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ORIGINAL ARTICLE

Strength training elevates HSP27, HSP70 and αB-crystallin levels in musculi vastus lateralis and trapezius

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Abstract A single bout of high-force exercise has been shown to increase the muscle levels of heat shock proteins (HSPs). Here, changes in the levels of HSPs after 2 and 11 weeks of strength training with either one or three sets per exercise were examined. Fifteen young men (27 ± 6 years, 182 ± 8 cm and 82 ± 13 kg) were randomized to train either one set in lower-body exercises and three sets in upper-body exercises (1L-3UB), or three sets in lower-body exercises and one set in upper-body exercises (3L-1UB). Biopsies from vastus lateralis and trapezius were obtained before, during (2 weeks) and after 11 weeks of strength training (3 bouts per week). The biopsies were analysed for HSP27 (cytosolic and cytoskeletal fractions) and HSP70 and αB-crystallin (cytosolic fraction). No evidence for an effect of training volume (1 vs. 3 sets) on the HSP response was found. For all subjects combined, HSP27 [186 ± 69% (mean ± SD)], HSP70 (146 ± 51%) and αB-crystallin (184 ± 82%) increased in the cytosolic fraction of vastus lateralis after 11 weeks of training. In the trapezius, the only observed increase was for HSP27 in the cytosolic fraction after 2 weeks of training (149 ± 59%). However, the trapezius contained somewhat higher levels of HSP70 and αB-crystallin than vastus lateralis at baseline. The HSP27 levels in the cytoskeletal compartment did not increase significantly in either muscle. In conclusion, strength training resulted—independent of training volume—in elevated levels of HSP27, HSP70 and αB-crystallin in the cytosolic compartment of the vastus lateralis. In the trapezius, only the cytosolic HSP27 levels were increased with training.

Keywords Stress proteins · Resistance exercise · Hypertrophy · Adaptation to training

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Introduction

Heat shock proteins (HSPs or stress proteins) are functionally versatile proteins that protect cells against damage during stress situations and assist in the synthesis of new proteins (Locke 1997). Single bout ("acute exercise") experiments, applying high force (Thompson et al. 2001, 2003; Feasson et al. 2002; Paulsen et al. 2007) or endurance-type protocols (Puntschart et al. 1996; Febbraio and Koukoulas 2000; Khassaf et al. 2001; Febbraio et al. 2002; Morton et al. 2006; Fischer et al. 2006; Tupling et al. 2007), have demonstrated increased levels of HSP27 and αB-crystallin (the small HSPs) and, especially, HSP70. In short training periods (≤6 weeks), high-intensity exercise has been shown to up-regulate the muscular HSP70 levels in athletes [rowers; (Liu et al. 1999, 2000, 2004)] and in untrained subjects exposed to high-intensity endurance training [mRNA levels; (Vogt et al. 2001)]. In humans,

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changes in the HSP27 levels over a training period have only been investigated in one study, showing an increase (Gjøvaag and Dahl 2006). Changes in αB-crystallin due to training have so far not been studied in humans, although, in contrast to HSP27, αB-crystallin has been found in higher levels in aerobically trained than untrained men (Morton et al. 2008).

Up-regulation of HSPs seems to be a general response to physiological stress, e.g. increased temperature, reduced pH and changes in osmotic pressure, as well as pathological conditions (Locke 1997; Pockley 2003; Nishimura and Sharp 2005). Thus, elevated HSP levels in response to training are intuitively positive, as intensive exercise may stress the working myofibres through high mechanical forces and/or metabolic stress (e.g. reduced pH). Liu et al. (2000) have, in fact, showed that exercise intensity dictated the HSP70 response in trained individuals.

Previous studies on rodents and humans have indicated that the αB-crystallin and HSP27 accumulate (translocate) in cytoskeletal/myofibrillar structures during and/or after high-force exercise (Koh and Escobedo 2004; Paulsen et al. 2007). Paulsen et al. (2009) observed that the small HSPs accumulated on the borders of, and in, disrupted sarcomeres after maximal eccentric exercise with the arm flexors. As the accumulation persisted for days into the recovery period, it was suggested that these HSPs were part of the remodelling process. This assumption has support in data from animal studies that show that small HSPs seem to be involved in both remodelling and hypertrophy of skeletal muscles (Huey 2006; Murlasits et al. 2006; Kawano et al. 2007).

