Human skeletal muscle HSP70 response to training in highly trained rowers

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Rowing is a force- and endurance-demanding type of exercise, and the training in rowing is complex (24), varying in both intensity and duration. During adaptation to exercise, training not only may cause muscular hypertrophy and change of the muscular composition (9) but also may induce HSP70 production. This study investigated the human skeletal muscle HSP70 response to 4 wk of rowing training.

METHODS

Subjects. Ten male rowers, aged 18 yr, qualified for the German National Team, with eight chosen to be coxswains and the other two serving as replacements. Their height and body mass were \(192 \pm 5\) cm and \(85 \pm 7\) kg, respectively. After being informed about the study purpose and procedure, including the risks of a muscle biopsy, the subjects gave their written informed consent. This study was approved by the ethics committee of the Medical Faculty of the University of Ulm (Ulm, Germany).

Training. A 4-wk training program was conducted in preparation for the World Championships. This program consisted of four 1-wk phases (Table 1). Before and at the end of the training, a multiple-step rowing ergometry was performed until subjective exhaustion so that the power at 4 mmol/l of lactate (11) and relative maximum power (the maximum power output reached during this exercise test) could be obtained.

Muscle biopsy. The muscle biopsy taken at rest in the morning was performed in the middle of the belly of the right musculus vastus lateralis before the training and at the end of each training phase. A 16-gauge biopsy needle (Manan Medical Products, Northbrook, IL) was punctured 2 cm into the muscle belly, and the biopsy gauge was shot twice to attain \(-3\) mg of muscle tissue for each sample. The tissue was immediately frozen in liquid nitrogen and stored at \(-80^\circ\)C.

SDS-PAGE. Frozen muscle tissue was homogenized in 200 µl of protein-extraction buffer (5) with help of an ultrasonic homogenizator, and protein concentration was determined (19). The sample solution was added to the sample buffer according to Laemmli (16). The discontinuous gel system was used for the SDS-PAGE: 4.5% stacking gel and 8.75% separation gel. The 5 µg of protein from each muscle sample were electrophoretically separated by using Bio-Rad MiniProtein II gel system at 60 V/gel for 45 min in running buffer (16). A series of standard HSP70 (H 8778, Sigma Chemical, St. Louis, MO) was punctured 2 cm into the muscle belly, and the biopsy gauge was shot twice to attain \(-3\) mg of muscle tissue for each sample. The tissue was immediately frozen in liquid nitrogen and stored at \(-80^\circ\)C.

Protein transfer and immunodetection. Proteins were transferred from gels to polyvinylidene fluoride membranes (8) by using the LKB 2117 Multiphor II-Electrophoresis-Chamber (Pharmacia Biosystem, Freiburg, Germany) and a so-called semidry system at 200 V and 1.2 mV/cm2 membrane for 60 min. The membranes were then blocked by 5% bovine serum albumin. Immunodetection for HSP70 was performed according to the protocol of enhanced chemiluminescence (ECL) Detection System (Amersham Life Science, Amersham International). Primary monodonal antibody (mouse anti-human HSP70) in animals. The purpose of this study was to investigate human skeletal muscle HSP70 response to rowing training. Ten male rowers trained for 4 wk with different forms, durations, and intensities of exercise. Biopsy was performed in the right musculus vastus lateralis before training and at the end of each week. HSP70 in 5 µg of total protein from the muscle sample was determined by using Western blot and immunodetection with chemiluminescence technique, by means of laser densitometer referring to a series of known standard HSP70. Compared with pretraining (100%), HSP70 increased during training (181, 405, 456, and 363%) from the first to fourth training week, respectively) with the maximum HSP70 production at the end of second training week. Thus HSP70 is induced in highly trained human muscle by long-term training.

