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Different skeletal muscle HSP70 responses to high-intensity strength training and low-intensity endurance training

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Abstract Heat shock protein, e.g. HSP70, can be induced in human skeletal muscle undergoing exercise training, and plays important role in adaptation to stress. This study was designed to investigate the effects of high-intensity strength training and low-intensity endurance training on the HSP70 response to exercise, bearing in mind whether HSP70 is induced in the well-trained muscle during low-intensity endurance training. Six well-trained rowers (male, aged 18 years) underwent a training program which consisted of 3 weeks high-intensity training (HIT) and 3 weeks low-intensity endurance training (ET), followed by 1 week of recovery each (R1 and R2, respectively). HSP70 (2.5 µg total protein loaded) was determined by Western blot with reference to a series of known amount of standard HSP70. HSP70 mRNA was analyzed by RT-PCR, and the relative percentage change was referred to the baseline level (before training). HSP70 increased significantly at the end of HIT (from 51 to 73 ng), decreased at the end of R1 (66 ng), and remained unchanged throughout ET and R2. HSP70 mRNA increased significantly after HIT (257%) and decreased gradually afterwards (194%, 166%, and 119% for R1, ET, and R2, respectively). It can be concluded that: (1) HSP70 was induced by high-intensity training, but not by endurance training at low intensity, and (2) there was a discrepancy in terms of HSP70 regulation between the protein and mRNA levels, suggesting that posttranscriptional regulation may play a role in HSP70 expression in human skeletal muscle in response to exercise.

Keywords Exercise · Heat shock protein · Human skeletal muscle · Rowing · Stress protein

Introduction

As a stress protein, heat shock protein with 70 kDa molecular mass (HSP70) has been extensively investigated because of its important role as a “molecular chaperone” in facilitating cellular adaptation to stresses (Liu and Steinacker 2001). There are a number of data showing that HSP70 can be induced by many stressors including physical and chemical factors, metabolic challenges, etc. (Linguist 1986). Exercise-induced physiological and biochemical changes are included in those factors which can induce HSP70 (Locke and Noble 1995). The meaning of HSP70 induction may be its universal role in cellular protection against stresses since all eukaryotic cells examined have been proven to be able to produce HSP70 in response to stressful situation (Hunt and Morimoto 1985). By capturing the denatured proteins which may disturb cellular functions (Hightower 1991; Moseley 1997) and by stabilizing the newly synthesized proteins, HSP70 may play an important role in maintaining cellular functions. Meanwhile, the HSP70 response may serve as a marker of cellular stress (Baba et al. 1998), thus HSP70 changes in cells may provide valuable information for monitoring training in terms of exercise intensity and volume.

Though there are studies on the response of HSP70 to exercise and it is generally accepted that physical exercise is a sufficient stimulus for HSP70 induction in the stressed muscle (Locke 1997; Naito et al. 2001; Neuffer et al. 1998; Ryan et al. 1991), only a few studies have been carried out on human skeletal muscle in terms of the HSP70 response (Khassaf et al. 2001; Thompson et al. 2001). In a previous study, we reported that HSP70 was induced significantly in human skeletal muscle during physical training, and the response seemed to be associated with the total amount of training (Liu et al. 1999). However, it was not clear in that study whether

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the HSP70 response was dependent on the exercise volume or intensity. Therefore, a second study was carried out to investigate the relationship between HSP70 response and exercise intensity. It can be demonstrated that, in accordance with our first study, the HSP70 response was associated with total exercise volume; however, this relation was mainly attributed to the dependence of HSP70 induction on exercise intensity (Liu et al. 2000). Since most of the training in the second study was endurance exercise at relatively low intensity, and HSP70 induction depended on exercise intensity, the question is raised as to whether HSP70 in well-trained skeletal muscle is induced by endurance exercise at a relatively low intensity.

