Effect of exercise and heat stress before hindlimb suspension on prevention of the skeletal muscle atrophy

Hiromitsu Tasaki,[1,2] Takako Matsubara,[3] Akinori Miki,[4]

Effect of exercise and heat stress applied before hindlimb suspension on prevention of muscle atrophy was examined in the rat soleus muscle. Male Wistar rats were randomly divided into 4 groups: 1) control (C), 2) hindlimb suspension for 2 weeks (HS), 3) exercise before HS (Ex-HS), and 4) heat stress before HS (Heat-HS). Before hindlimb suspension, the rats in the Ex-HS were exercised by running on an incline treadmill, and those in the Heat-HS were placed 4 times in a week, in a heat chamber. After 2-week hindlimb suspension, the soleus muscles were isolated and their wet weight was examined. Cross sections of each muscle were cut in and stained for ATPase to distinguish fiber types, and alkaline phosphatase to visualize the capillaries. Muscle wet weight, fiber diameter, ratio of type I fiber, and capillary-to-fiber (C/F) ratio in the HS were significantly smaller than the C. The muscle wet weight, diameter and ration of type I fiber and C/F ratio in the Ex-HS and heat-HS were significantly larger than the HS. These findings suggested that the exercise and heat stress applied as pre-emptive countermeasures have considerable benefits to inhibit muscle atrophy and regression alterations.

Key words

muscle atrophy; pre-emptive treatment; hindlimb suspension; muscle fiber; capillary

Introduction

It has been well known that mechanical unloading on the skeletal muscle results in muscle atrophy. In human beings, bed rest and non-weight bearing lead to rapid loss of skeletal muscle mass, size and function. These changes were also reported in an animal model by hindlimb suspension or cast fixation in numerous literatures. It was shown that the ratio of type I fiber decreased after 1-week suspension (1), and Capillary-to-fiber (C/F) ratio decreased in the atrophied muscle (2). Therefore, many different countermeasures to skeletal muscle atrophy have been studied extensively for repair of muscle atrophy following unloading and/or immobilization. Actually, it is confirmed that the loss of skeletal muscle mass, size and function are attenuated by various treatments, e.g., mechanical loading, heat stress and stretching. These additional treatments imposed on skeletal muscle

1 Graduate school of Health Sciences, Kobe University
2 Department of Rehabilitation Science, Physical Therapy, Faculty of Human Health, Nagoya Gakuin University
3 Department of Social Healthcare and Business Management, Faculty of Healthcare Management, Nihon Fukushi University
4 Faculty of Health Sciences, Kobe University School of Medicine

can induce adaptations in their size and phenotype and capillary supply.

The prevention of muscle atrophy is very important for inhibition of dysfunction progress to keep activities in daily living. Thomason et al. (3, 4) reported that myofibril protein synthesis in soleus muscle begins to decrease within the first five hours of unloading, and then increases in protein degradation. Thus, it is necessary to immediately perform countermeasures to muscle atrophy. Naito et al. (5) demonstrated that heat stress before hindlimb unweighting can reduce the rate of disused muscle atrophy. However, the effect of treatment applied before unloading to prevent the muscle atrophy has not yet been examined. Moreover, any countermeasure that completely prevents the muscle atrophy has not yet been developed. In the present study, therefore, the effect of exercise training and heat stress before hindlimb suspension on prevention of the skeletal muscle atrophy was examined in the rat soleus muscle.

Materials and Methods

Animals

Twenty-eight male Wistar rats weighting 225-310g (8 weeks old) at beginning of the experiments were randomly divided into control (C; n=7) and experimental groups. In the experimental group, the rats were further divided into three groups: hindlimb suspension for 2 weeks (HS; n=7), exercise before hindlimb suspension (Ex-HS, n=7) and heat stress before hindlimb suspension (Heat-HS; n=7). The rats in the C group were not imposed to any stimulation or suspension. These rats were housed individually in conventional plastic cages and given pellet food and water ad libitum. They were acclimatized at a room temperature of 22℃ with a 12:12-h light-dark cycle for 1 week before the experiments. The maintenance and condition of the animals and the experiments received authorization from the Ethic Reviews Committee for Animal Experimentation in our institute.