Heat shock protein; stress protein; exercise; Western blot

Several stressors induce production of the so-called heat shock protein (HSP), which is also called a stress protein (18). The HSP family contains a series of proteins with different molecular weights, and one of the prominent inducible HSPs in humans is HSP70 with a molecular weight of 72 (17). The production of HSP70 depends on the intensity and/or duration of a stimulus (23). Exercise-induced muscular ischemia, glycogen depletion, and oxidative free radicals might also be involved in HSP induction. Because changes in the exercise intensity or its duration may exert different effects on the exercise-induced muscular ischemia, glycogen depletion, and oxidative radicals, etc., it might be assumed that different exercise forms, intensities, and volumes have different impacts on HSP70.

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from the subsequent biopsies was elevated. With the pretraining HSP70 taken as 100%, the maximum level reached in the third week of training demonstrated an increase of about fivefold (Fig. 3A). The differences between the two consecutive biopsies are depicted in Fig. 3B, which may serve as an indirect indicator of HSP70 production.

**DISCUSSION**

The present study was conducted to observe the human skeletal muscle HSP70 response to training in rowing, and a clear increase of HSP70 in exercised human skeletal muscle was demonstrated. Quantification of HSP70. Most studies on HSP70 are performed semiquantitatively (22). In this study, HSP70 was quantified by referring to a series of known amounts of HSP70. A prerequisite for comparing the HSP70 of each muscle sample is the determination of the protein concentration. We used a well-established method (19) and examined the reproducibility (coefficient of variability = 0.72%).

Another important factor influencing the comparability of the muscle sample is the electrotransfer of protein (26). According to prior experiments, we chose 5 µg of total protein for analysis, which can be completely transferred and simultaneously completely received by the membrane under our experimental conditions.

In the analysis of the ECL films by laser densitometer, we found that not only the optical density but also the area of each sample band varied with the amount of HSP70 (Fig. 1). With the parameter integral, which combines the density with the area, a more linear relationship to HSP70 could be demonstrated (Fig. 2). Therefore, the densitometric integral was taken to calculate the HSP70 of each sample.

HSP70 response to training. Exercise can induce HSP70 production in muscles of animals (18), whereas a significant increase of HSP70 was found within 3 h after a bout of exercise in muscles of humans, although the concentration of HSP70 mRNA did increase (20). In the present study, HSP70 production increased to reach a maximum of five times the pretraining level.

During the period of training, HSP70 accumulation did not keep the same level. In the first week of training there was already an increase. During the second phase, in which the total exercise amount increased, HSP70 increased further (123%). With the exercise volume decreasing, the increase of HSP70 from the second to third training phase was reduced (62%). The increase from the second to third training phase was only 11.5%. In the fourth phase, in which the rowers trained with a reduced amount of exercise, the HSP70

Table 1. Training program and physiological parameters for the training

<table>
<thead>
<tr>
<th>Training Phase</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total training time, min/day</td>
<td>210</td>
<td>230</td>
<td>150</td>
<td>130</td>
</tr>
<tr>
<td>Time rowed, min/day</td>
<td>101</td>
<td>127</td>
<td>112.6</td>
<td>77.5</td>
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<tr>
<td>Distance rowed, km/day</td>
<td>25.4</td>
<td>28.5</td>
<td>24.5</td>
<td>15.2</td>
</tr>
<tr>
<td>Training for force development, min/day</td>
<td>60</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exetime, min/day</td>
<td>98</td>
<td>117</td>
<td>106</td>
<td>70</td>
</tr>
<tr>
<td>HIE time, min/day</td>
<td>3</td>
<td>10</td>
<td>6.6</td>
<td>7.5</td>
</tr>
<tr>
<td>Lifts of training (gymnastics, etc.), min/day</td>
<td>49</td>
<td>58</td>
<td>37.4</td>
<td>52.5</td>
</tr>
<tr>
<td>CK, U/I</td>
<td>186 ± 98</td>
<td>120 ± 64</td>
<td>92 ± 44</td>
<td>82 ± 38</td>
</tr>
</tbody>
</table>

Values are means ± 2SE for 10 men. Exe, extensive endurance training in rowing (mean blood lactate 6.4 mmol/l); HIE, high-intensity endurance training in rowing (mean blood lactate 0.45 mmol/l); HIE, high-intensity endurance training in rowing (mean blood lactate 6.4 mmol/l); CK, creatine kinase.