It is known that the expression of HSP70 is regulated at both the transcriptional and the translational level; however, there seem to be no data on HSP70 mRNA in terms of response to training. It would therefore be interesting to investigate whether the HSP70 response at the mRNA level to high-intensity strength training is different from that to low-intensity endurance training, and whether there is a discrepancy between the mRNA and protein levels with regard to the HSP70 response to exercise training.

Methods

Subjects

Six male, well-trained rowers were enrolled in the study. Their topographic data are summarized in Table 1. The subjects were well-informed about the study purpose and procedure, they gave their informed consent prior to the study. This study was approved by the Ethics Committee of the Medical Faculty of University of Ulm (Ulm, Germany).

Training

A classic rowing training program is usually composed of strength training with bench pulls and leg press, rowing and unspecific training such as swimming and gymnastics. The training program done in the study consisted of two phases, each of 3 weeks, followed by a 1-week recovery period, respectively. The study design and protocol are depicted in Fig. 1 and the training data are summarized in Table 2. The first training phase emphasized strength training (bench pulls and leg press) at high intensity (HIT). The work load was individually set at 50% of the subjects' one

Table 1 Topographic data of the subjects studied. ($\dot{V}O_{2max}$ Maximal O_2 uptake, $\dot{V}O_{2max/kg}$ $\dot{V}O_{2max}$ per kg body mass, P_{max} maximal power output)

Parameter	Value
<i>n</i>	6
Age (years)	19 (3)
Height (cm)	190 (9)
Body mass (kg)	83 (15)
$\dot{V}O_{2max}$ ($l \cdot min^{-1}$)	5.3 (0.6)
$\dot{V}O_{2max/kg}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	64.8 (6.2)
P_{max}	468 (68)

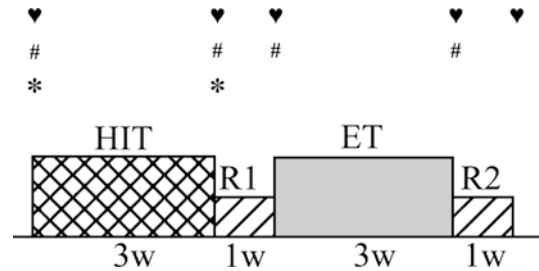


Fig. 1 Study protocol. The training program is composed of a high-intensity training (HIT) period and endurance training at low intensity (ET) followed by a 1-week recovery each (R1 and R2, respectively). *Time points for measurement of arm strength by bench pulls; #time points for peak oxygen uptake by ramp test as well as a multiple-stage test for submaximal power output, and performance and heart rate at 4 mmol l^{-1} blood lactate on a rowing ergometer. *Time points of muscle biopsy sampling. For details see text

repetition maximum (1-RM), and the mean workload was 45.7 kg for the bench pulls. The blood lactate concentration was monitored at regular intervals during the training, and was between 4.7 and 11.3 mmol l^{-1} from 139 measurements. The second training phase was mainly endurance rowing training at low intensity (ET), and the blood lactate concentration was between 1.3 and 3.0 mmol l^{-1} from 57 measurements. Only a small part of the training was strength training in the second training phase.

A ramp test to determine peak oxygen uptake ($\dot{V}O_{2max}$ by K4, Cosmet, Rome, Italy) and peak blood lactate concentration (La_{max} by YSI 2300 Stat plus, Yellow Springs, Morrisville, Vt., USA), and a multiple-stage test to determine submaximal power output (P_{max}), and performance and the heart rate at 4 mmol l^{-1} blood lactate (P_{la4} and HR_{la4} , respectively) were performed on a rowing ergometer (Concept II, Ohio, USA) before and at the end of each training phase. Strength of the arms expressed as maximum repetitions at 50% of 1-RM was determined by bench pulls before and after HIT.

Muscle biopsy

The muscle biopsy was done at rest in the morning before any training on the day. Muscle samples were taken from the middle of the belly of the right musculus vastus lateralis. The details are described elsewhere (Liu et al. 1999, 2000).