Experimental protocol

The experimental groups were subjected to tail-suspension for 2 weeks, according to the Morey technique (6, 7). The rats tails where wrapped in an antiallergic orthopedic tape and secured to an overhead swivel that mounted at the top of the cage and permitted free 360° rotation of the animal. The rats were unloaded at -30° head-down angle to mimic fluid shifts characteristic of weightlessness.

In the Ex-HS group, the rats performed uphill running on a treadmill (MK-680S, MUROMACHI KIKAI, Tokyo, Japan) set to an elevation of 20 degrees at 20 m/min for 25 min before hindlimb suspension. In the Heat-HS group, the rats were placed in an environmentally controlled heat chamber (STAC-P-450F, SHIMADZU, Tokyo, Japan) at a temperature of 42.0 degrees for 60 min, 4 times for a week before the hindlimb suspension. During the heat exposure, their colonic and soleus muscle temperature was recorded with a calibrated thermistor probe. Immediately after the heat exposure, the rats were quickly returned to a cage in a 22℃ climate-controlled room and given water and rat chow ad libitum.

At the end of 2-week hindlimb suspension period, the animals of the experimental groups and the C group at the corresponding age were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), right soleus muscles were extracted, and their wet weight was examined.

Morphological examinations

Each muscle was immediately mounted in tragacanth gum and frozen at -160℃ in isopentane cooled in liquid nitrogen. Cross sections 7 μm in thickness were cut at the midbelly of the muscles in a Cryostat microtome (CM1850, Leica, Germany) at -20℃. For classification of fibers by ATPase activity (following pre-incubation at pH10.8), type I fibers

appeared light, type II fibers appeared dark (Fig.1). To visualize the distribution of capillaries, sections were stained for alkaline phosphatase by using an indoxyl tetrazolium method (Fig.2).

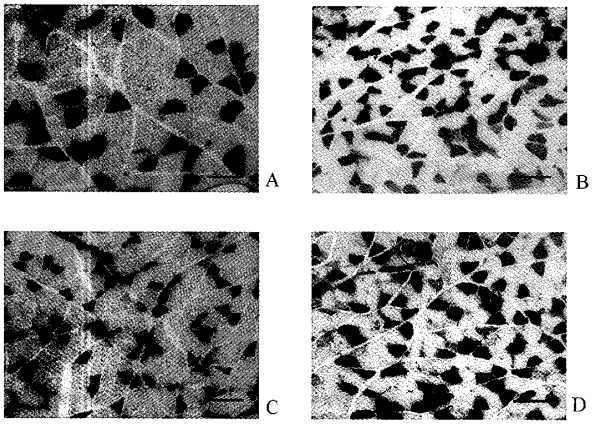

Fig. 1. Cross section of rat soleus muscles, stained for myosin adenosinetriphosphatase, after pre-incubation at pH10.8.
Type I fibers appeared light, type II fibers appeared dark. A: control rats, B: hindlimb suspension rats, C: exercise before hindlimb suspension rats, D: heat stress before hindlimb suspension rats. Bar=100 μ m.

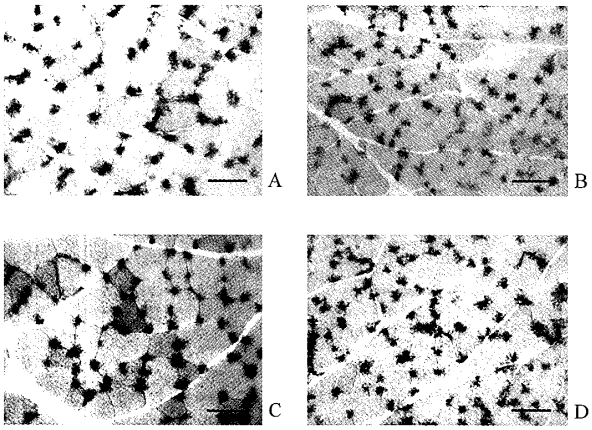

Fig. 2. Cross section of rat soleus muscles, stained for alkaline phosphatase to depict capillaries. Capillaries were appeared as dark blue circles or lines. A: control rats, B: hindlimb suspension rats, C: exercise before hindlimb suspension rats, D: heat stress before hindlimb suspension rats. Bar=50 μ m.

