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Yosuke Shima[1], Katsuhiko Kitaoka[1], Yumiko Yoshiki[2], Yoshinobu Maruhashi[1], Takeshi Tsuyama[1], and Katsuro Tomita[1]

[1]Department of Orthopedic Surgery, Kanazawa University, Kanazawa, Japan; and [2]Department of Environmental Bioremediation, Tohoku University, Sendai, Japan

Abstract: The mechanisms of the protective effect conferred by heat shock preconditioning (HS) are currently unknown. The purpose of this study was to determine the effect of HS on muscle injury after downhill running and to address the mechanism of the effect. Female Wistar rats were assigned to three groups: heat shock preconditioning (HS), downhill running (E), and downhill running after heat shock preconditioning (HS+E). HS and HS+E rats were placed in a heat chamber for 60 min (ambient temperature 42±1.0°C) 48 h before downhill running. Reactive oxygen species (ROS) scavenging activity was determined by electron spin resonance (ESR) and heat shock protein 72 (HSP72) mRNA expression was measured in rat quadriceps femoris. Leukocyte infiltration and degenerated muscle fibers were determined histopathologically. ROS scavenging activity significantly increased at 3 days after HS (151±18%) and HSP72 mRNA expression increased immediately after HS (1750±1914%). No decrease in ROS scavenging activity was observed in the HS+E rats at 2 days after exercise compared with the E rats (102±9% vs. 79±5%). Degenerated muscle fibers in HS+E rats were significantly less than in E rats at 2, 3 and 7 days after exercise (0.8±1.0 vs. 2.8±1.6, 0.8±1.0 vs. 1.8±1.6, 0 vs. 0.3±0.6, respectively). These data demonstrated that HS can reduce muscle injury after downhill running and this effect may be mediated by increased ROS scavenging activity. Furthermore, HS may protect the antioxidant defense system in skeletal muscle by enhancing the adaptive HSP72 mRNA response.

Key Words: heat shock protein, leukocyte infiltration, muscle, reactive oxygen species, scavenging activity

Unaccustomed eccentric exercise induces muscle injury [1]. The involvement of reactive oxygen species (ROS) in exercise-induced muscle injury is increasingly apparent [2], and oxidative stress during exercise could be of importance in intense eccentric exercise because mechanical expansions are important activators of plasma membrane systems for the generation of superoxide and nitric oxide [3].

Although various prophylactic or treatment measures have been proposed to reduce exercise-induced muscle injury, delay the onset of muscle soreness (DOMS) and facilitate recovery from the muscle injury, their efficacy remains largely unproven [4-6]. Recent papers have proposed that heat shock proteins (HSPs) can prevent exercise-induced muscle injury and play a role in skeletal muscle recovery and remodeling/adaptation processes after high-force eccentric exercise [7-9]. Heat shock preconditioning (HS) has been shown to reduce tissue injury induced by a variety of insults [10-13], and several investigations in rat skeletal muscle have demonstrated that HS protected muscle from disuse atrophy [14] and from oxidant damage during reloading after immobilization [15]. Nosaka et al. [16] first reported that passive hyperthermia treatment 1 day prior to eccentric exercise-induced muscle injury had a prophylactic effect in a human clinical study. However, the mechanisms by which HS reduces exercise-induced muscle injury are not well understood.

In addition, skeletal muscle contains a certain antioxidant system that seems to be closely related with the HSPs [3]. Mn-SOD induction, not HSP72 induction, plays a major direct role in the heat shock-induced acquisition of tolerance to hypoxia-reoxygenation in rat cardiac myocytes [17]. On the other hand, Smolka et al. [18] demonstrated that HSP72 provided increased resistance to oxidative stress during exercise. Furthermore, Selsby et al. [15] suggested that oxidative stress attenuation by heat stress was not due to endogenous upregulation of antioxidant enzymes. Since the effect of HS on antioxidant capacity in skeletal muscle is still an unsettled issue, we focused on the effects of HS on subsequent ROS scavenging activity of muscle tissue and exercise-induced muscle injury. Scavenging activity represents the capacity of the tissue for a reduction of ROS.