HSP70 quantitation

Muscle tissue was homogenized in protein-extraction buffer, and the concentration of total protein in the homogenates was determined in order to exactly load 2.5 μg total protein for protein separation on SDS-PAGE. The protein detection was performed with Western blot using specific monoclonal antibody against the

Table 2 Training data

Parameter	Intense training	Endurance training
Total training time (min/day)	144 (12)	120 (18)
Strength training time (min/day)	78 (6)	10 (3)
Rowing time (min/day)	37 (7)	90 (12)
Distance rowed (km/day)	7.3 (1.5)	17.1 (2.4)
Unspecific training (min/day)	30 (6)	18 (6)

HSP70 inducible form (SPA 810, Stressgen, Biomol, Hamburg, Germany). The quantitation of HSP70 followed densitometric determination of the protein bands with reference to the known amount of standard HSP70 (Fig. 2a). The details are described elsewhere (Liu et al. 1999).

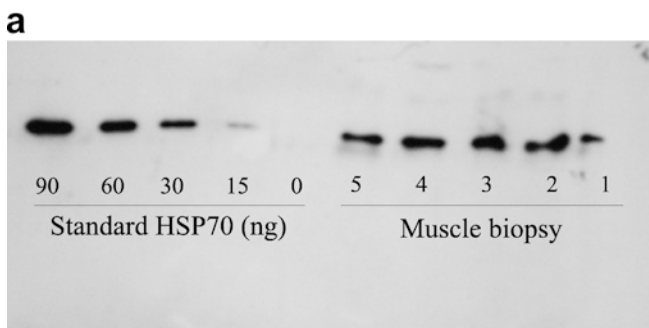
RT-PCR

About 3 mg muscle tissue was taken to extract total RNA using phenol extraction (RNAClean System, AGS, Heidelberg, Germany), and total RNA was dissolved in a final volume of 10 μ l for each 1 mg muscle tissue. Oligo (dT) primed synthesis of cDNA was performed using MuLV reverse transcriptase (Perkin Elmer, Roche Molecular System, Branchburg, New Jersey, USA) according to the standard protocol of the provider. Amplification of cDNA for HSP70 was performed according to the method reported by Taggart et al. (1997). Simultaneously, PCR amplification for skeletal muscle α -actin was done according to the method reported by Peuker and Pette (1995). The product of RT-PCR for α -actin served as an internal reference for HSP70 mRNA. The expected products of RT-PCR for HSP70 and α -actin are 200 bp and 367 bp segments, respectively (Fig. 3a). The RT-PCR products were densitometrically measured on 3% agarose gel containing ethidium bromide.

Data analysis

Samples were run in duplicate. The calculation of HSP70 followed the linear regression between densitometric values and known standard HSP70 amounts (Liu et al. 1999). HSP70 mRNA is calculated by its RT-PCR product in relation to the corresponding RT-PCR product for α -actin (i.e. ratio of RT-PCR products HSP70/ α -actin) and is expressed by percentage changes taking the baseline (before training) as 100%. Data are expressed as mean (SD), and the difference was tested with one-way ANOVA and Newman-Keuls test and assumed to be significant if $p < 0.05$.

Fig. 2a, b Determination of HSP70 by Western blot. **a** An example of a Western blot. The *left five lanes* were loaded with a series of known amount of standard HSP70 (ng), and the *right five lanes* were loaded with the serial muscle samples from one subject studied. The muscle biopsy *numbers 1–5* indicate the times when muscle samples were obtained (before HIT, at the end of HIT, before ET, at the end of ET, and at the end of the recovery following ET, respectively). **b** HSP70 results from all subjects studied. HSP70 increased significantly after HIT ($P < 0.01$), and decreased during the recovery period following HIT, and then kept constant during ET and the following recovery period



Results

Each subject accomplished the training program, and all muscle samples could be analyzed successfully.