Statistical analysis

Fiber type ratio and short diameter fibers were individually traced in randomly selected areas of the ATPase stained sections at least 200 muscle fibers from each soleus muscle. The incidence of fiber type was expressed as the number of each fiber type relative to the total number of fibers. The capillary-to-fiber (C/F) ratio was determined by dividing the number of capillaries by the number of fibers. The quantitative data was analyzed with a personal computer (PowerMac G4, Apple computer) and analysis software (NIH Image 1.62, NIH, Bethesda, MD), and presented as means ±standard error of each group. The relative difference of fiber diameter, distribution of fiber type and C/F ratio among all groups were analyzed with a one-way analysis of variance (ANOVA). If a significant F ratio was noted, a Fisher's (least significant difference) test was carried out post hoc. Statistical significance was accepted at $p < 0.05$.

Results

Colonic and soleus muscle temperature

Before the expose to heat stress, the colonic and soleus muscle temperature was 36℃ and 33.7℃. On exposure to heat stress, colonic and muscle temperature gradually increased over time, reaching peak temperature of 42.3℃ and 41.8℃ at the end of heating period in Fig.3.

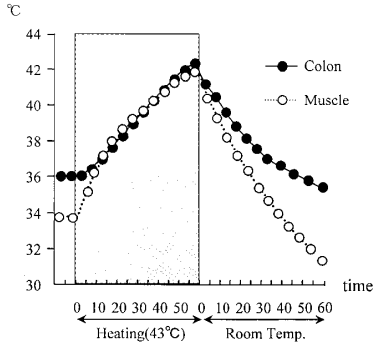

Fig. 3. Colonic and muscle temperature of rats exposed to heat stress.

Muscle wet weight

Changes in wet weight of the soleus muscle are presented in Table 1. The weight in the HS, Ex-HS and Heat-HS groups after 2-week hindlimb suspension significantly decreased to 82%, 85% and 87% respectively, in comparison with the C group (statistically significant at $p < 0.05$). While the wet weight loss in the Ex-HS and Heat-HS groups was 8.9% and 9.5% less than in the HS group ($p < 0.05$).

	C	HS	Ex-HS	Heat-HS
B.W(g)	333±6	274±7*	284±9*	292±3*
M.W.W(mg)	168±5	112±4*	127±9*	128±7*

Table. 1. Body Weight (B.W) and Muscle Wet Weight (mg).
Values are means±SE.
* ; significant difference from C ($p < 0.05$).

Ratio of fiber type

Changes in the fiber type ratio after 2-week hindlimb suspension are presented in Fig. 4. The ratio of type I in the HS group was significantly less than the C group ($p < 0.05$). But, statistically there was no difference in the ratio of type I fibers among the Ex-HS, Heat-HS and C groups.

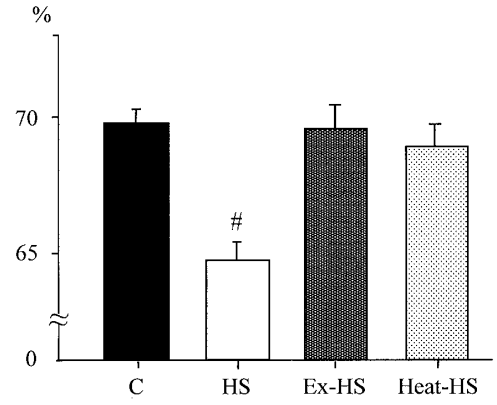

Fig. 4. Percentage distribution of type I fiber in soleus muscle.
Values are means±SE.
#; Significant different from C ($p < 0.05$).

Muscle fiber diameter

Changes in the muscle fiber diameter after 2-week hindlimb suspension are presented in Fig. 5. Diameter of type I and type II muscle fibers in the HS, Ex-HS and Heat-HS groups were significantly smaller than in the C group ($p < 0.05$). As to the type I fibers, the diameter in the Ex-HS and Heat-HS groups was significantly larger than in the HS group. In contrast, as to the type II fibers, although the diameter in the Heat-HS groups was larger than in the HS group, there was no difference between the Ex-HS and HS groups.

