Exercise-induced elevation of HSP70 is intensity dependent

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Exercise-induced elevation of HSP70 is intensity dependent. J Appl Physiol 93: 561–568, 2002. First published May 3, 2002; 10.1152/japplphysiol.00528.2001.—Exercise induces expression of the protective heat shock protein, HSP70, in striated muscle. To characterize the relationship between induction of this protein and exercise intensity in muscles exhibiting different recruitment patterns, male Sprague-Dawley rats were assigned to a sedentary control or one of seven exercise groups for which treadmill running speed varied between 15 and 33 m/min (n = 8/group). Twenty-four hours after a single 60-min exercise bout, hearts, red and white portions of the vastus (RV and WV, respectively) muscles, and soleus (Sol) muscles were harvested and analyzed for both relative and absolute HSP70 content. Cardiac HSP70 was significantly elevated only when animals were exercised at 24 m/min and beyond. Similarly, HSP70 was elevated at running speeds above 24 m/min but did not increase in WV until 27 m/min. In contrast, HSP70 content was initially elevated in the Sol but subsequently declined at the highest running speeds. The observed patterns of HSP70 expression in skeletal muscle were in general accordance with known muscle recruitment patterns and suggest that alterations in muscle loading, resulting from changes in exercise intensity, are an important component of exercise-induced increases in HSP70 content.

heart; skeletal muscle; rat; treadmill running; stress proteins; heat shock protein 70

MATERIALS AND METHODS

Animals

Use and treatment of laboratory animals was approved by the University of Western Ontario Council on Animal Care according to the guidelines of the Canadian Council on Animal Care. Adult male Sprague-Dawley rats (~220 g and 8 wk of age) were obtained from Charles River Laboratories and housed in triplicate in standard shoe box rat cages. The vivarium was maintained at constant temperature and humidity with a 12:12-h light-dark cycle. All rats were fed LabDiet 5P00 standard rat chow and water ad libitum.

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Acute-Exercise Protocol

After being housed for 1 wk, the rats were randomly assigned to either a sedentary control group (Con; n = 8) or one of seven exercise groups (15, 18, 21, 24, 27, 30, and 33 m/min on a 2% incline; n = 8/group). All rats were familiarized with treadmill running for 10 min on 3 alternating days in the week leading up to the acute exercise bout. Familiarization consisted of 2 min running at 15 m/min, 4 min at 24 m/min, 2 min at 30 m/min, and 2 min at 15 m/min, all at a 2% incline. Rats were encouraged to run with a gentle air blast that blew on their hindquarters when the rats broke a photosensor beam near the rear of the treadmill. Although the air blast was generally sufficient to keep the rats running, if they stopped on the grid at the back of the treadmill, they were further encouraged to run by the administration of a brief electric shock. Neither the familiarization nor the stimuli used to encourage running elicited a significant heat shock or stress response (Noble EG, Ho R, and Dzialoszynski T, unpublished observations). Each exercise bout consisted of continuous treadmill running at room temperature (~21°C) at one of the designated speeds for a period of 60 min. Animals were weighed, and rectal temperature was measured immediately before the exercise bout by using a thermometer inserted 5 cm into the rectum. Rectal temperature was then measured every 15 min during the exercise bout and immediately after the run. Con rats were handled similarly to their exercising counterparts without being placed on the treadmill. All animals completed the full hour of their respective exercise bouts.

Blood Collection

Blood was collected by tail clipping exactly 5 min after each rat had finished its exercise bout. Whole blood was centrifuged at 17,000 g for 10 min, after which the serum was carefully pipetted out and transferred to microcentrifuge tubes for later analysis. Blood was taken from Con rats at similar times during the day and treated as indicated. Duplicate measurements of serum lactate were made on a Yel- lar Springs Instruments 2300 STAT Plus glucose and lactate analyzer and expressed as millimoles per liter.

Heart Rate Measurements

A separate group of animals, of comparable weight and age to those above (n = 3), was used to determine resting, maximum, and exercise heart rates at various running speeds. These animals were given a surgical dose of pentobarbital sodium (65 mg/kg), and then an electrode was implanted under the skin at the nape of the neck. One week after surgery, resting and exercise heart rates were determined. Heart rates were measured at rest while the animals stood on the nonmoving treadmill. Exercise heart rates were measured by running the animals for 2 min intervals (at which point heart rates had stabilized) at progressively increasing speeds. Maximum heart rate was determined when a plateau in HR was reached even after running speed was increased.