Previous methods to determine antioxidant activity (e.g. colorimetric method and chemiluminescent method) have some limitations, including development of side reactions such as production of hydrogen peroxide and hydroxyl radical and low selectivity for superoxide anions. In contrast, ROS measured by electron spin resonance (ESR) with spin-trapping has very high selectivity for superoxide anions and is not influenced by the color of the specimen [19]. To our knowledge, this is the first study that has applied ESR with spin-trapping to evaluate the effect of HS on ROS scavenging activity in skeletal muscle.

We hypothesized that HS would reduce muscle injury after downhill running and this would be associated with increased ROS scavenging activity in a rat model. To test this hypothesis, we induced HS and investigated changes over time in the scavenging activity converted into SOD activity against superoxide anions determined by ESR with spin-trapping. Moreover, we also examined intramuscular HSP72 mRNA expression and histopathological findings to evaluate muscle injury.

Materials and Methods

Animals and Experimental protocol. Female Wistar rats (n=94; Japan SLC, Shizuoka, Japan) that were 7 weeks old (body weight; 140-209 g) were housed in a temperature-controlled room (22±2.0°C) with 12 h periods of light and darkness. A standard diet (Rat Chow; Oriental Yeast, Tokyo, Japan) and water were provided ad libitum. All procedures were approved by and followed the guidelines of the institutional animal care and use committee at Kanazawa University.

Because treadmill exercise training diminishes trauma to muscle from an acute bout of exercise, untrained rats were used in all experiments [20]. Rats were divided into a control (C) group (n = 6), a group that underwent downhill running (E; n = 30), a group exposed only to heat shock preconditioning (HS; n=28), and a group that underwent downhill running 48 h after HS (HS+E; n = 30). Using the rat downhill running model, preferential damage of the quadriceps femoris, especially the deep vastus intermedius has been previosuly reported [1,21]. Therefore, samples of the quadriceps femoris muscles were obtained from both hindlimbs of rats in each group. Muscles from 2 C rats, 10 E rats, and 10 HS+E rats were analyzed histopathologically. Rats were exercised by running on a treadmill (Treadmill for Rats and Mice, Model MK-680; Muromachi Machine, Tokyo, Japan) with variable velocity and inclination; since the treadmill has a variable setting for uphill slopes only, the treadmill was elevated by boards to obtain a downhill slope. Downhill slopes were used for eccentric exercise as a physiologic method of inducing muscle injury [1]. E and HS+E rats were exercised by running down a 15° incline at a speed of 20 m/min for 150 min (30 min×5 sets; intervening interval, 30 min). At each intervening interval, diet and water were provided ad libitum. Some electrical stimulation was used to spur the rats, particularly at the beginning of the run, but this was held to a minimum. Rats were sacrificed at 0, 1, 2, 3, 4, 5, and 7 days after HS in the HS group and at 0, 1, 2, 3, and 7 days after termination of exercise in the E and HS+E groups. After the rats were anesthetized with diethyl ether and ketamine/xylazine (50 mg/kg and 4 mg/kg i.m.), the quadriceps femoris muscles were isolated and freed of connective tissue. Portions of the vastus intermedius (20-30 mg) were removed immediately at a point 5 mm proximal to the superior pole of the patella and treated with 10 volumes of an RNA stabilization reagent (RNA*later*; QIAGEN, Tokyo, Japan) for measurement of HSP72 mRNA. Remaining specimens were weighed and stored in a freezer at -80°C until measurement of ROS scavenging activity.

Heat shock preconditioning (HS). HS and HS+E rats were placed in an environmentally controlled heat chamber (WI-50; AS ONE, Osaka, Japan) for 60 min (ambient temperature, 42±1.0°C) without anesthesia and were weighed immediately before and after HS. Our preliminary experiments using the same method showed that the colonic temperature of rats reached the target temperature (>40°C) at 40 min of HS and then reached a plateau (Fig. 1). The rats in the E group were also placed in the chamber for 60 min, but at an ambient temperature of only 22±2.0°C. A rectal probe (ME-PDK061; Terumo, Tokyo, Japan) inserted 5 cm beyond the anal sphincter into the colon was used for colonic temperature monitoring with a thermometer (CTM-303; Terumo). Immediately after HS, rats were quickly returned to a cage in a temperature-controlled room (22±2.0°C) and provided diet and water ad libitum. In this study, we performed HS at 48 h before downhill running, according to the protocol of Yamashita et al. [22], in which whole-body heat stress at 41°C induced maximal cardioprotection and Mn-SOD activity at 48 h after HS.

