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Cool Water Immersion after Downhill Running Suppresses Exercise-induced Muscle Damage in the Rat Soleus Muscle

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Abstract. [Purpose] Cryotherapy has been used to treat acute skeletal muscle damage, but there are some controversies about the effects of cryotherapy on exercise-induced muscle damage. In present study, we investigated the effect of cool water immersion on exercise-induced muscle damage. [Methods] Twelve-week-old male Wistar rats were divided into a control (Cont) group, exercise (Ex) group, and cool water immersion after exercise (Ex+W) group. Rats in the Ex and Ex+W groups performed downhill running at 16 m/min on a −16 degree incline, for 90 minutes. The rats of the Ex+W group were immersed in cool water (20 degrees C for 30 minutes) immediately after exercise. The soleus muscles were removed at 24, 48, and 72 hours after exercise, cross-sectional areas of muscle were stained with hematoxylin-eosin, and glucose-6-phoshate dehydrogenase (G6PD) activity was measured. [Results] Muscle damage was observed in both the Ex and Ex+W groups. The percentage of damaged muscle fibers in the Ex+W group was lower than that in the Ex group at 72 h. G6PD activity in the Ex+W group was lower than that in the Ex group at 48 and 72 h. [Conclusion] These results suggest that cool water immersion after downhill running suppresses exercise-induced muscle damage.
Key words: Cool water immersion, Muscle damage, Exercise

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INTRODUCTION

Strenuous and unaccustomed exercise can induce skeletal muscle damage, and this is particularly true of exercise including eccentric contraction, such as downhill running[1)]. Skeletal muscle damage has been thought to cause delayed-onset muscle soreness (DOMS). DOMS typically occurs 1–2 days after unaccustomed exercise[2–4)] and generally resolves within a week of the inciting activity[5)]. Skeletal muscle damage causes microcirculatory dysfunction, inflammation, spasm, edema, and pain. Skeletal muscle damage is evident as disruption of the normal banding patterns of skeletal muscle and broadening or complete disruption of sarcomere Z lines[6)]. Edema, as a result of production of prostaglandin E2 and increased capillary permeability, has been observed in eccentrically exercised muscle at 24, 48, and 72 h[7–9)]. Prostaglangin E2 is also responsible for transmitting dull aching pain to the central nervous system[10)]. Muscle damage contributes to strength deficits, reduction of range of motion, and pain. These morbidities may cause sedentary lifestyle, activity limitation, and prevent continuation of theraputic exercise.

Cryotherapy is a physical agent often used by physical therapists to treat acute skeletal muscle damage and DOMS, and to facilitate the recovery of muscle damage. Cryotherapy reduces blood flow, edema and inflammation. Skeletal muscle damage induces microcirculatory dysfunction and it causes failure of oxygen supply to muscular cells. Failure of oxygen supply to muscular cells causes further muscle damage. Cryotherapy reduces skin and deep tissue temperatures, and suppresses cellular metabolism[11)] and oxygen consumption[12)]. Therefore, cryotherapy is thought to suppress further muscle damage and DOMS. However, there are some controversies about the effect of cryotherapy on exercise-induced muscle damage. Some studies have reported that cryotherapy has no effect on the alleviation of DOMS[4)]. Nosaka et al. reported that DOMS is a poor reflector of eccentric exercise-induced muscle damage, and muscle damage and inflammation do not necessarily accompany DOMS[13)]. Howatson et al. reported that cryotherapy had no effect on DOMS, but reduced the appearance of plasma creatine kinase[14)]. We hypothesized that cryotherapy would not be effective for DOMS, but would reduce of exercise-induced muscle damage.

In present study, we investigated the effect of cool water immersion on exercise-induced muscle damage, via observation of muscle cross-sectional areas stained with hematoxylin-eosin and measurement of glucose-6-phospatase activity.

SUBJECTS AND METHODS

Twelve-week-old male Wistar rats were used in this study. The rats were housed in a temperature controlled room at 22 ± 2 °C on a 12 h light-dark cycle and allowed free access to food and water. This study was performed in accordance with the Japanese Association for Laboratory Animal Science Guidelines for the care and use of animals.

Rats were divided into a control (Cont, n=5) group, exercise (Ex, n=15) group, and cool water immersion after exercise (Ex+W, n=15) group. Rats in the Ex and Ex+W groups were forced to run downhill on a treadmill at 16 m/min on a −16 degree incline, for 90 minutes. The rats in the Ex+W group were immersed in water (20 degrees C for 30 minutes) immediately after the downhill running. It has been reported that cryotherapy at temperatures below 15 °C may have deleterious effects[15], and metabolism is at its lowest when the ambient temperature is 20 °C [16]. We considered that 20 °C was safe and the most effective water temperature for suppressing muscle damage after downhill running. Rats were sacrificed by an overdose of sodium pentobarbital and bilateral soleus muscles were removed at 24, 48, and 72 hours after exercise (n=5 / time point). Soleus muscles were immediately frozen in isopentane cooled by liquid nitrogen and stored at −80 °C until use.

For the assessment of the soleus muscle fibers, 10 μm thick frozen sections were cut using a cryostat at −20 °C. These frozen sections were fixed in 10% formalin solution for 15 min at 4°C, and stained with hematoxylin-eosin. Damaged muscle fibers were observed under a microscope (20x) and estimated using 4 randomly selected fields per section. Damaged muscle fibers were expressed as a percentage of damaged muscle fibers.