Tissue Collection

Twenty-four hours after the acute exercise bout, animals were anesthetized (65 mg/kg pentobarbital sodium) and weighed, and the heart, red and white portions of the vastus (RV and WV, respectively), and soleus (Sol) were harvested, rapidly frozen in liquid nitrogen, and stored at ~70°C until further analysis. The heart represents an organ that may particularly benefit from increased HSP70 content (37), and the RV, WV, and Sol represent muscles that exhibit different fiber-type profiles and recruitment patterns (1, 2, 3). Sol is a posturally active muscle, rich in slow oxidative (SO) fibers [87% SO and 13% fast oxidative glycolytic (FOG) fibers]. In contrast, RV and WV are composed of 9 and 0% SO, 56 and 3% FOG, and 35 and 97% fast glycolytic (FG) fibers, respectively (1), and are sequentially recruited at increasing running speeds (13).

HSP70 ELISA

To complement and compare the observations made with Western blotting, changes in HSP70 content were also assessed by using a competitive ELISA as per Gutierrez and Guerrero (6). Briefly, 25 ng/well of purified HSP70 (StressGen SPP-755) in a carbonate buffer (15 mM Na2CO3, 35 mM NaHCO3, and 30 mM NaNO3, pH 9.6) were added to 96-well microtiter styrene plates and allowed to incubate overnight.
at 4°C. Serially diluted standards (from 12.5 to 800 ng/100 μl) and homogenates used for Western blotting (27–35 μl) were brought to a final concentration of 1% SDS and boiled for 3 min. An adequate amount of antibody buffer [10 mM Tris (pH 7.4), 0.15 M NaCl, 30 mM NaN₃, and 1% Triton X-100] to bring the samples to a final volume of 200 μl was then added. Samples without homogenate served as controls. Two hundred microliters of HSP70 antibody (StressGen SPA-812 diluted 1:1,000) were added to both standards and unknown samples, which were then incubated overnight at 4°C.

The next day, wells were blocked for 15 min in 10 mM Tris (pH 7.4), 0.15M NaCl, 30 mM NaN₃, and 0.25% Tween 20, and 100 μl each of standards and samples were subsequently added to blocked wells and incubated for 3 h at room temperature. After three washes with cold blocking buffer, 100 μl of the secondary antibody (goat anti-rabbit alkaline phosphatase conjugate diluted 1:2,500) were added to each well and incubated at room temperature for 2 h. Plates were then washed three times with TBS and 100 μl of freshly made p-nitrophenyl phosphate (1 mg/ml) in developing buffer (100 mM triethanolamine, 1 mM MgCl₂, and 30 mM NaN₃, pH 9.8) and incubated at 37°C until absorbance, measured at 405 nm, in wells without competitor reached 1.0. Amount of HSP70 in samples was calculated from a curve of absorbance vs. log of the standards.

**Statistics**

Statistical analysis was performed by using Sigma Stat for Windows Version 2.03. For the comparison of HSP70 levels among treatment groups, a one-way analysis of variance was employed to determine significance (P < 0.05), Dunnett’s post hoc test was employed to determine significant differences from the Con group.

**RESULTS**

**Heart Rate**

In a separate group of animals, heart rates were measured to provide estimates of the exercise intensities at the different running speeds (Table 1). These data are similar to those previously recorded (3) and show that, from the lowest intensity of 15 m/min to the highest at 33 m/min, the animals were exercising at ~87–94% of their maximum heart rate.

**Serum Lactate**

Serum lactate data are presented in Fig. 1. Lactate values were similar after exercise at all intensities except 33 m/min, indicating that at most treadmill speeds the animals were exercising below the lactate threshold.

**Animal Weight and Temperature Measurements**

Animal weight and temperature measurements are presented in Table 2. Peak temperature (T_p) was approached by the 15th minute of exercise and was then maintained throughout. Increasing the intensity of exercise tended to result in lower postexercise body weights and higher T_p.

**HSP70 Expression**

Both Western blotting and ELISA protocols generated similar results with regard to HSP70 expression, although in some instances the ELISA assay proved more sensitive and had the additional advantage of allowing absolute quantification of changes in HSP70 content.

