significantly different between the level and control groups. In this experiment, the running speed corresponded to about 50 to 60% of the lactate

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Table 1. Muscle wet weights, relative weight ratios and fiber cross-section minor axes (mean±SEM)

| Group          | Muscle wet weight (mg) | Relative weight ratio (mg/g body weight) | Fiber cross-section minor axis (µm) |
|----------------|------------------------|---------------------------------------|-----------------------------------|
| Control group  | 146.3 ± 6.1            | 0.46 ± 0.02                           | 50.0 ± 0.25                       |
| Level group    | 157.9 ± 4.6            | 0.47 ± 0.01                           | 49.6 ± 0.24                       |
| Downhill group | 178.4 ± 4.4 *†         | 0.55 ± 0.01 *†                        | 55.9 ± 0.24 *†                    |

* p < 0.01, Significant difference the control group.
† p < 0.01, Significant difference the level group.

Fig. 1. Histology of the rat soleus muscle (HE staining). Bar=50 µm. (a); Control, (b); Level, (c); Downhill
threshold in the rats\textsuperscript{(10)}, which could be described as a very low intensity exercise. Low intensity exercise effectively increases endurance capacity, but it does not effectively induce hypertrophy of the skeletal muscle\textsuperscript{(15)}. Therefore, the level group did not show a gain in hypertrophy of the soleus muscle. In contrast, the muscle wet weights, relative weight ratios, and muscle fiber cross-section minor axes of the downhill group were significantly larger than those of the control and level groups. These results indicate that a low intensity exercise using an eccentric contraction in downhill running may be an effective method of inducing hypertrophy of skeletal muscle.

The muscle blood flow decreases when a muscle contracts, and expressions of growth hormone and insulin-like growth factor-I increase\textsuperscript{(6, 17)}. Therefore, protein synthesis in the muscle may be stimulated\textsuperscript{(6, 17)}. Previous reports\textsuperscript{(5–7, 16, 17)} have shown that, compared to a concentric contraction, an eccentric contraction elicits a different response to the mechanical stress and metabolic response in the muscle. In an eccentric contraction, the activated muscle is forcibly lengthened, and the mechanical stress in the muscle created by an eccentric contraction is greater than that of a concentric contraction\textsuperscript{(5–7, 16, 17)}. For this reason, an eccentric contraction exercise produces greater muscle hypertrophy than a concentric contraction exercise\textsuperscript{(5)}. However, a high-intensity eccentric contraction is also known to cause an increase in muscle fiber damage, induce delayed-onset muscle soreness, and cause a temporary decrease in muscular strength\textsuperscript{(18)}. In the current study, no abnormal findings, such as fiber necrosis or inflammatory cell infiltration, were noted in the muscles of the level and downhill groups. The treadmill speed of our experiment, 16 m/min, provided a low-intensity exercise. Therefore, we consider that the mechanical stress experienced by the downhill group was sufficient to induce muscle hypertrophy without any over-stressing that might have produced muscle fiber damage.

It has been reported that in resistance training, training should be done two or three times per week\textsuperscript{(2–9, 19)}. Thus, we used this training frequency our exercise frequency in this experiment\textsuperscript{(2–9, 19)}. The results of exercising at this frequency and low intensity indicate that a low intensity exercise using an eccentric contraction is effective at increasing muscle mass. In addition, our findings suggest that a low intensity exercise using an eccentric contraction may be suitable for the treatment of the elderly, because low intensity exercise exerts less stress on cardiovascular function and articular structures\textsuperscript{(5, 20)}.

The mechanism of muscle hypertrophy induction through low intensity eccentric exercise is not clearly understood, and investigation using biochemical analysis is needed in future studies. Furthermore, an exercise protocol used for animals cannot always be directly applied to humans. Re-examination of the exercise protocol with regard to intensity and frequency is required before clinical application.

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