Glucose-6-phosphate dehydrogenase (G6PD) activity in skeletal muscles has previously been shown to be associated with damage, inflammation and repair processes[17, 18], and is known to increase in locomotory muscles following an acute bout of exercise. Thus, G6PD activity in skeletal muscle appears to serve as a reasonably sensitive biochemical marker of degenerative processes. In the present study, we used this enzyme as a direct marker of muscle damage. For the analysis of G6PD activity, soleus muscles were homogenized in Tris-HCl (pH 7.4). After centrifugation, the supernatant was collected as the measurement sample. The protein content in these samples was measured by the Lowry Method. G6PD activity was assayed by spectrophotometrically measuring the change in light absorption at 340 nm following the reduction of NADP+, as previously described[19]. The enzyme activities were expressed as mIU/mg protein.

All data are expressed as means ± standard deviation. Percentages of damaged muscle fibers and G6PD activities at each time point in the same group compared to the Cont group were subjected to statistical analysis using Kuraskal-Wallis analysis followed by the Shirley-Williams post hoc test. When the Shirley-Williams test showed a significant difference at a certain time point, we used the Mann-Whitney U-test to identify significant differences in the data between the Ex and Ex+W groups at that time point. These analyses were performed using Excel Statistics 2008 (Social Survey Research Information Co. Ltd., Tokyo, Japan). The determination for acceptance of statistical significance was $p < 0.05$.

RESULTS

The percentage of damaged muscle fibers was significantly larger in both the Ex and Ex+W groups at 24, 48, and 72 h after exercise than in the Cont group (all $p < 0.05$). A significant increase in percentage of damaged fibers was observed in the Ex group at 72 h after exercise compared with the Ex+W group ($p < 0.05$) (Table 1).

G6PD activity was significantly greater in both the Ex and Ex+W groups at 24, 48, and 72 h after exercise than in the Cont group (all $p < 0.05$). Significant elevations in G6PD activity were observed in the Ex group at 48 and 72 h after exercise compared with Ex+W group ($p < 0.01$ and $p < 0.05$, respectively) (Table 2).

DISCUSSION

In the present study, we investigated the effect of cool water immersion on exercise-induced muscle damage. Our findings indicate that cool water immersion after exercise suppresses exercise-induced muscle damage.

Damaged muscle fibers were not observed in the Cont group, but were present in both the Ex and Ex+W groups, and G6PD activity was elevated in both the Ex and Ex+W groups compared with the Cont group. G6PD activity is the major factor contributing to increased flux thorough the pentose phosphate pathway during the initial stages of muscle regeneration. Enhancement of this pathway is important for anabolic processes in the initial stages of skeletal muscle regeneration[19]. G6PD has been associated with elevated activity of the pentose phosphate pathway, and probably reflects accelerated glucose utilization in the production of nucleic acid[20]. G6PD activity provides a simple means of quantifying inflammatory or degenerative processes. Therefore, the present findings indicate that muscle damage occurred in the rat soleus muscle in both the Ex and Ex+W groups. Muscle damage includes initial damage and secondary delayed damage. The most direct evidence of secondary delayed damage is the increase between 1 and 3 days in the extent and severity of the morphological evidence of injury observed by either microscopy[21, 22] or electron microscopy[6, 23]. The percentage of damaged muscle fibers in the Ex+W group was similar at 24 and 48 h, but it was decreased at 72 h after exercise compared with the Ex group. Moreover, G6PD activity in the Ex+W group was similar levels at 24 h compared with the Ex group, but it was at lower levels at 48 and 72 h after exercise than that in the Ex group. These findings indicate that cool water immersion prevents secondary delayed damage. Reactive oxygen

Table 1. Percentage of damaged muscle fibers (%)

	Cont	post-exercise		
		24h	48h	72h
Ex	0.00 ± 0.00	2.50 ± 0.26*	2.40 ± 0.53*	3.03 ± 0.28*
Ex+W		2.37 ± 1.07*	2.50 ± 1.04*	2.20 ± 0.57*,#

* significant difference compared with Cont ($p < 0.05$).
# significant difference from the Ex group at the same time point ($p < 0.05$)

Table 2. G6PD activity (mIU/mg protein)

	Cont	post-exercise		
		24h	48h	72h
Ex	1.27 ± 0.34	4.59 ± 1.41*	15.48 ± 2.45*	9.56 ± 1.58*
Ex+W		4.93 ± 2.05*	10.09 ± 1.42*,##	7.90 ± 0.54*,#

* significant difference compared with Cont ($p<0.05$)
# significant difference from the Ex group at the same time point ($p<0.05$)
significant difference from the Ex group at the same time point ($p<0.01$)

species are reportedly enhanced in skeletal muscle by acute and eccentric exercise[24]. Reactive oxygen species[25] and the inflammatory processes[26] have been implicated in the secondary delayed damage. In the present study, cool water immersion after downhill running suppressed inflammation, cellular metabolism, and oxygen consumption. We consider that the above-mentioned effects reduced the production of reactive oxygen species, thereby suppressing secondary delayed muscle damage.

Skeletal muscle damage can affect performance through reduction in joint range of motion, pain and peak torque. These morbidities discourage patients from continuing with the theraputic exercise. We have previously reported that thermal preconditioning induces heat stress proteins and attenuates exercise-induced muscle damage[27]. This preconditioning may have a preventive effect on initial damage induced by mechanical stress. In the present study, we demonstrated that cool water immersion suppresses exercise-induced muscle damage. A combination of these physical modalities, before and after strenuous exercise, might result in a larger effect than either of these modalities before or after exercise alone. The prevention of muscle damage is important for allowing patients to continue rehabilitation. Therefore, further investigations should be carried out to seek other methods of prevention of muscle damage.

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