Histopathological analysis of muscles. Specimens of each quadriceps femoris muscle were fixed in 10% formalin, dehydrated, and embedded in paraffin. Sections were cut with a cryostat as a complete cross-section near the middle of the belly at a thickness of 10㎛. The sections were stained with hematoxylin and eosin (H&E), and examined using a light microscope (Nikon Eclipse, TE2000-U; Nikon Instech, Kanagawa, Japan). The number of leukocytes that infiltrated the muscles and degenerated muscle fibers were counted directly in 10 random fields in each section at 400× magnification by an observer blinded to the corresponding experimental group. Degeneration of muscle fibers is normally evaluated by morphological changes such as rounding of muscle fibers, nuclear centralization, caliber variation, and changes in staining intensity. Therefore, in the present study, degenerated muscle fibers in cross-section were identified by a rounded appearance and intense staining. The mean number of leukocytes and degenerated muscle fibers per section in the HS+E and E groups were determined and compared.

Electron spin resonance (ESR) measurement of intramuscular ROS scavenging activity against superoxide. An ESR instrument (JES-TE25X; JEOL, Tokyo, Japan) was used to measure ROS scavenging activity against superoxide based on Masuda's method [23]. ESR settings were: frequency, 9.4190 GHz; power, 4.00 mW; field, 334.5 mT; sweep time, 1.0 min; modulation, 0.079 mT; and time constant, 0.3 s. Specimens of the quadriceps femoris muscle were homogenized in 1.15% KCl homogenizing buffer at a 1:10 ratio. Muscle scavenging activity was determined directly against superoxide anions derived from a xanthine oxidase-hypoxanthine reaction by measuring the inhibition rate shown by ESR signals in a mixture of muscle homogenate and the superoxide-generating system. The ESR spectrum allows the original reactive radical to be identified and quantified. The spectrum of the reaction without muscle was recorded as a control, and a standard curve for SOD activity was constructed based on spectra with known SOD concentrations of 5, 10, 15, 20, 25, 30, 40, and 50 U/ml. The reaction mixture consisted of 50 µl of homogenate, 50 mM phosphoric acid buffer, 2 mM hypoxanthine (6-hydroxypurine), 0.4 U/ml xanthine oxidase, and 20 µl of 9.2 M 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as a spin-trap. The ESR spectrum 45 s after initiation of the reaction by addition of xanthine was recorded at room temperature (22±2.0°C). The signal intensity was expressed as a ratio of the peak located at the lowest magnetic field of the four-line DMPO-OOH signal to the signal intensity of a Mn^{2+} internal standard. Scavenging activity was calculated from the SOD activity based on the standard curve.

Measurement of mRNA expression for intramuscular HSP72. HSP72 was chosen for analysis because of its biologic importance and its greater temperature sensitivity than that of other HSPs. Harvested muscle tissue was homogenized in 300 µl of cold RLT buffer (QIAGEN, Tokyo, Japan) using an ultrasonic disruptor (UD-201; TOMY Seiko, Tokyo, Japan). Total RNA was extracted by an RNeasy kit (QIAGEN), subjected to proteinase K digestion, eluted in RNase-free water, and quantified by spectrophotometry at 260 nm. Total RNA was reverse-transcribed using a high-capacity cDNA archive kit (Applied Biosystems, Foster City, CA). First-strand cDNAs were amplified in a 25 µl reaction volume containing 12.5 µl TaqMan 2×Universal PCR Master Mix, 1.25 µl target cDNA specific primers and probe and 1 µl undiluted first-strand cDNA sample. Fluorescence was measured in an ABI PRISM 7700 Sequence Detector (Applied Biosystems) according to the manufacturer's instructions. Glyceraldehyde-3-phosphate dehydrogenase (GAPD) was used as a reference gene. Relative expression of HSP72 mRNA was normalized to the amount of GAPD in the same cDNA, using a relative standard curve method described by the manufacturer. Predeveloped primers and TaqMan MGB probes (6-FAM dye-labeled) for rat HSP72 and for GAPD were purchased from Applied Biosystems (HSP72: Rn00583013_s1, GAPDH: Rn99999916_s1). All probes were tagged at their 3'-end with nonfluorescent quencher.

