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Original Article

Influence of preliminary exercise training on muscle damage indices in rats after one bout of prolonged treadmill exercise

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Abstract. [Purpose] The purpose of this study was to investigate the effects of exercise on muscle damage indices in male Sprague-Dawley rats. Two groups of rats were trained in either moderate- or high-intensity treadmill running for 4 weeks. Subsequently, the concentrations of creatine kinase, lactate dehydrogenase, and high-sensitivity C-reactive protein were examined following a single bout of prolonged (3-h) intensive exercise. [Subjects and Methods] The study included forty 6-week-old male Sprague-Dawley rats weighing 150–180 g each. The aerobic exercise group was divided into high-intensity (28 m/min) and moderate-intensity (15 m/min) subgroups. Both subgroups were trained for 35 min daily for 6 days per week (excluding Sunday) over a 4-week period. Following training, the high- and moderate-intensity exercise groups and a non-exercise group performed one bout of prolonged (3-h) treadmill exercise for 3 hours at a speed of 15 m/min. [Results] Creatine kinase and lactate dehydrogenase levels differed significantly among the groups. [Conclusion] The preliminary exercise groups showed lower muscle damage and inflammatory response levels than the non-exercise group after the bout of prolonged intensive exercise.
Key words: Muscle damage, Treadmill exercise, Preliminary exercise

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INTRODUCTION

Muscle fatigue in human performance is defined as any exercise-induced decrease in maximal voluntary force or power produced by a muscle or muscle group[1)]. Muscle fatigue is disruptive to exercise since it causes dysfunction. More moderate-intensity exercise is recommended to enhance aerobic capacity[2)]. Nevertheless, the beneficial effects of training could be obtained by exercising at higher intensity and adapting this exercise stimulus. Thus, physical adaptation to fatigue is important to the enhancement of aerobic capacity.

After their first session of moderate-intensity exercise, individuals often experience muscle soreness because they do not have sufficient time to adapt to various factors such as increased mitochondrial activity leading to malondialdehyde (MDA) production from lipid peroxidation of the plasma membrane[3, 4)]. This phenomenon can cause muscle damage, leakage of creatine kinase (CK)[4, 5)] into the circulation, and inflammation[6, 7)].

Exhaustive and/or unfamiliar exercises (particularly those involving high-intensity muscle contractions) are known to induce temporary muscle damage. This damage consists of a series of events: damage to the sarcolemma and the muscle cell membrane; and the release of biochemical markers of muscle damage, including lactate dehydrogenase (LDH), CK, and myoglobin (Mb)[8, 9)].

CK and LDH are the metabolites produced within the human body during exercise, and an increase in exercise intensity and duration is known to result in a substantial corresponding increase in cardiac and skeletal muscle activity. The serum CK

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level is the most important indicator of muscle tissue damage[10], while the LDH level is a specific indicator of fatigue[11, 12]. In addition, the level of hs-CRP, an acute inflammatory marker, increases rapidly after exercise[13]. In physical tissue damage and other inflammatory conditions, hs-CRP is the main acute-phase protein and a very sensitive and objective indicator[14].

The purpose of this study was to investigate differences in the concentrations of CK, LDH, and hs-CRP in male Sprague-Dawley rats during one bout of prolonged (3-h) treadmill exercise following different training/non-training regimens.

SUBJECTS AND METHODS

Forty male Sprague-Dawley rats (age 6 weeks; weighing 150–180 g each) were used in this study. All animals were housed (three rats per cage) at 23 ± 2 °C and 50 ± 5% humidity with a 12-hour light/dark cycle. The rats were given a 7-day period for adapting to the environment before the experiment. Pellet feed and water were periodically provided during the experimental period. The rats were randomly divided into the following four groups (n=10 each): control, high-intensity exercise, moderate-intensity exercise, and non-exercise. This study was approved by the Institution of Animal Care and Use Committee of Daegu University, and the experimental procedure complied with the management guidelines for experimental animals.

The exercise intensity levels used in this study were set on the basis of the results of a study by Shepherd and Gollnick (1976) in which the maximal oxygen uptakes of white Sprague-Dawley rats were measured using a metabolic rate-measuring instrument and a treadmill. As a result, the levels were set at 15 m/min, i.e., approximately 65% of the maximal oxygen uptake; and 28 m/min, based on the reference data that defined the relevant velocity as being approximately 82% of the maximal oxygen uptake[15]. This study's aerobic exercise groups first performed a preliminary aerobic adjustment exercise for 20 min daily for 4 days and then performed their main treadmill training for 35 min/day, 6 days per week (except Sunday) for 4 weeks. The high- and moderate-intensity exercise groups maintained intensity levels of 28 m/min and 15 m/min, respectively. After the training, the high- and moderate-intensity exercise groups and the non-exercise group simultaneously performed one bout of prolonged treadmill exercise at 15 m/min for 3 h. Following this bout of prolonged treadmill exercise, the animals were weighed; they were anesthetized using ether as an inhalation anesthetic; the abdominal cavity was dissected immediately, and 10 ml of blood was collected from the main artery using a syringe. The collected blood was put into an Eppendorf tube and centrifuged at 18,000 rpm at 4 °C for 18 min. The collected sera were stored in a freezer at −70 °C prior to analysis. CK, LDH, and hs-CRP were measured using a TSA-200FR NEO (Toshiba, Japan), CK and LDH were measured using an enzyme method, and hs-CRP was measured using immunoturbidimetry.