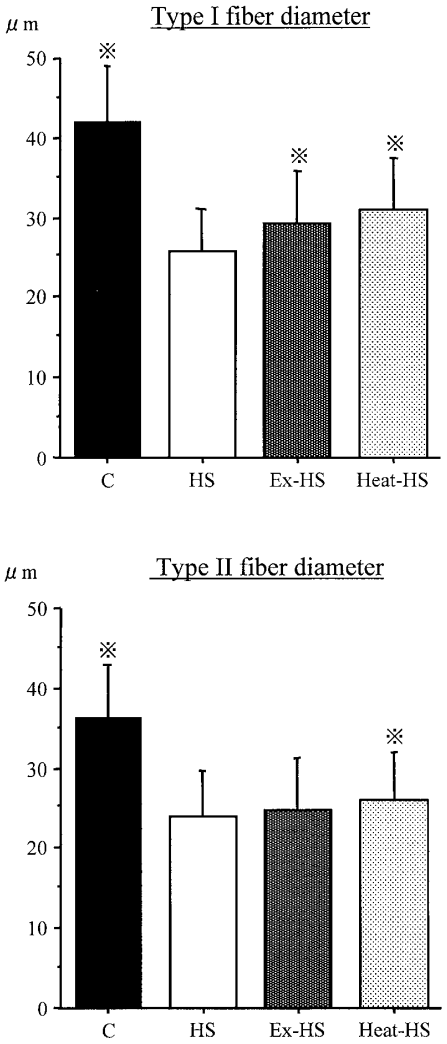

Fig. 5. The fiber diameters in soleus muscle.
Values are means±SE.
※; Significant different from HS ($p < 0.05$).

Capillary-to-Fiber (C/F) ratio

Changes in the C/F ratio after 2-week hindlimb suspension are presented in Fig. 6. Though the C/F ratio in the HS and Ex-HS groups was significantly smaller than in the C group, the C/F ratio in the Ex-HS group was larger than in the HS group. There was no difference in the ratio between the Heat-HS and C groups.

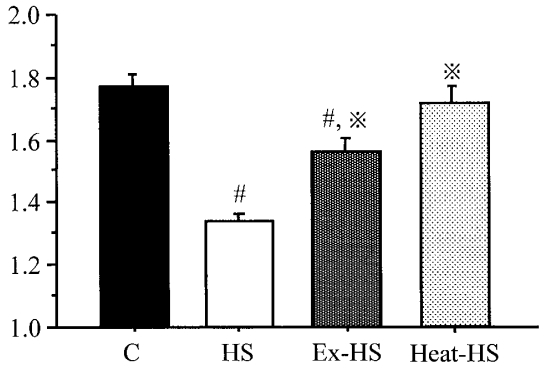

Fig. 6. Capillary/Fiber ratio in soleus muscle.
Values are means±SE.
#; Significant different from C ($p < 0.05$).
※; Significant different from HS ($p < 0.05$).

Discussion

In the present study, the effect of the running exercise and heat stress applied before hindlimb suspension on muscle atrophy was morphologically examined in the rat soleus muscle. It is well known that unloading induces several phenomena of muscle atrophy, such as decrease of muscle wet weight, fiber size and ratio of type I fibers (1, 2, 4, 8, 9, 10, 11). In the present study, muscle wet weight of the soleus muscle decreased 33.4% after 2-week hindlimb suspension, and the diameter of type I and type II fibers decreased 38.9% and 33.7%, respectively. It was reported that the loss of myofibril protein was due to both decreased myofibril protein synthesis and increased myofibril protein degradation (3, 4). In the present study, the ratio of type I fiber decreased from 78.8% to 63.7% after 2-week hindlimb suspension. Desplanches et al. (1) reported that ratio of type I fibers in the rat soleus muscle decreased by 13% after 1-week suspension, and Leterme et al. (12) reported that it decreased by 19% after 2-week suspension. Therefore, our results were consistent with previous studies. Histochemically, muscle fiber type is divided into 2 types, type I and type II. The type II is further divided into three subtypes, namely type IIa, IIb and IIc. In the unloading muscle, the percentage of slow isoenzyme reduced, the intermediate isoenzyme increased, but the fast isoenzyme unchanged (1). This might be one of the reasons why slow fibers (type I) decrease during unloading (10). In the present study, decrease in ratio and size of type I fibers were noted in the atrophied soleus muscle after hindlimb suspension.