**Cardiac muscle.** In cardiac tissue, HSP70 levels were similar between Con and exercise groups up to a running speed of 21 m/min (Fig. 2). At speeds of 24 m/min and greater, myocardial HSP70 content (ELISA data in Fig. 2) of exercised animals was significantly increased (P < 0.05). This increase became especially pronounced after exercise at 33 m/min, an exercise intensity that exceeded the lactate threshold (Fig. 2).

**Skeletal muscle.** Like the heart, the RV (Fig. 3), a mixed muscle high in FOG fibers (1), demonstrated a progressive rise in HSP70 content from the slowest speed of 15 m/min. This increase achieved significance at exercise intensities of 24 m/min and above (P < 0.05). When assessed with use of ELISA, HSP70 levels rose to approximately sixfold those of nonexercised values at the highest intensity (P < 0.05). In contrast, the WV (Fig. 3), a muscle previously shown to have a majority of FG fibers (1), exhibited similar HSP70 values between Con and all exercise groups up to 24 m/min, inclusive. Exercise at 27 m/min caused a 2.5-

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**Table 1. Rat heart rate responses to treadmill running at different speeds**

<table>
<thead>
<tr>
<th>Exercise Speed, m/min</th>
<th>Rest</th>
<th>12</th>
<th>14</th>
<th>16.5</th>
<th>28</th>
<th>37.5</th>
<th>45</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>366 ± 16.0</td>
<td>506 ± 20.7</td>
<td>516 ± 20.0</td>
<td>529 ± 20.5</td>
<td>553 ± 13.3</td>
<td>568 ± 8.9</td>
<td>580 ± 8.2</td>
<td>596 ± 12.6</td>
</tr>
<tr>
<td>Maximum heart rate, %</td>
<td>61.3 ± 2.7</td>
<td>84.9 ± 3.5</td>
<td>86.4 ± 1.9</td>
<td>88.7 ± 3.5</td>
<td>92.6 ± 2.2</td>
<td>95.3 ± 1.5</td>
<td>97.2 ± 1.4</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Values are means ± SE for 3 rats/group.

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**Fig. 1. Blood serum lactate concentrations measured in rats 5 min after 60 min of treadmill exercise at various running speeds. ** P < 0.05. Significantly greater than sedentary control (Con) and 15, 18, 21, 24, 27, and 30 m/min, P < 0.05.
fold increase in HSP70 \( (P < 0.05) \), an increase that was further enhanced (to almost 6 times that of Con values), after running at 33 m/min \( (P < 0.05) \).

The Sol (Fig. 3), composed predominantly of SO fibers (1), showed a different pattern of HSP70 expression to the other skeletal muscles examined. HSP70 content tended to be elevated at the lower exercise intensities between 15 and 27 m/min \( (P < 0.05) \), as assessed from Western blots, whereas after exercise at 30 m/min and above, HSP70 content declined to approach Con levels.

With use of either ELISA or SDS-PAGE (when data were normalized for the total amount of protein loaded on each gel; see MATERIALS AND METHODS), the constitutive expression of HSP70 was highest in Sol and least in the WV (Fig. 4). When the change in HSP70 content between Con, a low exercise intensity (18 m/min), and the highest exercise intensity (33 m/min) was examined, the magnitude of the maximal absolute increase in HSP70 also followed the pattern of Sol \( (0.342 \, \text{ng/\mu g total protein at 18 m/min}) > \text{RV} \, (0.2024 \, \text{ng/\mu g total protein at 33 m/min}) > \text{WV} \, (0.0612 \, \text{ng/\mu g total protein at 33 m/min}) \).

**DISCUSSION**

Exercise is associated with several mechanisms that may afford cardioprotection (7, 38, 40). However, recent observations that specific antisense oligonucleotide ablation of exercise-induced elevations in cardiac HSP70 resulted in loss of protection against ischemia-reperfusion injury (29), coupled with the positive effects of HSP70 overexpression in transgenic (21, 31) and chronically exercised models (8) on this condition, suggest that the HSP70 plays a prominent role in exercise-induced myocardial protection. In a recent study in which Noble et al. (27) employed intense treadmill training vs. voluntary free wheel running, only treadmill running resulted in an increase in cardiac HSP70. At the time, these investigators speculated that the induction of myocardial HSP70 may exhibit an intensity-related threshold. The findings of the present investigation confirm this speculation and extend the previous observations of Liu et al. (16) with regard to skeletal muscle.