Strength training is well known for inducing muscle growth (Wernbom et al. 2007), but little is known about the myocellular HSP response to this mode of exercise and training in humans. Nonetheless, Folkesson et al. (2008) obtained tissue samples from the vastus lateralis and found signs of HSP27 accumulation on myofibrillar structures after a single bout of traditional strength training exercise (10 × 8 reps at 70% of 1RM), and Liu et al. (2004) have reported increased levels of HSP70 in the vastus lateralis after 3 weeks of resistance exercise (i.e. exercising with 50% of 1RM) in well-trained rowers. Gjøvaag and Dahl (2006) conducted a study in which they measured changes in HSP27, GRP75 and HSP72 in the triceps brachii of beforehand untrained subjects over a 12 week training period (isolated elbow-extension training). When pooling all subjects (training with either 30 or 60% of 1RM and with either low or high volume), an increase was found for all the investigated HSPs. Furthermore, a normalization of the HSP values was found with detraining. In another study, Gjøvaag et al. (2006) compared the HSP response to high-force eccentric training with high-force concentric training with the arm flexors in well-trained subjects. In contrast to the studies mentioned above, decreased levels of HSP70 in the biceps brachii were observed after concentric training, whereas no significant changes occurred after eccentric training. Thus, previous investigations have applied rather unconventional strength training programs (i.e. low-intensity and only concentric or eccentric muscle actions), which means that hitherto the HSP response to traditional strength training with heavy loads (>60% of 1RM) is largely unknown.

Different skeletal muscles may respond dissimilarly to exercise and training. Indeed, the magnitude of muscle damage appears to be greater in the elbow flexors than the knee extensors after eccentric exercise (Jamurtas et al. 2005), and the muscle growth rate is generally higher in the elbow flexors than the knee extensors during strength training (Wernbom et al. 2007). The upper trapezius muscle has been reported to contain hypertrophied fibres in well-trained subjects [cross-sectional study on power lifters (Kadi et al. 1999)], but no previous studies have investigated the myocellular responses in this muscle during a period of strength training in healthy, young male subjects.

In the present study, we aimed at investigating the response of HSP27, HSP70 and αB-crystallin in a lower (vastus lateralis) and an upper (trapezius) body muscle of previously untrained subjects exposed to 2 and 11 weeks of strength training. We hypothesized that (1) strength training would induce muscle growth and increased strength and that these physiological adaptation processes would be accompanied by increased myocellular levels of HSP27, HSP70 and αB-crystallin, and (2) three sets per exercise would result in a stronger response than one set per exercise (i.e. a dose–response relationship). Furthermore, because myofibrillar remodelling appeared to occur already after the initial bouts of high-force exercise (Yu et al. 2003a; Woolstenhulme et al. 2006), we postulated that the HSP27 levels in the cytoskeletal structures would be increased after 2 weeks of training.

Methods

Participants and study design

Fifteen healthy untrained men were randomly assigned to two groups (Table 1). One group performed single sets in the leg exercises and three sets in the upper-body exercises (1L-3UB), while the other group performed three sets in the leg exercises and single sets in the upper-body exercises (3L-1UB). Muscle biopsies were collected from m. vastus lateralis and m. trapezius before and after 2 and 11 weeks of training. The biopsies were analysed for HSP27, HSP70 and αB-crystallin.

Table 1 Characteristics of the subjects

	1L-3UB ($n = 7$)	3L-1UB ($n = 8$)
Age (year)	24 ± 4	28 ± 7
Height (cm)	183 ± 10	181 ± 8
Weight (kg)	82 ± 8	82 ± 16

The study was approved by the Regional Ethics Committee of Southern Norway and complied with the standards set by the Declaration of Helsinki.

Training protocol

In the 11 weeks of training period, participants performed three workouts per week. Each workout consisted of leg press, leg extension, leg curl, seated chest press, seated rowing, latissimus pull-down, biceps curl and shoulder press. All participants were supervised by one of the investigators on all workouts during the three first few weeks and thereafter at least once a week during the entire training period.

Both groups trained three times a week on non-consecutive days. Training load (number of repetition maximum, RM) was altered similarly for the two groups. During the first 2 weeks, both groups trained with 10 RM sets in all exercise, during the third and fourth training weeks they increased the load to 8 RM sets, and during the final 6 weeks they trained with 7 RM sets. Participants were encouraged to continuously increase their RM loads during the intervention and were allowed assistance on the last repetition. Participants were allowed to complete no more than one bout of endurance training per week during the intervention. This was controlled with a training diary.

Strength and whole muscle cross-sectional area

In brief, strength (1RM) was measured in all leg and upper-body exercises before training and after 3 and 11 weeks of training [for details, see (Rønnestad et al. 2007)].

The cross-sectional area (CSA) measures of m. quadriceps were performed before training and after 11 weeks of training. The cross-sectional area was measured on resonance tomography (MR) or computer tomography (CT) images (for details, see Rønnestad et al. 2007).

Biopsy sampling

The biopsies were collected 7–10 days before the training period, 1–5 days (2.8 ± 1.2 days) after the sixth exercise session (2 weeks) and 3–5 days (3.5 ± 0.6 days) after the final exercise session (11 weeks).