It has been reported that the density of capillary in the muscle closely correlate with the oxidative capacity of the muscle (13), thus the C/F ratio has been widely used to estimate the oxidative supply capacity (14). It was demonstrated that C/F ratio decreased in unloading muscles (2) and increased after exercise in the atrophied muscle (15, 16). Displanches et al. (17) showed that in the severely atrophied rat soleus muscle after 5-week hindlimb suspension, the C/F ratio reduced 37% in comparison with the normal rat soleus muscle. In our data, 2-week hindlimb suspension resulted in a reduced 36% in the C/F ratio. It has been reported that since the oxygen supply of muscles depends largely on the density of vascular network, regression of capillaries means the decrease of the blood flow and oxygen demand after unloading (18). Considering above mentioned facts, the results of the present study suggested that the oxygen demand decreased after unloading due to disuse as well as to muscle atrophy.

Thomason et al. (3) described that the myofibril protein synthesis began to decrease within the first 5 hours of unloading, and deg-

radation of the protein began to increase 48 hours after the beginning of unloading and reached a peak on the 15th day of unloading. This finding suggests that some suitable countermeasures are necessary to prevent muscle atrophy as early as possible. Although, various countermeasures have been applied during unloading and/or immobilization to attenuate muscle atrophy, sufficient effects have not yet been reported. Kirby et al. (19) reported that eccentric exercise for only 0.035% of the total hindlimb suspension time period inhibited the loss of soleus muscle protein content. Similarly, it has been indicated that heat stress for only 0.005% of the total hindlimb suspension time period attenuated the loss of soleus muscle protein and heat shock protein content (5). Therefore, in the present study, effects of running exercise and heat stress applied before the unloading as a countermeasure was morphologically examined. In our preliminary study, a single bout of heat stress had no effect on atrophic muscle, but in the present study, four times heat stress (for only 0.012% of the total suspension time period) and a single bout of running exercise (for only 0.0012% of the total suspension time period) before hindlimb suspension reduced the atrophic changes occurring in the atrophied muscles. According to our data, the reduction of muscle wet weight in the Ex-HS and Heat-HS was 8.9% and 9.5% less than in the HS. The ratio and diameter of type I fibers and C/F ratio in the Ex-HS and Heat-HS were significantly larger than in the HS. These results suggested that the exercise and heat stress applied before the hindlimb suspension had considerable benefits as countermeasures for muscle atrophy, even if the treatment period was very short. The atrophic alterations in the muscle might be caused by 1) reduction of myofibril protein contents; 2) apoptosis of the muscle fiber and capillary; and 3) reduction of blood supply. It was reported that the heat stress could elevate the heat shock protein (e.g., HSP72) which plays some roles on protecting the striated muscle against a variety of insults, including as a chaperone of nascent peptides during translation (20), and the higher cellular level of HSP72 induced by heat stress could reduce the rate of proteolysis during unweighting (5). It was also suggested that mechanical stress, e.g., running exercise increases not only the content of HSP70 in skeletal muscle (21), but also insulin-like growth factor (IGF) which is important for the maintenance and growth of skeletal muscle. Although recent data demonstrated that the unloading caused apoptotic change to the muscle fibers and capillaries, both of HSP72 and IGF-I play an important role to protect cell death and inhibit the apoptotic change of muscle fibers and capillaries induces by unloading. According to these findings, there are possibilities that the treatment in present study restrained probably through the production of HSP and IGF 1) reduction of myofibril protein contents; 2) apoptosis of the muscle fiber and capillary. Additionally, the adequate restitution of blood supply to the disused muscle was a prerequisite for normalization of the muscle fiber, their size and internal structure, and it is reported that C/F ratio increased after the exercise for muscle atrophy (15, 16). Therefore, as a result of the treatment in the present study, capillary remodeling was likely to occur for adequate blood supply. However, we cannot confirm the mechanism responsible for the observed protection of heat stress and exercise training against muscle atrophy during hindlimb suspension. Thus, further research is needed.

The present study showed that running exercise and heat stress applied before the hindlimb suspension, even though for a short time could considerably reduce atrophic changes, such as decrease of wet weight, diameter of type I fiber, distribution of type I fiber and C/F ratio, which occur during unloading in the soleus muscle. The results suggested that these pre-emptive countermea-

sures might be useful to reduce the muscle atrophy and regression alteration, if they were applied before cast fixation, non-weight bearing and at early stages of bed rest. To date, the mechanism and effective site of heat stress and running exercise applied before the unloading are far from clear.

