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Original Article

Aerobic interval exercise with an eccentric contraction induces muscular hypertrophy and augmentation of muscular strength in rats

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Abstract. [Purpose] The purpose of this study was to examine whether an aerobic interval exercise using an eccentric contraction would result in skeletal muscular hypertrophy and augmentation of muscular strength in rats. [Subjects and Methods] Twenty-one female Wistar rats were used in this study. The rats were randomly divided into three groups. The control group performed no exercise. The aerobic endurance exercise group ran for 90 min. The aerobic interval exercise group ran for a total of 90 minutes in 5 minute bouts separated by 2 minute rest periods. The exercise groups ran on a downhill treadmill incline, once every three days, for a total of twenty sessions. [Results] The muscle wet weights, the muscle fiber cross-section minor axes, and the tetanus tension results of the aerobic endurance and aerobic interval exercise groups were significantly larger than those of the control group. [Conclusion] These results indicate that aerobic interval exercise may be an effective method of inducing hypertrophy and augmenting muscular strength in skeletal muscle.
Key words: Aerobic interval exercise, Muscular hypertrophy, Augmentation of muscular strength

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INTRODUCTION

Decrease of muscle strength and muscle mass is caused by aging and decrease of physical activity[1, 2)]. The American College of Sports Medicine[3)] and the American Heart Association[4)] recommend that in resistance training, training should be done two to three times per week to prevent a decrease in muscle mass and muscle strength. To obtain augmentation of muscular strength and muscular hypertrophy, it has been reported[1–5)] that a training intensity of more than 60 to 70% of maximum strength is required. However, high intensity exercise exposes the elderly to high risk, because it imposes a high burden on articular and cardiovascular functions[1, 6, 7)]. Accordingly, the elderly often perform low intensity exercises for safety reasons[1)], even though it is not anticipated to increase muscle strength and mass. Therefore, we consider that it is necessary to develop a safe and effective low intensity training program for the elderly.

An eccentric contraction has been shown to produce greater augmentation of muscular strength and muscular hypertrophy than a concentric contraction after resistance training[8)]. Furthermore, an eccentric contraction exercise tends to exert less stress on the cardiovascular system[9)]. When eccentric contractions are used, lower-intensity training can be more effective than concentric contractions at augmenting muscular strength and eliciting muscular hypertrophy. We recently observed that an aerobic intensity exercise of 50% lactate threshold[10)] using an eccentric contraction increased soleus muscle mass in rats[11)]. We employed a method of eccentric exercise using downhill running, since, from the point of view of soleus muscle activity, downhill running can be considered a low intensity exercise[12)]. In our previous study[11)], the rats performed an endurance exercise for a long period, but elderly persons would most likely find it difficult to perform an endurance exercise for such a long time. Clinically, elderly and cardiovascular disease patients perform interval exercises when they are indicated not to perform endurance exercises[13–15)]. Interval exercise involves repeated short to long bouts of rather high intensity exercise interspersed with recovery periods (light exercise or rest)[16)]. The methods of interval exercise have been developed mainly in the field of sports medicine, but interval exercise may also be suitable for the training of the elderly, because it has rest periods between exercise periods. Therefore, the primary

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aim of this study was therefore to compare the effect of 8 weeks of endurance exercise using an eccentric contraction versus aerobic interval exercise using an eccentric contraction on the muscle strength and mass of rats. Our hypothesis was that an aerobic interval exercise would increase muscle strength and mass to the same degree as endurance exercise.

SUBJECTS AND METHODS

Twenty-one female Wistar rats (10 weeks of age, 223.5 ± 5.7 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 seven rats each: the control group, the aerobic endurance exercise group, and the aerobic interval exercise group. The control group performed no exercise for 60 days. Armstrong et al.[17] studied eccentric contractions in rats performing downhill running, a widely used method of eccentric contraction training for rat skeletal muscles[17–19]. In light of this, in the present study, the aerobic endurance exercise and aerobic interval exercise group ran on a treadmill with a −16° incline at 16 m/min. The aerobic endurance exercise group ran continuously for 90 minutes. The aerobic interval exercise group ran using an interval protocol for a total of 90 minutes: eighteen 5-minute bouts separated by 2 minutes rest. A treadmill made for rats and mice (Exer-3/6, Columbus Co., Ltd., USA) was used. In general, resistance training for muscle hypertrophy is performed for about eight weeks[20, 21]. The aerobic endurance exercise group and the aerobic interval exercise group performed treadmill exercises once every three days for 60 days (a total of twenty sessions). The aerobic endurance exercise load for the eccentric exercise used in our previous study[11] of muscle hypertrophy induction was used.