Statistical analysis. All data are expressed as means ± SD. HSP72 mRNA expression and ROS scavenging activity of each time period in the same group compared to the C group were subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by a Fisher's protected least significant difference (PLSD) test. When the PLSD test showed a significant difference in a certain time period, we then used Student's *t*-test to identify significant differences in the data between the E and HS+E groups at that time period. The above analyses were performed using StatView version 5.0 (SAS, Cary, NC). The chosen level for statistical significance was $P < 0.05$.

Results

Weight of rats

We gave careful consideration to weight loss due to HS. The HS group had an immediate weight reduction after HS that was significant compared with the C group (146.5±6.9 g vs. 164.8±6.9 g, respectively; *P*=0.0054). However, the HS group regained the lost weight at 1 day after HS (160.8±1.5 g vs. 164.8±6.9 g, respectively; *P*=0.3355). Since rats in the HS+E group underwent downhill running 48 h after HS, we did not need to account for differential loading of the muscle in favor of the lighter rats.

Colonic temperature

Before the exposure to HS, the colonic temperature of the rats was 37.9±0.3°C (37.0-38.5°C). On exposure to HS, the colonic temperature gradually increased, reaching a peak temperature of 41.6±0.7°C (40.4-43.7°C) after completion of the 60 min heating period (Fig.1). The average colonic temperature was 3.6±0.6°C (2.4-5.6°C) higher than the temperature before exposure to HS.

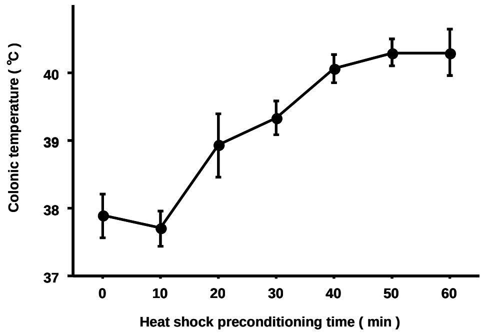

Fig. 1. Colonic temperature of rats every 10 min during heat shock preconditioning. Colonic temperature gradually increased, reaching a peak temperature of 41.6±0.7°C (40.4-43.7°C) on completion of the 60 min heating period. Data are given as means ± SD (n=3 for all data points, except 0 min, in which n=18).

Intramuscular ROS scavenging activity measured by ESR

ROS scavenging activity converted into SOD activity in the quadriceps femoris muscle at 3 days after HS in the HS group was 74.3±8.9 U/ml; this activity was significantly higher than the activity of 49.1±4.7 U/ml in the C group (151±18%, *P*=0.0012), but it normalized at 4 days after HS (Fig. 2). In the E group, ROS scavenging activity at 2 days after exercise was lower than in the C group (nearing statistical significance, *P*=0.0559), but it normalized at 3 days after exercise (Fig. 3a). No decrease of ROS scavenging activity was observed at 2 days after exercise in the HS+E group (Fig. 3b). A significant difference in ROS scavenging activity was observed between the HS+E and E groups at 2 days after exercise (102±9% vs. 79±5%, respectively; *P*=0.0053) (Fig. 3).

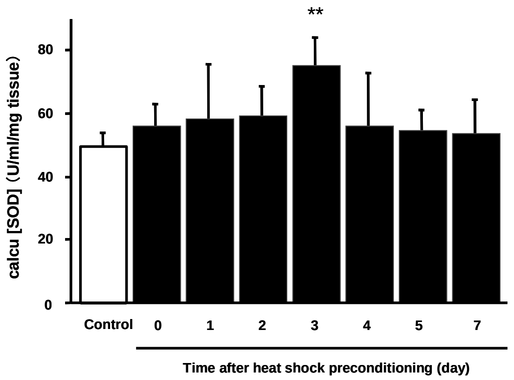

Fig. 2. In the HS group, heat shock preconditioning significantly increased intramuscular ROS scavenging activity at 3 days after preconditioning compared to activity in the C group (151±18%, $^{**}P<0.01$).