Grants

This study was supported in part by Grants-in-Aid for Young Scientists (16700432) and Research Grants from Nagoya Gakuin University.

References

1. Desplanches D, Mayet MH, Sempore B, Flandrois. Structural and functional responses to prolonged hindlimb suspension in rat muscle. J Appl Physiol 63(2): 558-563, 1987.
2. Tyml K, Mathieu-Costello O, Cheng L, et al. Differential microvascular response to disuse in rat hindlimb skeletal muscles. J Appl physiol 87: 1496-1505, 1999.
3. Thomason DB, Booth FW. Atrophy of the soleus muscle by hindlimb unweighting. J Appl Physiol 68(1): 1-12, 1990.
4. Thomason DB, Biggs RB, Booth FW. Protein metabolism and β-myosin heavy-chain mRNA in unweighted soleus muscle. Am J Physiol Integrative Comp Physiol 257: R300-R305, 1989.
5. Naito H, Powers SK, Demirel HA, et al. Heat stress attenuates skeletal muscle atrophy in hindlimb-unweighted rats. J Appl Physiol 88: 359-363, 2000.
6. Morey ER. Spaceflight and bone turnover: correlation with a new rat model of weightlessness. Bioscience 29: 168-172, 1979.
7. Morey ER, Sabelman EE, Turner RT, et al. A new rat model simulating some aspects of flight. Physiologist 22: S23-S24, 1979.
8. McNulty AL, Otto AJ, Kasper CE, et al. Effect of recovery mode following hindlimb suspension on soleus muscle composition in the rat. Int J Sports Med 13(1): 6-14, 1992.
9. Brown M, Hasser EM. Weight-bearing effects on skeletal muscle during and after simulated bed rest. Arch Phys Med Rehabil 76: 541-546,1995.
10. Templeton GH, Sweeney HL, Timson BF, et al. Changes in fiber composition of soleus muscle during rat hindlimb suspension. J Appl Physiol 65(3): 1191-1195, 1988.
11. Falempin M, Soumeya F. Influence of brief daily tendon vibration on rat soleus muscle in non-weight-bearing situation. J Appl Physiol 87(1): 3-9, 1999.
12. Leterme D, Cordonnier C, Mounier Y, et al. Influence of chronic stretching upon rat soleus muscle during non-weight-bearing condition. Eur J Physiol 429: 274-279, 1994.
13. Anderson P, Henriksson J. Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. J Physiol 270: 677-690, 1977.
14. Schmidt-Nielsen K, Pennycuik P. Capillary density in mammals in relation to body size and oxygen consumption. Am J Physiol 200: 746-750, 1961.
15. Kannus P, Laszlo J, Teppo LNJ, et al. Free mobilization and low- to high-intensity exercise in immobilization-induced muscle atrophy. J Appl Physiol 84(4): 1418-1424, 1998.
16. Mathieu-Costello O, Ellis CG, Potter RF, et al. Muscle capillary-to-fiber perimeter ratio: morphometry. Am J Physiol Heart Circ Physiol 261: H1617-H1625, 1991.

17. Displanches D, Kayar SR, Sempore B, et al. Rat soleus muscle ultrastructure after hindlimb suspension. J Appl Physiol 69: 504-508, 1990.
18. Fujino H, Kohzuki H, Takeda I, et al. Regression of capillary in atrophied soleus muscle induced by hindlimb unloading. J Appl Physiol 98: 1407-1413, 2005.
19. Kirby CR, Ryan JR, Booth FW. Eccentric exercise training as a countermeasure to non-weight-bearing soleus muscle atrophy. J Appl Physiol 73(5): 1894-1899, 1992.
20. Ku Z, Yang J, Menon V, et.al. Decreased polysomal HSP-70 may slow polypeptide elongation during skeletal muscle atrophy. Am J Physiol Cell Physiol 268: C1369-C1374, 1995.
21. Milne KJ, Noble EG. Exercise-induced elevation of HSP70 is intensity dependent. J Appl Physiol 93: 561-568, 2002.