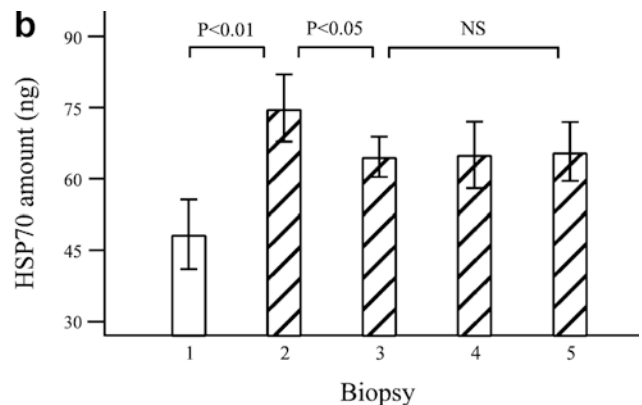
To estimate the training effects, several parameters were determined (Table 3). $\dot{V}O_{2\max}$ did not change significantly from before to the end of HIT, and it also remained constant over the endurance training period. There was a small but not significant decrease of $\dot{V}O_{2\max}$ during the recovery period following HIT. The sub-maximal power output (P_{\max}) in the multiple-stage test did not change during HIT and decreased somewhat at the end of the recovery phase following HIT (not significant); however, P_{\max} at the end of ET was increased significantly compared with that before ET. There was no significant change with regard to the maximum lactate level in the ramp test, the performance and heart rate at 4 mmol l^{-1} lactate during the multiple-stage test. The strength expressed as repetitions at 50% of 1-RM increased significantly at the end of HIT compared to that before HIT.

HSP70 increased significantly from about 51 ng before HIT to 73 ng at the end of HIT ($P < 0.01$); it decreased during the recovery phase following HIT ($P < 0.05$) and then remained unchanged (Fig. 2b).

HSP70 mRNA expressed as the ratio of HSP70/ α -actin increased clearly ($P < 0.01$), and reached its highest level (257%) at the end of HIT, and then decreased gradually afterwards ($P < 0.05$, Fig. 3b).

Discussion

The skeletal muscle HSP70 response may take place during physical training (Liu et al. 1999), and seems to be related to exercise intensity. In a previous study, it was demonstrated that the HSP70 response is mainly dependent upon exercise intensity rather than exercise volume (Liu et al. 2000). Generally, exercise volume during training is mainly constituted by endurance training rather than high-intensity training. Therefore, the exercise-intensity-dependent manner of the HSP70 response raises the question of whether endurance exercise at low intensity can induce HSP70 upregulation