A 5 mm Pelomi needle (Albertslund, Denmark) with manual suction was used to obtain tissue samples (3 × 30–100 mg) from the midsection of the vastus lateralis and from the upper part (pars superior) of the trapezius. Participants lay in a supine position while the procedure was performed under local anaesthesia (Xylocaine adrenaline, 10 mg/ml + 5 µg/ml; AstraZeneca, Södertälje, Sweden). During the biopsy procedure, each needle insertion was placed 3 cm from the previous insertion to avoid affected tissue from earlier biopsies. Muscle samples were rinsed in saline and divided into smaller pieces selected for immunohistochemistry and homogenization (protein measurements). Thereafter, the pieces were frozen in isopentane on dry ice and stored at −80°C until further analyses.

Fibre typing and fibre area

Five micrometer-thick cross sections were cut using a cryostat microtome (LEICA CM 3050, Mannheim, Germany) and mounted on glass slides. The cross sections were air dried at room temperature overnight and then stored at −80°C. Skeletal muscle fibre types and areas were characterized by immunohistochemistry using monoclonal antibodies against myosin heavy chains specific to humans (all from Santa Cruz Biotechnology, Inc.). The combination of A4.951 [fibre type I and I/II), N2.261 (fibre type I/II, IIa and IIax (weakly)] and A4.74 [fibre type IIa, IIax (weakly) and IIx (weakly)], applied on three serial cross sections, allowed for the recognition of type I, I/II (hybrid), IIa, IIax (hybrid) and IIx fibres. Images were captured with the Spot software (3.4 for Windows) operating the camera (Spot, Insight Color, Diagnostic Instruments, Inc.) and a microscope (Nikon Eclipse E4000). For fibre-type distribution, all fibres on the sections were included; and for the fibre area analysis, 100 fibres of each type were included.

Western blotting of heat shock proteins

Muscle samples (50 mg) were homogenized after the supplier's prescription using a subcellular extraction kit (ProteoExtract® Subcellular Proteome Extraction Kit, Merckbiosciences, cat. no. 539790, Darmstadt, Germany) to obtain two different fractions, containing proteins from the cytosol and the cytoskeleton. Protein content was determined using the RC/DC protein assay kit 1 (Bio-Rad, cat. no. 500-0121, San Diego, CA, USA).

Equal amounts of protein from the cytosolic fraction (approximately, 15 µg per well) were separated on 10% SDS-PAGE gels for 35 min at 200 V and transferred for 90 min at 30 V to PVDF membranes (Bio-Rad, cat. no. 162-0177) in NuPAGE Transfer buffer (20×, cat. no. NP 0006-1) with 10% methanol and 500 µl NuPAGE Antioxidant (cat no. NP0005).

After transfer, membranes were stained with PonceauS and gels were stained with Coomassie Blue to evaluate loading and transfer conditions. The membranes were thereafter blocked with 5% non-fat milk in TBS-T (0.1% Tween 20) overnight. After washing, the membranes were incubated for 2 h with a primary antibody against HSP70 (monoclonal; SPA810, Stressgen Bioreagents, Ann Arbor, MI, USA) and αB-crystallin (monoclonal; SPA222, Stressgen). Blots were washed and then incubated with a horseradish peroxidase-conjugated secondary antibody. After a final wash, protein bands were detected using chemiluminescence (Super Signal West Dura, Pierce Biotechnology cat. no. 34076, Rockford, IL, USA). Finally, signal density was measured using a Kodak image station (Kodak 2000R), with Kodak 1D analysis software (version 3.6.1), Rochester, NY, USA).

ELISA of HSP27

HSP27 in the cytosolic and cytoskeletal fractions was measured with a home-made double-antibody sandwich ELISA, utilizing a monoclonal capture antibody (25 ng/well; SPA800, Stressgen Bioreagents, Ann Arbor, MI, USA), a polyclonal detection antibody (SPA803, Stressgen) and a horseradish peroxidase-conjugated secondary antibody. The assay was performed in high-binding polystyrene microtiter plates (no. 3590, Costar, Corning, NY). Tetramethylbenzidine (no. CL07 Calbiochem, Merck KGaA, Darmstadt, Germany) was used as substrate, and 2 N sulphuric acid was used as stop solution. Recombinant HSP27 (SPP715, Stressgen) was used as standard (0.78–25 ng/ml). All samples were analysed in triplicate and diluted 1:100 or 1:300 (1–3 µg total protein) and optical density (OD) was read at 450 nm (analytic CV <10%).