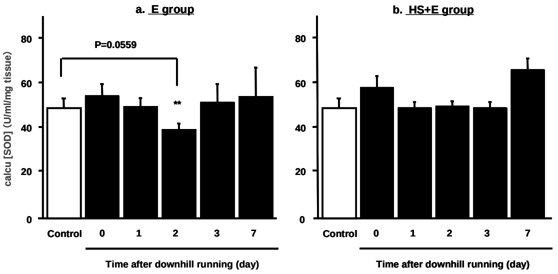

Fig. 3. Intramuscular ROS scavenging activity was lower (nearing statistical significance, *P*=0.0559) at 2 days after downhill running in the E group than in the C group (**a**), with no obvious decrease in the HS+E group (**b**). A significant difference was observed between the HS+E and E groups at 2 days after downhill running (102±9% vs. 79±5%, $^{**}P<0.01$).

Intramuscular HSP72 mRNA expression

A significant increase in HSP72 mRNA expression was evident in HS rats immediately after HS (1750±1914%, *P*<0.0001); expression returned to the same level as in the C group at 4 days after HS (Fig. 4). Although there were no statistically significant differences, intramuscular HSP72 mRNA expression in HS rats increased by more than 2-fold from the C group until 3 days after HS. HSP72 mRNA expression was significantly increased in both E and HS+E groups compared with the C group immediately after downhill running (*P*<0.0001 for both). Although a significant increase compared to expression in the C group persisted in the HS+E group until 3 days after exercise, the E group subsided at 2 days after exercise (Fig. 5).

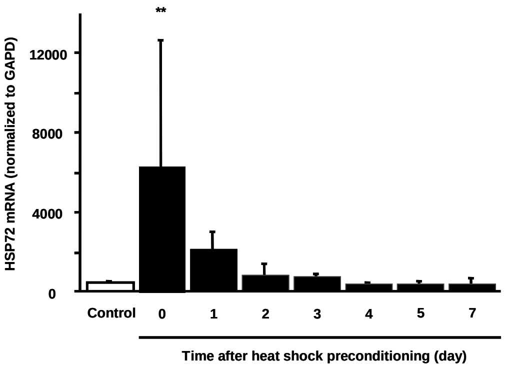

Fig. 4. In the HS group, heat shock preconditioning significantly increased intramuscular HSP72 mRNA expression immediately after preconditioning compared to expression in the C group (1750±1914%, ***P*<0.01).

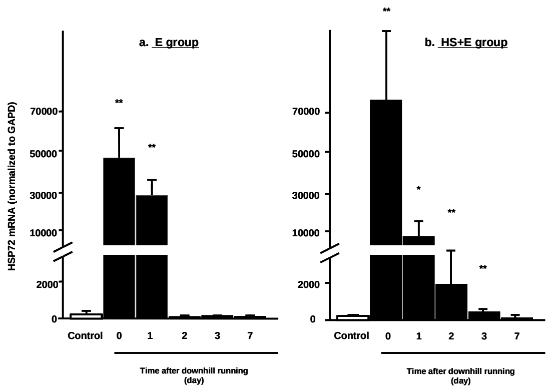

Fig. 5. (**a**). A significant increase in HSP72 mRNA expression was observed in the E group immediately and at 1 day after downhill running and returned to the same level as in the C group at 2 days after downhill running (***P*<0.01). (**b**). A significant increase in HSP 72 mRNA expression was observed until 3 days after downhill running in the HS+E group (**P*<0.05, ***P*<0.01).

Histopathology

Approximately 100 muscle fibers were observed in each miscroscopic field. A significant increase in leukocyte infiltration (Fig. 6a) compared with the C group was observed in both the E and HS+E groups from 1 to 7 days after exercise (*P*<0.05 in all), although a significant suppression compared with the C group was observed in the HS+E group immediately after exercise (*P*<0.0001; Table 1). Leukocyte infiltration was significantly greater in the E group than in the HS+E group immediately, and at 2 and 7 days after exercise (*P*<0.0101, *P*<0,0001, and *P*=0.0059, respectively; Table 1). Findings consistent with degenerated muscle fibers (Fig. 6b) were observed in both the E and HS+E groups from 1 to 3 days after downhill running, with significantly more cells in the E group than in the HS+E group at 2 days, 3 days, and 7 days after exercise (*P*<0.0001, *P*=0.0153, and *P*=0.0492, respectively; Table 1).