A linear relationship was found between the known HSP70 and the densitometric integrals: densitometric integral = 37.4·HSP70 + 1.1 (r = 0.98, P < 0.01; Fig. 2A), whereas the relationship between the known HSP70 and the optical densities of the protein bands was curvilinear (Fig. 2B).

On the basis of this linear relationship, HSP70 of each muscle sample was calculated. In comparison with the pretraining level (biopsy 0), HSP70 derived

![Fig. 1. Heat shock protein 70 (HSP70) blot depicting standard HSP70 and HSP70 obtained from subject 1. Lanes 1–5 represent standard HSP70 of 0, 0.05, 0.1, 0.15, and 0.20 µg, respectively. Lanes 6–10 are HSP70 taken from biopsies 1–5; HSP70 of each muscle sample (5 µg of total protein) was calculated to be 0.047, 0.096, 0.182, 0.217, and 0.193 µg, respectively.](http://jap.physiology.org/)
decreased. In Fig. 3B, HSP70 response is more clearly illustrated by the HSP70 difference between consecutive biopsies. The maximum increase of HSP70 was found at the end of the second training week when the maximum exercise amount was undertaken. Thus the HSP70 response to training seems related to the total exercise amount. However, in the present study it cannot be answered whether HSP70 response depends more on form, intensity, or volume of exercise.

In this study, we could not calculate HSP70 production because HSP70 kinetics in human muscle have not been investigated. Figure 3 indicates that there may be a time delay between HSP70 production and HSP70 accumulation. This time delay may be, at least partly, responsible for the results of Puntschart et al. (20). With the time delay, we may explain why the maximum HSP70 level happened at the end of the third training phase, although the maximum increase of HSP70 happened at the end of the second training phase. One might even speculate that the peak HSP70 level was hidden between the second and third training phases.

Mechanisms of HSP70 induction. Hyperthermia causes a HSP70 response (25). Because hyperthermia occurs during exercise (2), a “heat-shock” effect might play a role, but exercise-induced HSP70 can be independent of changes in the body temperature (23). Metabolic stress induces HSP70 (13), which may be at least partly responsible for HSP70 production in exercised muscles. During the training program, blood lactate frequently reached 4–8 mmol/l, causing a decrease of blood pH, which can induce HSP70 production (27). High-volume exercise demands a high-energy supply provided by substrate oxidation, producing glycogen depletion and oxidative free radicals, which induce HSP70 production (17).

Meanings of HSP70 production. HSP70 production is considered to protect the vital function and to prevent damage (12). Currie et al. (3) demonstrated that in myocardium pretreated with HSP70 induction (by hyperthermia) not only was the cardiac function less impaired by ischemia and could recovery more rapidly but also a reduction of myocardial infarct size could be produced (4). Conversely, failure to produce HSP70 leads to an exaggerated organic damage (10). We observed the change of creatine kinase (CK) during the training period. At the beginning of training, CK was elevated, and with the training CK decreased. Because CK is associated with muscular injury, HSP70 accumulation might be partly responsible for lowering CK level (6) and provide a protective effect on musculature (14).

Another important role HSP70 plays in muscular adaptation to exercise is the so-called “molecular chaperone” (7). Because, in the training performed in this study.
study, physiologically hypertrophic changes in the stressed muscle will happen, procedures involving new protein synthesis and protein folding will take place, in which HSP70 may play a role (1). An augmented endurance capacity was observed at the end of training. Simultaneously, we observed the change of contractile proteins of muscle and found that they increased distinctly (unpublished data) so that synthesis of new contractile proteins may be assumed to take place.

It can be concluded that the HSP70 level increased in response to rowing training in the stressed muscle of highly trained subjects.

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