Statistical analyses

All values presented in the text and the figures are mean ± SD. A two-way ANOVA (time and group) was used to investigate group changes (1 vs. 3 sets) in 1 RM strength and whole muscle cross-sectional area. For all subjects (groups combined; $n = 15$), changes in fibre type distribution and myofibre cross-sectional area were analysed with a one-way repeated measure ANOVA (Dunnett's post-test), while all HSP data were analysed with Friedmans test (Dunns post-test) to test for changes from pre- to 2 and 11 weeks of training. The HSP pre-values in the vastus lateralis and trapezius were compared with a paired *t* test. Correlation analyses were performed by the methods of Pearson or Spearman (depending on the distribution/normality of the data). The level of significance was set at $p \leq 0.05$. Microsoft Excel 2003, GraphPad Instat and Prism (San Diego California USA, http://www.graphpad.com) were used for the statistical analyses.

Results

During 3 and 11 weeks of resistance exercise, both the 1Leg-3Upper-Body (1L-3UB) group and the 3L-1UB group increased strength (1RM) and the cross-sectional area of m. quadriceps (Table 2). The 3L-1UB group increased 1RM in leg extension significantly more than the 1L-3UB group, but not in shoulder press. The cross-sectional area of quadriceps tended to increase more in the 3L-1UB group than the 1L-3UB group (group differences: $p = 0.15$). In the study by Rønnestad et al. (2007), in which six more subjects were included (in addition to the 15 reported on herein), the quadriceps cross-sectional area in the 3L-1UB group was reported to increase significantly more than in the 1L-3UB group (11 ± 4 vs. 8 ± 4%, $p < 0.05$; $n = 10$ and 11, respectively).

Exercising leg muscles with three sets were more effective than one set in promoting strength and muscle growth (tendency), but group differences in the protein levels of HSP27, HSP70 or αB-crystallin were found in neither in m. vastus lateralis nor m. trapezius (Table 3).

Before training, the cytosolic protein levels of HSP70 and αB-crystallin tended to be higher in the trapezius than vastus lateralis (Fig. 1). This was not as apparent for HSP27 (Fig. 2).

For all subjects combined (1L-3UB and 3L-1UB), we found increased levels of cytosolic HSP27, HSP70 and αB-crystallin in the vastus lateralis (Figs. 2, 3). For trapezius,

Table 2 Percentage changes (from pre-values) in 1RM and quadriceps cross-sectional area (CSA) for the 1L-3UB and the 3L-1UB groups

	1L-3UB		3L-1UB	
	3 weeks	11 weeks	3 weeks	11 weeks
1RM shoulder press (%)	7 ± 2**	33 ± 11**	12 ± 6**	33 ± 10**
1RM leg extension (%)	10 ± 6**	31 ± 10**	16 ± 4**	49 ± 13**‡
CSA-quadriceps (%)	–	7 ± 4**	–	11 ± 4**

** Difference from pre-values, $p < 0.01$

‡ Group difference, $p < 0.01$

Table 3 Percentage changes (from pre-values) of the cytosolic protein levels of HSP27, HSP70 and αB-crystallin in the m. trapezius (pars superior) and m. vastus lateralis for the 1L-3UB and the 3L-1UB groups

	HSP27		HSP70		αB-crystallin	
	2 weeks	11 weeks	2 weeks	11 weeks	2 weeks	11 weeks
Trapezius						
1L-3UB (%)	144 ± 39	123 ± 40	158 ± 65	82 ± 35	138 ± 51	213 ± 258
3L-1UB (%)	153 ± 75	171 ± 111	126 ± 73	140 ± 88	153 ± 126	190 ± 199
Vastus lateralis						
1L-3UB (%)	176 ± 76	196 ± 84	197 ± 138	154 ± 49	218 ± 132	201 ± 107
3L-1UB (%)	136 ± 36	177 ± 57	137 ± 25	140 ± 54	145 ± 48	170 ± 58