On the 60th day after the start of the experiment, all of the rats' body weights were measured, and then they were anesthetized with pentobarbital (50 mg/kg, ip). The left soleus muscle was excised. The wet weight of the soleus was measured on a precise balance scale. The excised soleus muscles were quick-frozen and cooled by dry ice in acetone. The right soleus muscle was excised along with the bony origin part, and mounted on a force recording device (MlTF-500/ST, AD Instruments Co., Ltd., USA). The muscle was placed vertically in a chamber filled with Ringer solution which was then oxygenated with a mixture of 95% O_2 and 5% CO_2, and the muscle was stretched to 110% of its resting length. The muscle was stimulated with a 1 msec square wave delivered by two parallel platinum electrodes using an electric stimulator (SEN-3401, NIHON KOHDEN Co., Ltd., Japan), and the peak twitch tension was measured. Tetanus tension was elicited for a 1 msec duration, 100 Hz, 1 sec train and with 130% peak twitch tension voltage. Peak twitch tension and tetanus tension data were recorded using analysis software (LabChart 7 Japanease, AD Instruments Co., Ltd., USA).

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 using image analysis software (NIH Image J), randomly targeting 300 fibers in each muscle sample. The cross-section minor axes of the muscles were measured according to the method reported by Brooke et al[22].

The body weight, muscle wet weight, cross-section minor axis of the muscle and the twitch tension were analyzed using one-way analysis of variance (ANOVA). When a significant difference was present, a multiple comparison test was performed using the Tukey test. The tetanus tension was analyzed using the Kruskal-Wallis test, and Scheffe's post-hoc test was then performed. Significance was accepted at $p<0.05$. Values are presented as mean ± SD.

RESULTS

Mean body weights at the beginning of the experiment were 224.1±4.3 g in the control group, 222.9±5.8 g in the aerobic endurance exercise group, and 223.4±7.5 g in the aerobic interval exercise group. Mean body weights at the end of the experiment were 322.9±18.2 g in the control group, 326.4±7.8 g in the aerobic endurance exercise group, and 331.9±11.3 g in the aerobic interval exercise 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 weights and fiber cross-section minor axes are shown in Table 1. The muscle wet weights of the aerobic endurance exercise and aerobic interval exercise groups were significantly larger than those of the control group (aerobic endurance exercise, $p<0.0001$; aerobic interval exercise, $p=0.007$). There was no significant difference between the muscle wet weights of the aerobic

Table 1. Results of muscle wet weights and fiber cross-section minor axes

Group	Muscle wet weight (mg)	Fiber cross-section minor axis (µm)
Control group	146.7 ± 13.6	50.0 ± 10.6
Aerobic endurance exercise group	177.4 ± 10.0**	55.5 ± 10.3**
Aerobic interval exercise group	168.1 ± 10.6**	54.9 ± 10.4**

**p < 0.01. Significant difference from the control group. Mean±SD endurance exercise and aerobic interval exercise groups. The muscle fiber cross-section minor axes of the aerobic endurance exercise and aerobic interval exercise groups were significantly wider than those of the control group (both, p<0.0001). There was no significant difference between the muscle fiber cross-section minor axes of the aerobic endurance exercise and aerobic interval exercise groups. Histologically, no abnormal findings, such as fiber necrosis or inflammatory cell infiltration, were noted in any of the muscles of the three groups (Fig. 1).

The results for twitch tension and tetanus tension are shown in Table 2. The twitch tension of the aerobic interval exercise group was significantly larger than that of the control group (p=0.016). The tetanus tension results of the aerobic endurance exercise and aerobic interval exercise groups were significantly larger than that of the control group (aerobic endurance exercise, p=0.036; aerobic interval exercise, p=0.016). There was no significant difference between the tetanus tension results of the aerobic endurance exercise and aerobic interval exercise groups.

DISCUSSION

The current study investigated whether an aerobic interval exercise using an eccentric contraction in downhill running would increase soleus muscle strength and mass to the same degree as an aerobic endurance exercise. The muscle wet weights and fiber cross-section minor axes of the aerobic endurance exercise group were significantly larger than those of the control group. The aerobic endurance exercise group performed the exercise at the same load as in our previous study[11] and ran continuously on a treadmill with a −16°incline at 16 m/min for 90 minutes. The results of this study are similar to those of our previous study[11], reconfirming that an aerobic endurance exercise using an eccentric contraction in downhill running induces hypertrophy of the soleus muscle. The tetanus tension of the aerobic endurance exercise group was significantly greater than that of the control group. Therefore, the aerobic endurance exercise using an eccentric contraction in downhill running induced muscle hypertrophy, and, moreover, it also resulted in increased muscle strength.