One-way analysis of variance was performed using PASW (version 18.0 for Windows) to investigate intergroup differences. The Bonferroni post-hoc test was used to perform the post-hoc comparisons. The significance level was set at 0.05.

RESULTS

Table 1 presents the weights of the rats measured for each exercise group after one bout of prolonged treadmill exercise. CK levels differed significantly among the groups: 97.3 ± 18.5 IU/l for the control group, 344.6 ± 52.8 IU/l for the high-intensity exercise group, 430.2 ± 80.2 IU/l for the moderate-intensity group, and 713.9 ± 123.4 IU/l for the non-exercise group ($p<0.05$). The post-hoc analysis showed that the high- and moderate-intensity exercise groups as well as the non-exercise group had significantly higher CK levels than the control group ($p<0.05$), while the high- and moderate-intensity exercise groups had significantly lower CK levels than the non-exercise group ($p<0.05$) (Table 1).

LDH levels differed significantly among the groups: 172.3 ± 33.6 IU/l for the control group, 282.4 ± 73.7 IU/l for the high-intensity exercise group, 478.2 ± 160.01 IU/l for the moderate-intensity exercise group, and 661.5 ± 115.2 IU/l for the non-exercise group ($p<0.05$). The post-hoc analysis showed that the moderate-intensity exercise group and the non-exercise group had significantly higher LDH levels than the control group ($p<0.05$), while the high- and moderate-intensity exercise groups had significantly lower LDH levels than the non-exercise group ($p<0.05$) (Table 1).

Following moderate- and high-intensity aerobic exercise, the high-intensity exercise, moderate-intensity exercise, and non-exercise groups showed significantly higher hs-CRP levels (0.02 ± 0.00 mg/dl) than the control group (0.01 ± 0.00 mg/dl) ($p<0.05$; Table 1).

Table 1. Results of prolonged intensive exercise in rats accustomed to high- and moderate-intensity training

	CG (n=10)	HG (n=10)	MG (n=10)	NG (n=10)
Weight (g)	230	220	220	230
CK (IU/L)	97.3 ± 18.5	344.6 ± 52.8*†	430.2 ± 80.2*†	713.9 ± 123.4*
LDH (IU/L)	172.3 ± 33.6	282.4 ± 73.7†	478.2 ± 160.01*†	661.5 ± 115.2*
hs-CRP (mg/dL)	0.01 ± 0.00	0.02 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*

*$p<0.05$.
CG: control group; HG: high-intensity group; MG: moderate-intensity group; NG: non-exercise group.
*Significantly different compared with CG ($p<0.05$); †Significantly different compared with NG ($p<0.05$)

DISCUSSION

Following moderate- and high-intensity aerobic exercises, the high- and moderate-intensity exercise groups as well as the non-exercise group showed significantly higher CK levels than the control group, while the high- and moderate-intensity groups showed significantly lower CK levels than the non-exercise group. It was previously reported that an exercise-induced increase in CK resulted in increased serum CK activity following long-distance running[16, 17], which reflects the findings of the present study. However, in the present study, following long-distance exercise, the high- and moderate-intensity exercise groups showed significantly lower CK levels than did the non-exercise group. This confirms that preliminary training can inhibit skeletal muscle damage. Following moderate- and high-intensity aerobic exercises, the moderate-intensity and non-exercise groups showed significantly higher LDH levels than the control group, while the high- and moderate-intensity groups showed significantly lower LDH levels than the non-exercise group. The findings confirm increases in LDH levels after intense exercise in the moderate-intensity exercise group and the non-exercise group in accordance with the literature[18]. However, since the high-intensity exercise group showed no significant increase in LDH level, high-intensity training may inhibit LDH increases associated with skeletal muscle damage induced by intense exercise.

The high- and moderate-intensity exercise groups and the non-exercise group showed significantly higher hs-CRP levels than the control group. Several studies have reported that, unlike regular exercise, a single bout of intense exercise increased the hs-CRP level and the white blood cell count[19, 20]. The present study confirms that, compared to the control group, the moderate- and high-intensity exercise groups and the non-exercise group showed significant increases in hs-CRP level; however, no significant differences were found among the three groups, except for the control group.

A single bout of exercise causes muscle damage and an inflammatory response, while an increase in exercise intensity or duration may lead to a corresponding higher inflammatory response. Tartibian et al.[19, 20] instructed normal individuals to exercise for 30 minutes at 60% and 75% of the maximal oxygen uptake and reported no differences in muscle damage or the inflammatory response, not only immediately after the exercise but also during the recovery period. However, Gacrial et al.[21] reported that when the average duration of exercise was extended from 30 minutes to 87 minutes, despite low exercise intensity, there was a higher inflammatory response. In the present study, the most pronounced inflammatory response was noted in the non-exercise group, which did not undergo preliminary training. This may be because, compared to the other groups, this group found the 3-hour prolonged exercise period comparatively more intense. Wannamethee et al.[22] noted that the implementation of regular exercise reduced exercise-induced inflammatory responses; this was attributed to the adaptive responses to exercise and the anti-inflammatory effects.

In the present study, the groups that underwent moderate- and high-intensity preliminarily training showed lower muscle damage and inflammatory response levels than the untrained non-exercise group when they performed one bout of intense exercise. Compared to the non-exercise group, the moderate- and high-intensity groups may have decreased their muscle damage indices as their muscles acclimated to the repeated training and recovery process.

In conclusion, the present study found that, compared to the non-exercise group, those rats that underwent preliminary exercise showed less muscle damage and inflammatory responses during prolonged intense exercise; in particular, the effects were greater after one bout of intense exercise following high- than moderate-intensity training.

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