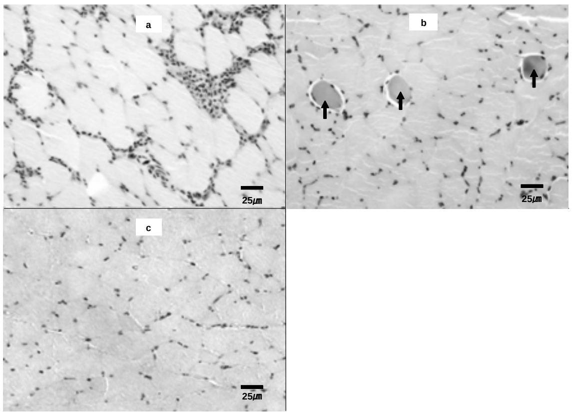

Fig. 6. Representative images of microscopic skeletal muscle alterations in rat quadriceps femoris muscle (H&E, 400×). (a): Leukocyte infiltration in the damaged region. (b): Degenerated muscle fibers (representative fibers shown by arrows). * These two images are from a rat in the E group at 3 days after downhill running. (c): Control (there are no degenerated muscle fibers, but some leukocytes are found occasionally).

Discussion

The present animal experiments demonstrated that heat shock preconditioning (HS) can inhibit the decrease in ROS scavenging activity in muscle tissue after downhill running and reduce exercise-induced muscle injury. There was a significant increase in ROS scavenging activity determined by ESR at 3 days after HS, and HSP72 mRNA expression was increased by more than 2-fold from the C group until 3 days after HS (Figs. 2 and 4). Although no

TABLE 1. Numbers of leukocytes and degenerated muscle fibers (Values are expressed as the mean ± SD).

Time after downhill running (day)	**Leukocytes**		**Degenerated muscle fibers**	
	E	**HS+E**	**E**	**HS+E**
Control	38.6 ± 16.1		0	
0	36.1 ± 12.6 **NS**	15.4 ± 4.8 ‡ **	0	0
1	55.0 ± 14.6 †	58.2 ± 14.8 ‡	0.3 ± 0.6	0.1 ± 0.2
2	104.6 ± 32.5 †	70.2 ± 41.6 ‡ **	2.8 ± 1.6	0.8 ± 1.0 **
3	111.7 ± 46.9 †	93.0 ± 62.7 ‡	1.8 ± 1.6	0.8 ± 1.0 *
7	95.4 ± 28.6 †	55.7 ± 18.0 ‡ **	0.3 ± 0.6	0 *

† Different from control group (*P*<0.05); ‡ different from control group (*P*<0.05); ***** different from E group (*P*<0.05); ****** different from E group (*P*<0.01); **NS**; not significant.

significant decrease in ROS scavenging activity was observed after downhill running in the HS+E group (Fig. 3b), histopathologic findings showed that HS reduced muscle injury after downhill running (Table 1).

The accumulation of leukocytes even in our C group (Table 1) supports previous findings that the presence of leukocytes in muscle tissue is necessary for both normal muscle function and tissue repair following injury [24]. Leukocyte infiltration to remove cell debris is important for recovery of skeletal muscle from oxidative stress after exercise [25]. However, Malm et al. [26] found that muscle adaptation can take place in the absence of an inflammatory response and concluded that DOMS is not related to inflammation.

At the present time, the mechanism of the significant decrease in leukocyte infiltration in the HS+E group observed immediately after exercise (Table 1) remains unclear. We speculate that it may have reflected a suppression of initial subcellular damage as a result of HS, although we have no direct evidence for suppression of initial subcellular damage.

Leukocyte infiltration in the E group at 2 days after downhill running sharply increased and almost doubled from day 1 (Table 1). Therefore, the borderline significant decrease in ROS scavenging activity at 2 days after downhill running in E group (Fig.3a) could be a consequence of increased ROS production in the muscle. On the other hand, no decrease of ROS scavenging activity was observed at 2 days after exercise in the HS+E group (Fig. 3b), giving rise to two different interpretations. One is that a suppression of initial subcellular damage after downhill running by HS resulted in less subsequent ROS production. The other is that an increase in ROS scavenging activity by HS counteracted an increase of subsequent ROS production. The present study suggests that HS may provide muscle protection against exercise-induced muscle injury involving increased ROS scavenging activity.