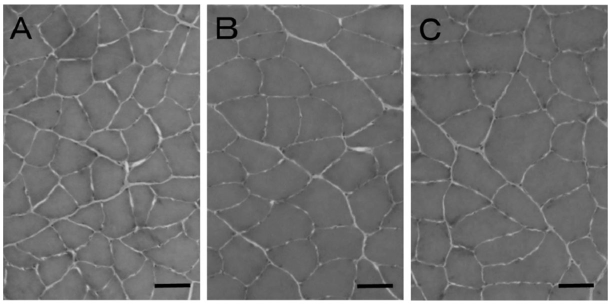

Fig. 1. Histology of the soleus muscle (HE staining)
Bar=50 µm.
Control (A), Aerobic endurance exercise (B), Aerobic interval exercise (C)

The muscle wet weights and fiber cross-section minor axes of the aerobic interval exercise group were significantly larger than those of the control group. There were no differences in the muscle wet weights, fiber cross-section minor axes, and tetanus tension results between the aerobic endurance exercise group and the aerobic interval exercise group. The results of this study show that an aerobic interval exercise using an eccentric contraction in downhill running increased soleus muscle strength and mass to the same degree as an aerobic endurance exercise.

Muscle blood flow decreases when a muscle contracts, and expressions of growth hormone and insulin-like growth factor-1 increase[23, 24], conditions which are favorable for protein synthesis 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[8, 17, 23, 24]. Armstrong et al.[17] and Schewane et al.[25] examined whether the rats skeletal muscle injury and plasma creatine phosphokinase of rats were increased by running on a treadmill at 16 m/min for 90 minutes with a change in incline for one session. Both studies reported that inflammatory muscle cell and plasma creatine phosphokinase increased after 48 to 72 hours when the rats ran downhill. In our study, no abnormal findings, such as fiber necrosis or inflammatory cell infiltration, were noted in the muscles of the endurance exercise group, which performed downhill running for 20 sessions using the exercise load reported by Armstrong et al[17]. We postulated that soleus muscle damage might occur in the initial stage of downhill running, as Armstrong et al.[17] and Schewane et al.[25] reported. However, it seems that excessive soleus muscle fiber damage did not occur, because the rats did not stop running, even though some slight damage that did not interfere with the rats' performance may have occurred. Moreover, the eccentric contraction training effect is the result of an increase in the number of sarcomeres in the muscle fibers, which allows the muscle fibers to operate at longer lengths[26, 27].

The present study showed that augmentation of muscular strength and muscular hypertrophy occurred in the rat

Table 2. Results of twitch tension and tetanus tension

Group	Twitch tension (g)	Tetanus tension (g)
Control group	11.4 ± 1.86	75.0 (62.9–81.5)
Aerobic endurance exercise group	13.4 ± 1.90	82.8 (70.8–85.3)*
Aerobic interval exercise group	14.1 ± 1.03*	81.4 (78.4–89.7)*

*p < 0.05. Significant difference from the control group. Mean±SD or median (minimum–maximum) soleus muscle through muscle fiber adaptations elicited by 20 sessions of eccentric contraction exercise. Paschalis et al.[28] showed that an equal volume of high-intensity and low-intensity eccentric exercise increased plasma creatine kinase after exercise. In addition, high-intensity eccentric exercise resulted in a greater decline of peak torque than low-intensity eccentric exercise. These findings suggest that a low-intensity eccentric exercise may be suitable for performing long-term exercises for augmentation of muscular strength and muscular hypertrophy.

Interval exercise effectively increases cardiovascular function, metabolic function, muscle strength and physical performance[13–16, 29]. In general, the protocol of interval exercise involves repeated short bouts of rather high-intensity exercise interspersed with recovery periods. However, interval exercise exposes the elderly to high risk, because it imposes a high burden on articular and cardiovascular functions by repetition of high-intensity exercise. The running speed of our study corresponded to about 50% of the lactate threshold[10], which could be described as a very low intensity exercise. We consider that an aerobic interval exercise using an eccentric contraction is effective at increasing muscle strength and mass by the same degree as an aerobic endurance exercise, even though rest periods are inserted between the exercise periods. In addition, our findings suggest that an aerobic interval exercise using an eccentric contraction may be suitable and safe for the treatment of the elderly, because low intensity exercise most likely exerts less stress on cardiovascular function and articular structures.

In this study, the eccentric contraction exercise performed by rats was downhill running. If this method were used for human subjects, there is a possibility that falls and injury would occur when running downhill on treadmill. In future studies, we will attempt to determine effective training methods for increasing muscle strength and mass via aerobic interval exercise using eccentric contraction.

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