The heart operates as a functional syncytium, whereby all fibers are recruited with each beat, but during exercise, the work of the heart is linearly increased with exercise intensity because of increases in both cardiac contractility and frequency. Cardiac HSP70 induction, however, does not exhibit this linear response; rather, it demonstrates an intensity-related threshold with significant increases in HSP70 being first noted at a speed of 24 m/min, corresponding to a heart rate of over 90% of maximum. A further dramatic increase in HSP70 is observed after exercise at 33 m/min when HSP70 content rose to nearly 22 times.

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**Table 2. Rat body weights and rectal temperatures before and after exercise**

<table>
<thead>
<tr>
<th>Exercise Speed, m/min</th>
<th>Control</th>
<th>15</th>
<th>18</th>
<th>21</th>
<th>24</th>
<th>27</th>
<th>30</th>
<th>33</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>269.7 ± 6.6</td>
<td>264.8 ± 6.3</td>
<td>275.0 ± 5.7</td>
<td>268.4 ± 6.1</td>
<td>271.6 ± 5.3</td>
<td>284.2 ± 5.3</td>
<td>269.7 ± 4.3</td>
<td>267.1 ± 6.5</td>
</tr>
<tr>
<td>Rectal temperature °C</td>
<td>38.2 ± 0.2</td>
<td>38.1 ± 0.2</td>
<td>38.3 ± 0.2</td>
<td>38.0 ± 0.1</td>
<td>37.8 ± 0.2</td>
<td>37.9 ± 0.1</td>
<td>38.6 ± 0.1</td>
<td>38.3 ± 0.2</td>
</tr>
<tr>
<td>Peak</td>
<td>39.2 ± 0.2</td>
<td>39.6 ± 0.2</td>
<td>39.7 ± 0.2</td>
<td>39.9 ± 0.1</td>
<td>39.9 ± 0.1</td>
<td>40.3 ± 0.1</td>
<td>40.3 ± 0.1†</td>
<td>40.3 ± 0.1††</td>
</tr>
</tbody>
</table>

Values are means ± SE of 8 rats/group. Control, sedentary control. In all groups, peak temperature was significantly elevated above resting levels, \( P < 0.05 \). *Significantly different from Control, 15 m/min, and 18 m/min, \( P < 0.05 \). †Significantly greater than 15 m/min, \( P < 0.05 \). ††Significantly greater than 18 m/min, \( P < 0.05 \).

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**Fig. 2. Heat shock protein 70 (HSP70) expression in rat heart measured by both Western blotting (A) and ELISA (B) (see MATERIALS AND METHODS) after 60 min of treadmill running at various speeds. Values are means ± SE. *Significantly greater than Con, \( P < 0.05 \).**
Con levels. Lactate concentrations obtained at various running speeds in the present study are in general agreement with the findings of Pilis et al. (30) and indicate that the largest increase in myocardial HSP70 content occurred when animals were exercising near or above their lactate threshold. Hence, it appears, that simply undertaking exercise per se is insufficient to induce this stress protein. To garner the potential cardioprotective effects of HSP70 (21, 29, 31), relatively intense exercise is required. These findings could help explain observations that the cardioprotective effect of exercise is more influenced by the intensity of the exercise rather than the total amount of physical activity (22, 23, 39), although total exercise volume (17, 34) and other factors such as temperature (7, 38) and gender (28, 29) may modulate the response.

Unlike the myocardium, skeletal muscle fibers do not function as one distinct unit but operate as motor units that are sequentially recruited as required. Therefore, to further evaluate the role of exercise intensity in eliciting HSP70 expression, we examined a variety of skeletal muscle types in the rat hindlimb known to vary with respect to fiber-type composition and recruitment patterns during treadmill running. In the vastus, the progressive recruitment of FOG fibers at all exercise intensities is contrasted by the recruitment of FG fibers only at high intensities. For example, by using periodic acid-Schiff staining of rat muscle cross sections, Armstrong et al. (2) observed lower glycogen content in FOG and SO fibers after treadmill running at 22.5 m/min, 0% grade, whereas glycogen content in FG fibers only decreased after exercise at

![Fig. 3. HSP70 expression in the red and white portions of the vastus (RV) and WV, respectively, and the soleus (Sol; C) measured by both Western blotting (left) and ELISA (right) after 60 min of treadmill running at various speeds. Values are means ± SE. *Significantly greater than Con, P < 0.05.](image)
38.9 m/min, 0% grade. Blood flow observations in the rat hindlimb confirmed these recruitment patterns (13). Similarly, although observing progressively larger increases in cytochrome c concentration in red RV after exercise training at low to moderate intensities, Dudley et al. (4) reported that the WV exhibited a training response threshold at and above 30 m/min. In the present study, HSP70 expression in the RV and WV closely mimicked these recruitment and adaptive patterns (Fig. 3).