HSP70 gene transcription in rat skeletal muscle is maximal between 0-2 h following exercise (mean running time; 64.9 ± 8 min) [27]. In addition, the magnitude of the HSP response has been shown to correlate well with the magnitude of the initial stress [28] and is enhanced by different stressors acting in a synergistic manner [29]. Since the present exercise protocol required 4 h and 30 min in total (30 min×5 sets; intervening interval, 30 min), it is consistent with the finding that significant and peak induction of HSP72 mRNA expression was observed immediately after downhill running in both the E and HS+E groups (Fig. 5). Regarding the effect of HS on HSP72 mRNA expression, a significant increase compared to expression in the C group persisted 2 more days in the HS+E group than in the E group (Fig. 5). That is, HS can enhance the downhill running-induced adaptive mRNA responses of HSP72. Smolka et al. [18] suggested that HSP72 is part of a secondary antioxidant defense system acting to provide fast additional protection when the main system is also attacked. It is possible that HS may protect the antioxidant defense system in skeletal muscle by enhancing the adaptive HSP72 mRNA response. [y1] It is suspected that the activation of heat shock factor 1 (HSF1) during HS leads to increased levels of HSP mRNA and HSP molecules, rendering the cell more resistant to future mechanical or oxidative stress by downhill running. Frier and Locke [30] showed that a single heat stress provided prior to overload resulted in inhibition of skeletal muscle hypertrophy. It was suggested that a prior increase in HSPs may have afforded some protection to muscle fibers during the initial stage of overload such that the stress experienced during overload may have been diminished.

Lepore et al. [31] noted that HSP72 alone cannot adequately explain and predict the protective effect of heat stress. It has been suggested that SOD is very important in the mechanism of tolerance, irrespective of HSP70 induction, and induction of HSP72 may promote the maturation of SOD [17, 22]. Suzuki et al. [32] showed that enhanced Mn-SOD activity during ischemia-reperfusion injury is a possible mechanism of HSP72-induced cardioprotection. Preconditioning and heat stress bring about complex biologic interactions enhancing a diverse network of gene expression and adaptive responses. However, it remains to be determined whether HSP72 and Mn-SOD are cooperative or interacting factors in acquired tolerance.

SOD activity in human skeletal muscle has been reported to increase significantly after training [33]. However, Patel et al. [34] reported that increasing muscle oxidative capacity by isometric electrical stimulation training did not protect muscle from eccentric contraction-induced injury. Selsby et al. [15] also reported that oxidative stress reduction by heat stress was not due to endogenous upregulation of antioxidant enzymes and suggested that alternative antioxidants must be considered. The contradictory results of these previous studies might be due to differences in the timing and methods used to measure SOD activity, differences in the exercise training protocols, or differences in the fiber composition of the muscles investigated. Maruhashi et al. [35] used the same exercise protocol and technique to assay ROS scavenging activity as in the present study; they reported that low-load eccentric training prevented intense eccentric exercise-induced muscle injury not through elevated ROS scavenging activity, but through a suppression of initial subcellular damage that triggered subsequent inflammatory cell infiltration and ROS production. Since the present study showed HS has the ability to increase ROS scavenging activity, there is a possibility that HS has a distinct mechanism to prevent exercise-induced muscle injury compared to low-load eccentric training.

Regarding the timing of HS, passive or active warming when HS is applied just before exercise apparently does not offer protection against DOMS and muscle injury [36, 37]. On the other hand, heat stress even in injured skeletal muscle can facilitate recovery by stimulating the proliferation of satellite cells and protein synthesis during regeneration [38]. Based on the present data of HS and downhill running on ROS scavenging activity, HS might be better applied 1 day before downhill running as was done by Nosaka et al. [16] (Fig. 2 and 3). However, the optimal timing of HS requires further investigation.

Comparisons between humans and rats indicate differences between these species in response to similar exercise protocols, and the prophylactic effect of HS may not be as strong as the repeated bout effect [16]. Thus, further investigations including human study should be carried out to seek the mechanisms by which HS protects against subcellular damage and the most suitable conditions of timing and frequency of HS.

The present study in rats demonstrated that HS can reduce muscle injury after downhill running and that this effect involves increased ROS scavenging activity. Furthermore, HS may protect the antioxidant defense system in skeletal muscle by enhancing the transcription of adaptive HSP72 mRNA.

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