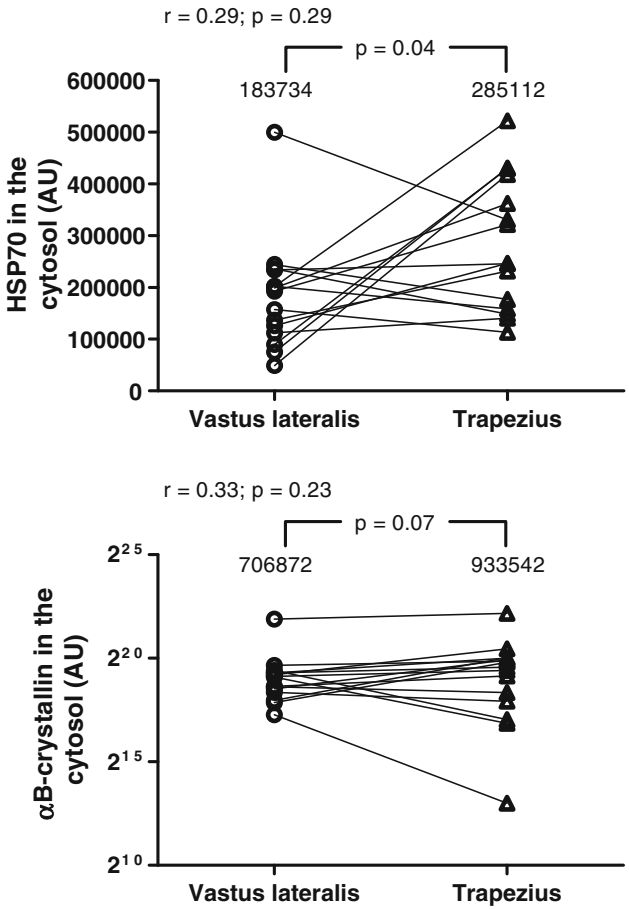

Fig. 1 Cytosolic protein levels (Western blotting; *AU* arbitrary units) of HSP70 and αB-crystallin in the m. vastus lateralis and m. trapezius (pars superior) before training (pre-values). Two data points connected with a line show individual data. The mean value for each muscle is given above each column of data points. The correlations between the protein levels in the two muscles are given by the *r* values. $N = 15$. Note the log2 scale *Y*-axis for αB-crystallin

the only detected increase was for HSP27 in the cytosolic fraction after 2 weeks of training (Figs. 2, 3). In the vastus lateralis, the cytosolic levels of HSP27 increased by 154 ± 59 and 186 ± 69% after 2 and 11 weeks compared to pre-values (both $p < 0.05$), while HSP70 increased by 165 ± 97% (ns) and 146 ± 51% ($p < 0.05$) after 2 and 11 weeks. αB-crystallin increased by 179 ± 100% (ns; 2 weeks) and 184 ± 82% ($p < 0.05$; 11 weeks).

HSP27 was measured in both the cytosolic and cytoskeleton fractions of samples from the vastus lateralis and trapezius (Fig. 2). No statistical significant changes were found in the cytoskeleton fraction (Friedmans test with Dunns post-test). However, some subjects seem to respond to training with an increase, especially after 2 weeks of training (Wilcoxon rank test: $p = 0.013$ and 0.14 for the vastus lateralis and trapezius, respectively; see individual values in Fig. 3).

The portion of type I fibres were higher in the trapezius than vastus lateralis ($p < 0.01$; Table 4). There were no statistical changes in the distribution of type I and type II fibres with training (Table 4). For all subjects combined, the numbers of hybrid fibres, i.e. I/II and IIa/IIx and IIx, were generally low. Combined, hybrid fibres and type IIx fibres accounted for only 1.3 ± 1.3 and 4.9 ± 5.4% of all the numerated fibres at baseline in the trapezius and vastus lateralis, respectively. After 11 weeks of training, hybrid fibres and type IIx fibres accounted for 0.8 ± 1.0% of the fibres in the trapezius and 1.2 ± 2.2% of the fibres in the vastus lateralis. The decrease in the vastus lateralis ($p < 0.01$) was mainly due to a reduction in the numbers of IIax and IIx fibres.

With no group differences, the mean cross-sectional area of type II myofibres from the vastus lateralis was increased after 11 weeks of training (20 ± 20%, $p < 0.05$), while no significant changes were found in the trapezius (Table 4).

No consistent inter-subject correlations were found between measurements for hypertrophy and the HSP response.

Discussion

Strength training for 11 weeks increased the cytosolic levels of HSP27, HSP70 and αB-crystallin in m. vastus lateralis. The changes in m. trapezius (pars superior) were less evident, with only a significant increase in cytosolic HSP27 after 2 weeks of training. However, the values of HSP70