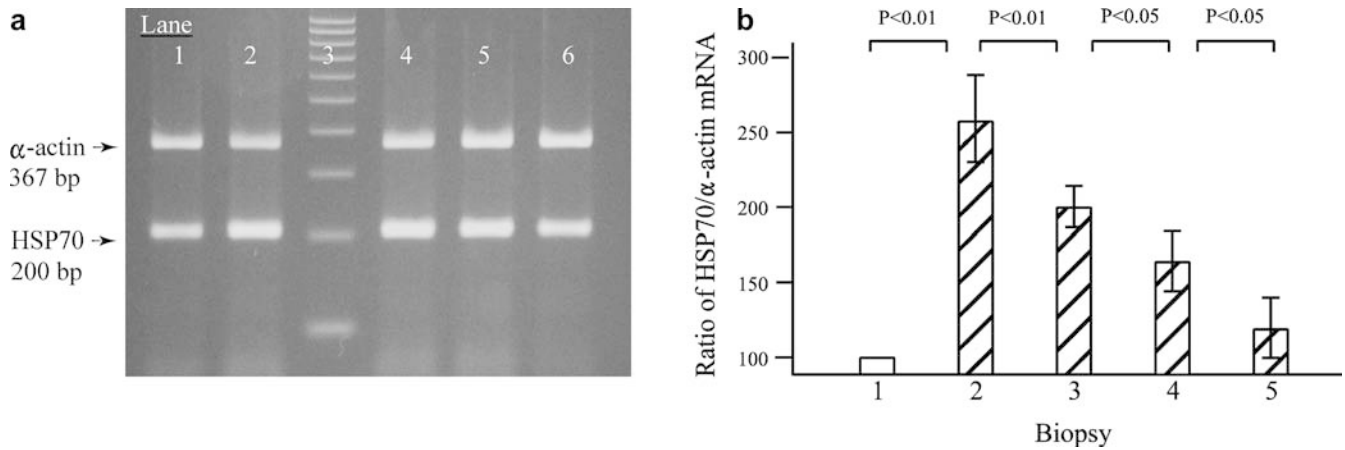


Fig. 3a, b Estimation of HSP70 mRNA. **a** An example of RT-PCR products for HSP70 (200 bp) and α -actin (367 bp). Lane 3 is the molecular mass marker, lanes 1, 2, and 4–6 are the RT-PCR products from muscle biopsy samples 1–5. The ratio HSP70/ α -actin was calculated from the densitometric values of each RT-PCR product for HSP70 and its corresponding product of α -actin. **b** The results derived from all subjects. HSP70 mRNA increased clearly at the end of HIT in comparison with that before HIT ($P < 0.01$), and decreased gradually afterwards ($P < 0.05$). The biopsy numbers indicate the same time points as when muscle samples were obtained as indicated in Fig. 2b

in well-trained skeletal muscle. The present study was thus conducted to investigate the possible different responses of HSP70 to different modes of training, i.e., strength training at high intensity and endurance training at low intensity. HSP70 expression was examined at both the protein and the mRNA level, and the results show that while the high-intensity strength training could lead to HSP70 production, the HSP70 level maintained constant during the low-intensity endurance training, and that there was a discrepancy between HSP70 and its mRNA.

Competitive rowing is a typical whole-body exercise which affords strength, movement velocity as well as endurance capacity to conquer the standard 2000-m distance (Steinacker 1993). Therefore, a rowing training strategy is usually constructed to improve both strength and endurance ability in addition to the rowing skills. Based on the facts mentioned above,

rowing training is usually complex consisting of several components. In the present study, we tried to construct the training program in two different phases with different aspects, i.e., strength training at high intensity and endurance training at low intensity. We were fully aware of the fact that in a real rowing training program it is hardly possible to clarify the effects of a single training component on the physiological and biochemical responses in working muscles. Our training data show a significant increase in the repetitions at 50% of 1-RM (unfortunately, we failed to repeat the strength measurements before and after ET because of a technical problem) and an augmentation of the submaximal power output after endurance training. However, no significant changes were observed for $\dot{V}O_{2\max}$, P_{\max} as well as P_{1a4} taken as parameters of endurance capacity. One explanation may be the fact that the subjects enrolled in this study were already well-trained, as shown by their relatively high values of the parameters measured. The significant increase of submaximal power output after ET is a training effect of endurance rowing, but is probably also due to a decrease before ET. We have also tried to match the exercise volume between both training phases by adjusting endurance rowing and unspecific training according to the subjects' experience and coaches' calculations, although we were aware that we were not likely to achieve this aim exactly.

Table 3 Training effects. (La_{\max} Blood lactate concentration measured in the ramp test, P_{\max} , P_{1a4} , HR_{1a4} submaximal power output, performance and heart rate at 4 mmol \cdot l $^{-1}$ blood lactate, respec-

tively, measured in the multiple-stage test. Strength expressed as the repetitions at 50% of one maximum repetition, measured on the arms with bench pulls

Parameter	Intense training		Endurance training	
	Before	End	Before	End
$\dot{V}O_{2\max}$ (l \cdot min $^{-1}$)	5.31 (0.62)	5.36 (0.89)	5.23 (0.57)	5.22 (0.61)
La_{\max} (mmol \cdot min $^{-1}$)	10.1 (1.7)	9.9 (2.3)	10.7 (3.6)	10.4 (1.8)
P_{\max} (watts)	468 (68)	467 (57)	448 (50)	484 (71)*
P_{1a4} (watts)	331 (63)	337 (60)	326 (62)	333 (58)
HR_{1a4} (beats \cdot min $^{-1}$)	180 (7)	181 (5)	176 (6)	180 (5)
Strength (reps)	86 (21)	150 (27)**		