The Sol, a postural muscle composed predominantly of SO fibers (1) and extensively recruited in rats during normal locomotion and standing (32), demonstrated a different pattern of HSP70 expression than that observed in the other skeletal muscles. The Sol showed modest exercise-induced changes in HSP70; nonetheless, when assessed via Western blots, HSP70 content in the Sol increased slightly at the lowest exercise intensity (15 m/min), but at speeds above 27 m/min, HSP70 content actually declined (Fig. 3). This response is reminiscent of observations by Dudley et al. (4), who found that the Sol demonstrated an increase in cytochrome c concentration as the intensity of an 8-wk treadmill running regimen was increased to ~80% maximal O_2 uptake, but at intensities greater than this, cytochrome c activity returned toward Con levels. This is also in keeping with observations made by Roy et al. (32), who found that with faster treadmill speeds, the total amount of activation of the rat Sol remained relatively constant and even exhibited a slight decrease as measured by electromyogram. It is likely that these observations reflect a functional unloading of the slower contracting Sol muscle when surrounding, faster contracting muscles are recruited at the higher running speeds.

The fiber-type differences in the constitutive or basal expression of HSP70 observed in the present study (Fig. 4) have been noted previously (9, 18). It has also been reported that the exercise-induced increase in HSP70 is inversely related to the basal levels of this protein in skeletal muscle. Hence, those muscles with the lowest constitutive expression of HSP70 demonstrate the greatest relative increases in response to exercise training (5, 10). A similar trend was observed in the present study, with the WV demonstrating a 6-fold increase in HSP70 and the Sol exhibiting only a 1.5-fold rise (Fig. 3). However, when data were normalized for the total amount of protein loaded on each gel during SDS-PAGE (see MATERIALS AND METHODS) and the change in HSP70 content between Con, a low exercise intensity (18 m/min), and the highest exercise intensity (33 m/min) was examined, the magnitude of the maximal absolute increase in HSP70 followed the pattern of Sol > RV > WV (Fig. 4). This expression pattern, which could result from the more frequent recruitment of muscles with higher oxidative capacity, is in accord with observations that heat shock results in greater and more rapid DNA binding of the factor responsible for HSP70 transcription (heat shock factor-1) in the Sol as opposed to the less oxidative white portion of the gastrocnemius muscle (19). It is interesting to speculate that the more robust response of the oxidative fibers may also be a factor in the reduced damage they experience after eccentric muscle contractions (15).

During exercise, a number of physiological and metabolic events occur within muscle cells. These include but are not limited to an increase in core and muscle temperature, oxidative stress, altered pH, and structural damage to muscle proteins. Many of these events are known to induce HSP70 (for review see Ref. 26). Although we do not provide evidence for a specific exercise stimulus that will elevate HSP70, it is likely that the previously mentioned factors, and possibly others, all contribute to the observed stress response to some degree. Increased body temperatures accompanying exercise are clearly a factor in the cardiac induction of HSP70 (7, 38); nonetheless, in the present study, the substantial increase in myocardial HSP70 content when the exercise intensity was increased from 30 to 33 m/min (Fig. 2), despite a similar elevation in core temperature (Table 2), suggests that other factors are also important. A whole body inflammatory or stress response may be involved (24); however, local conditions and indeed actual muscle loading plays a major role. If this were not the case, we would not expect to see the markedly different patterns of HSP70 expression after exercise in muscles in such close proximity to each other. In this regard, the observations that the Sol demonstrated a return toward Con HSP70 expression at the most intense workloads, despite these workloads...
resulting in the greatest elevations in body temperature, strongly suggests that factors associated with muscle recruitment are critical to eliciting a stress response. Whether individual motor units increase their expression of HSP70 on initial activation, or like cardiac tissue exhibit a threshold related to the frequency of activation, cannot be ascertained from this study.

In conclusion, the present results demonstrate that the exercise-induced increase in HSP70 exhibits an intensity-dependent threshold that in skeletal muscles is related to their known recruitment patterns. Because HSP70 has been demonstrated to protect striated muscle against a variety of insults, these observations may have implications regarding exercise prescription and the relative exercise intensity required to gain these benefits.

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