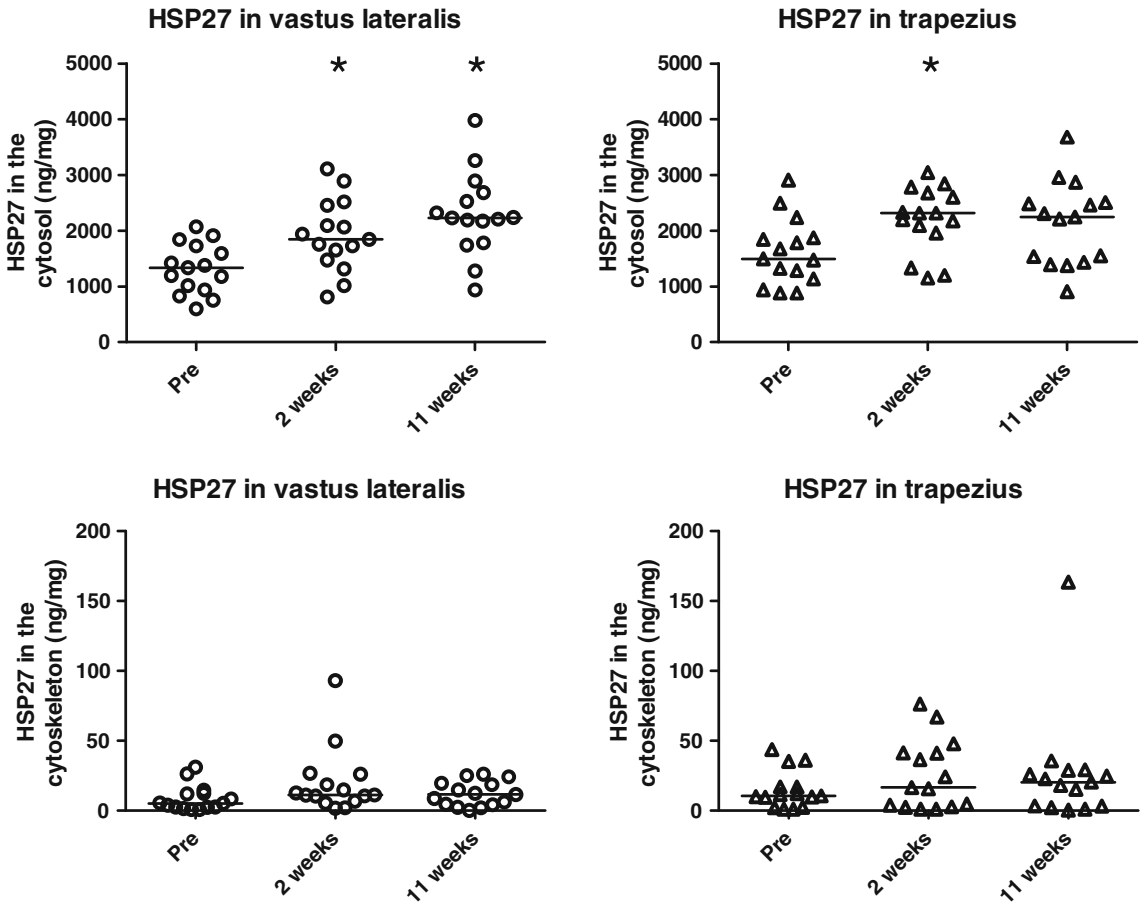

Fig. 2 Protein levels (ELISA) of HSP27 in the cytosolic and cytoskeleton fractions of tissue samples from the m. vastus lateralis and m. trapezius (pars superior) before (pre) and after 2 and 11 weeks of strength training. $N = 15$; *denotes differences from pre-values; $p < 0.05$

and αB-crystallin were slightly higher in the trapezius than in the vastus lateralis at baseline. In contrast to the cytosolic levels, there was no consistent increase of the HSP27 levels in the cytoskeleton. There seemed to be no significant effect of training volume on the HSP response (1 vs. 3 sets).

Based on previous studies with various types of training (Liu et al. 2006), we observed an expected increase of the HSP70 levels in the vastus lateralis after 2 and 11 weeks with strength training. In contrast, the HSP70 levels in the trapezius were unchanged. Hitherto, αB-crystallin has been found to be elevated in endurance trained males [cross-sectional study; (Morton et al. 2008)], in mice exposed to a period of running wheel training (Huey and Meador 2008) and in response to a single bout of unaccustomed eccentric exercise (Feasson et al. 2002; Paulsen et al. 2009). Thus, we report novel data in virtue of increased levels of αB-crystallin in the vastus lateralis after 11 weeks of strength exercise in humans. As for HSP70, the αB-crystallin levels did not increase in the trapezius. HSP27 did, however, increase in both the vastus lateralis and trapezius, although significantly only after 2 weeks in the trapezius. This latter finding is in accordance with that of Gjøvaag and Dahl (2006), showing elevated HSP27 levels in the triceps brachii after resistance training. Apparently, as HSP27 has been reported to be up-regulated in the recovery phase after single bouts of eccentric exercise (Thompson et al. 2001, 2003; Feasson et al. 2002; Paulsen et al. 2007, 2009), but not after endurance exercise (Morton et al. 2006, 2008, 2009a), HSP27 is most responsive to muscle damage and/or remodelling [Koh 2002; Koh and Escobedo 2004; Paulsen et al. 2009) and hypertrophy (observed in animal models; (Huey 2006; Murlasits et al. 2006; Kawano et al. 2007; Frier and Locke, 2007)]. Because significant remodelling and hypertrophy were expected during the first 2 weeks (Staron et al. 1994; Abe et al. 2000; Yu et al. 2003b; Miller 2007), we predicted accumulation of HSP27 in the cytoskeletal/myofibrillar structures; but no significant changes were found. After unaccustomed eccentric exercise, we have previously reported a strong accumulation of the small HSPs in cytoskeletal structures, lasting for more than 4 days (Paulsen et al. 2007). The biopsies after 2 weeks of training (6 sessions) were collected ~3 days after the last exercise session, which suggests that the degree of damage (myofibrillar disruptions) and/or the extent of remodelling