* $P < 0.05$, in comparison with that before endurance training

** $P < 0.01$, in comparison with that before the intense training

In the present study, HSP70 increased significantly after high-intensity training, and decreased during the recovery phase. This result is consistent with the results of our previous studies. However, HSP70 remained almost constant during the endurance training, implying that HSP70 was not increased by the endurance training at low intensity. The results of HSP70 mRNA showed that after the high-intensity training there was a steady down-regulation of HSP70 mRNA, and that no re-upregulation could be observed during the endurance training. Therefore, it seemed reasonable to assume that the HSP70 response to endurance training at low intensity was blunted in comparison with that to high-intensity training, and that endurance training at low intensity did not induce HSP70 production in the skeletal muscle in well-trained rowers.

Several factors might account for the reduced HSP70 response to endurance training at low intensity. First, the HSP70 response to endurance training was affected by the prior high-intensity training since the recovery phase between both training phases was too short for complete recovery. It is true that influences of the first training phase on the second phase must exist. However, this may not completely account for the blunted HSP70 response to endurance training since there was no re-upregulation of HSP70 mRNA during the second training and recovery phases. We chose such a short recovery period because of the well-trained status of the subjects studied, in order to elucidate the HSP70 response in well-trained muscle to low-intensity endurance training. Second, different training regimes cause different biochemical and physiological changes which lead to HSP70 induction. It is known that biochemical and physiological changes such as temperature variation, pH decline, lactic acidosis, glycogen depletion and oxidative free radical accumulation can induce HSP70 in stressed cells (Linguist 1986; Locke and Noble 1995). Therefore, the different training regimes performed in this study could have different effects on the biochemistry and physiology. For instance, during the high-intensity training program it was frequently observed that the blood lactate concentration increased clearly with a simultaneous decrease of blood pH and presumably these changes might be more exaggerated in stressed muscle. It is also likely that different training regimes had different impacts on the working muscle's temperature. In summary, high-intensity training may cause more abundant changes which can serve as stronger stimuli for the HSP70 response compared to those caused by endurance training at low intensity.

Interestingly, during recovery following HIT until the end of the study HSP70 remained constant while HSP70 mRNA decreased gradually, indicating a discrepancy between the HSP70 level and the HSP70 mRNA level. This discrepancy might be, on one hand, accounted for by the possibly different kinetics between HSP70 and its mRNA, which are not yet known. Actually, there are studies demonstrating that changes of HSP70 mRNA are not accompanied by

corresponding changes of HSP70, and it seems that the HSP70 mRNA response to stress may take place within hours while those of HSP70 occur within days (Khassaf et al. 2001; Nishi et al. 1993; Puntchart et al. 1996). Such results imply different kinetics at the protein and mRNA level in terms of the HSP70 response, which may be responsible for the observed discrepancy. On the other hand, a discrepancy suggests that post-transcriptional mechanisms may play an important role in regulating the HSP70 expression level. In the present study, it is shown that HSP70 decreased during the 1-week recovery period following HIT, suggesting that this period was long enough for down-regulation of HSP70. However, although HSP70 mRNA decreased steadily during ET and the subsequent recovery, the HSP70 level did not change significantly, and as mentioned above this period must be long enough for the down-regulation of HSP70 to occur, if this were the case. Thus, the posttranscriptional mechanisms must have an impact on the regulation of the HSP70 level. It is also likely that HSP70 synthesis is not only determined by the level of HSP70 mRNA, but also by translational efficacy (or number of copies).

In conclusion, while strength training at high intensity led to HSP70 upregulation, HSP70 was not induced by the endurance training at low intensity, suggesting a blunted HSP70 response in well-trained skeletal muscle to low-intensity endurance training. There is a discrepancy between HSP70 and its mRNA in terms of the HSP70 response to exercise, implying an important role of posttranscriptional mechanisms in the regulation of HSP70 expression.

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