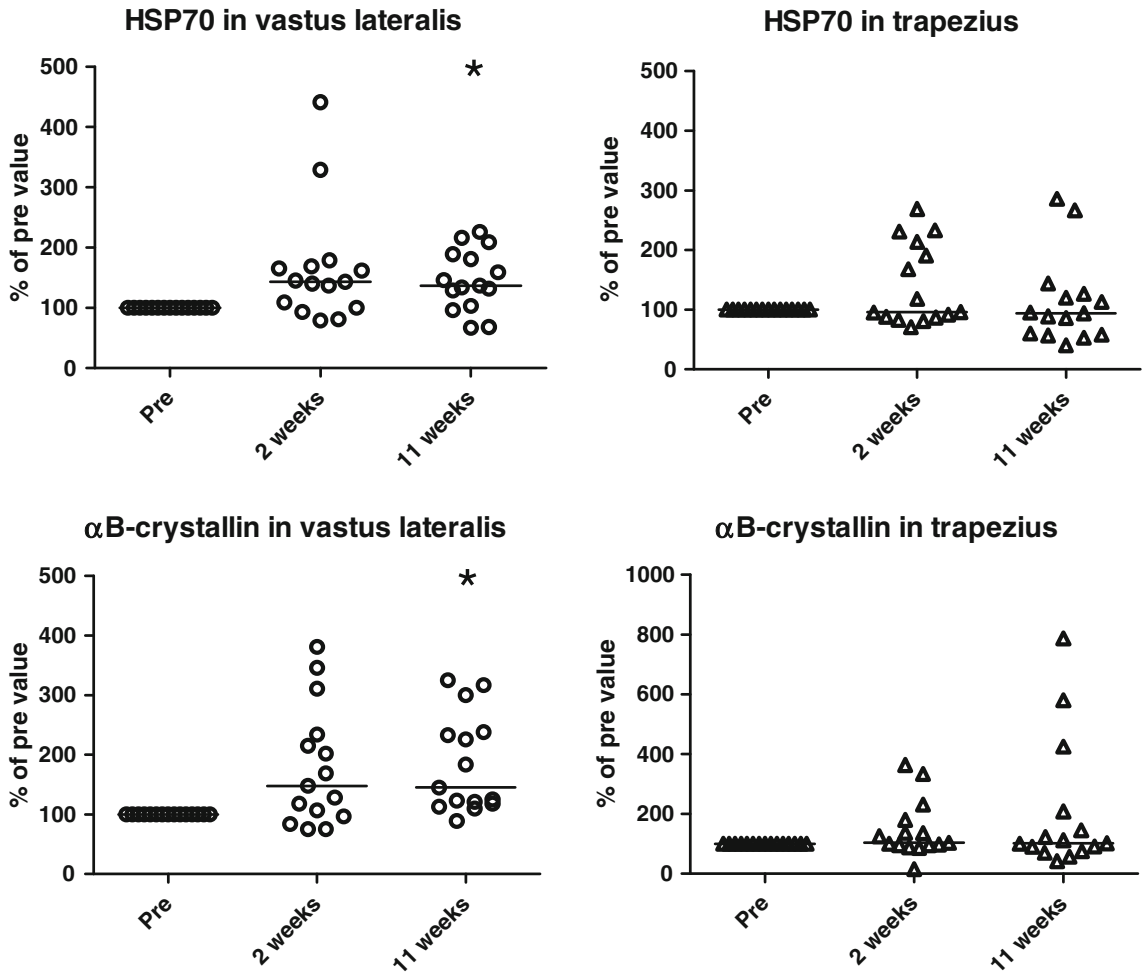

Fig. 3 Cytosolic protein levels (Western blotting) of HSP70 and αB-crystallin in the m. vastus lateralis and m. trapezius (pars superior) after 2 and 11 weeks of strength training. Note the different *Y*-axis on the graph for αB-crystallin in m. trapezius. The values are relative to the pre-values. $N = 15$; *denotes differences from pre-values; $p < 0.05$

Table 4 Proportion (%) of type I myofibres and mean cross-sectional area (CSA) of type I and II fibres in m. trapezius (pars superior) and m. vastus lateralis. $N = 15$

	Pre	2 weeks	11 weeks
Trapezius			
% type I fibres	53 ± 14	55 ± 13	53 ± 17
Type I CSA (μm^2)	5,252 ± 966	5,671 ± 1,291	5,368 ± 1,143
Type II CSA (μm^2)	6,017 ± 1,841	6,286 ± 1,789	6,826 ± 1,651
Vastus lateralis			
% type I fibres	45 ± 12	38 ± 12	39 ± 12
Type I CSA (μm^2)	5,599 ± 1,384	5,434 ± 1,637	5,735 ± 1,190
Type II CSA (μm^2)	6,687 ± 1,758	6,975 ± 2,450	7,792 ± 1,421*

* Difference from pre-values, $p < 0.05$

were minor at this time point. This is in fact in agreement with the repeated-bout phenomenon (McHugh 2003) and studies showing that the recovery time for a bout of resistance exercise is 1–2 days in trained individuals (Raastad and Hallen 2000; Judge and Burke 2010). Hence, any translocation of the small HSPs to the cytoskeleton during exercise is probably followed by a quite rapid shedding of the HSPs (relocation to the cytosol) when the degree of myofibrillar damage during exercise is sparse—in contrast to unaccustomed maximal eccentric exercise (Paulsen et al. 2007).

Huey (2006) used a rat overloading model to demonstrate that the HSP25 response was associated with hypertrophy (HSP25 in rats is homologues to HSP27 in

humans). We observed that the HSP27 levels in the vastus lateralis and trapezius increased during the first 2 weeks and seemingly levelled off towards 11 weeks. We identified, however, no strong inter-subject correlations between the HSP response and muscle growth. The lager increase in strength (1RM) and the tendency towards more muscle growth in the group that exercised with three sets on the lower body were not reflected in a stronger HSP response. However, the HSP response appeared stronger in the vastus lateralis than trapezius, which corresponded with the observed myofibre hypertrophy in vastus lateralis, but not in trapezius.

The lack of detectable myofibre hypertrophy and a minor HSP response in the trapezius, compared with vastus lateralis, are likely to be a reflection of the training loading. Indeed, pars superior of the trapezius—from where the biopsies were obtained—can be considered as a secondary muscle in both the rowing exercise and the shoulder press exercise, while vastus lateralis is clearly a prime mover in both the leg press and the knee-extension exercise. Thus, the degree of stress stimuli during the strength training sessions are seemingly important for both hypertrophy and the HSP response. The relationship between this adaptation processes is, however, unclear. Considering the indifferent HSP response to one and three sets per exercise, the HSP response appears to be more related to training intensity than volume.

Another plausible explanation for the stronger HSP response in the vastus lateralis than trapezius is the fact that the trapezius seemed to contain slightly higher basal levels of HSPs than vastus lateralis. The basal content of HSPs has been found to be negatively related to the increases in response to exercise and training (Gjøvaag and Dahl 2006; Morton et al. 2009b). Higher baseline levels may be linked to the fibre-type distribution, which differed between muscles: the trapezius contained more type I fibres than the vastus lateralis. Indeed, studies on rodents have demonstrated the highest basal levels of both αB-crystallin (Atomi et al. 2000; Golenhofen et al. 2004) and HSP70 (Locke et al. 1991; Bombardier et al. 2009) in type I dominated muscles.

A limitation in the present study is the low number of subjects in each group (1 vs. 3 sets). Thus, we have low statistical power and may have missed a true volume effect. On the other hand, our data are in line with observations of others: Liu et al. (2000) demonstrated that the HSP70 increase in the vastus lateralis was more related to training intensity than volume, while Gjøvaag and Dahl (2006) found no clear effect of either intensity or volume on the HSP27 and HSP70 response in the triceps brachii.

A challenge in the investigation of the HSP response to exercise is the generally high individual variability; e.g. we found rather large individual variations in the αB-crystallin response (see Table 3). This is a recognized phenomenon (Morton et al. 2009b), which could be explained by several factors, such as baseline levels and age. However, methodological variations in the applied assays (Western blotting) may also explain some of the variation. Hence, applying high-sensitive, quantitative ELISAs for HSPs, such as our HSP27 assay (Paulsen et al. 2007), is recommended for future studies of myocellular HSP levels.

Conclusion

Eleven weeks of strength training, exercising with either one or three sets, resulted in muscle growth and strength increase, which were accompanied with elevated levels of HSP27, HSP70 and αB-crystallin in the m. vastus lateralis. In m. trapezius (pars superior), only HSP27 increased. On the other hand, compared to vastus lateralis, trapezius contained slightly higher levels of HSP70 and αB-crystallin before exercise. The increased HSP27 levels seemed restricted to the cytosolic compartment, as no significant changes were found in the cytoskeleton.

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Conflict of interest None.

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