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Resistance training increases heat shock protein levels in skeletal muscle of young and old rats

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Abstract

Heat shock proteins (HSP) HSP72, HSC70 and HSP25 protein levels and mRNA levels of *HSP72* genes (*Hsp72*−1, *Hsp72*-2, *Hsp72*−3) and *HSC70* were examined in tibialis anterior muscles from young and old rats following 4.5 weeks of heavy resistance exercise. Young (3 months) ($n=10$) and old (30 months) ($n=9$) rats were subjected to 14 sessions of electrically evoked resistance training using stretch-shortening contractions of the left limb that activated the dorsiflexor muscle group, including the tibialis anterior muscle, while the right side served as the intra-animal control. Muscle wet weight of the left tibialis anterior increased by 15.6% in young animals compared to the untrained right side, while the aged rats demonstrated no significant hypertrophy based on muscle wet weight. There were no differences in mRNA expression between the control and experimental muscles in either the old or the young animals for any of the four genes examined. On the other hand, HSP72 levels as determined by Western blots were significantly ($p<0.01$) higher (968.8 and 409.1%) in the trained as compared to the contralateral control muscle in young and old animals, respectively. HSP25 expression was increased significantly ($p<0.01$) by training in muscles of young rats (943.1%) and old rats (420.3%). Moreover, there was no training by age interaction for HSP72, while a significant age and training by age effects were found in muscles for HSP25. There was no change in HSC70 protein expression in response to the training intervention in either age group. SOD-1 enzyme level increased by 66.6% in the trained muscles of the young rats, while this enzyme was 33% lower in trained muscles compared to the untrained control side in old rats. Moreover, a significant ($p<0.05$) training by age interaction was found for SOD-1 enzyme levels. This study suggests that fast contracting muscles in young and old animals are capable of increasing HSP expression in response to high intensity contractile stress. Furthermore, the data are consistent with the hypothesis that higher levels of oxidative stress in muscles of old animals limit HSP levels and/or function in response to high intensity contractile stress.

Keywords: Aging; Exercise; Chaperone proteins; Stress proteins; Oxidative stress

1. Introduction

Heat shock proteins (HSP's) are the products of highly conserved genes. These proteins are categorized by their molecular weights and show a high degree of homology among various species, which suggests that their function is essential for the normal physiology. They function mainly in cellular protection during stressful events, such as increased temperature, hypoxia, glucose deprivation, and cellular damage. Physical exercise has also been shown to increase HSP expression independently from other stressors (Kilgore et al., 1998; Locke and Noble, 1995; Locke et al., 1990).

The ability of old cells to respond to heat stress is diminished, leading to compromised cellular homeostasis (Fargnoli et al., 1990; Heydari et al., 1994; Liu et al., 1996; Powers et al., 2004). Furthermore, aging appears to attenuate the heat shock response in tissues from aged animals including the myocardium (Demirel et al., 2003; Locke and Tanguay, 1996; Powers et al., 2004). Nevertheless, these observations are not universal because Locke (2000) found that skeletal muscle of old animals retains the capacity to accumulate HSP72 to a similar extent as adult ones when subjected to heat shock. Moreover, aerobic exercise training was shown to induce a similar increase in HSP72 in the oxidative muscles of young

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and old rats, while the HSP response was reduced in glycolytic muscles of aged animals (Naito et al., 2001). The induction of HSP's in skeletal muscle of old animals in response to high intensity contractile stress has not been clearly established.

It is possible that while the acute stress response is reduced in muscles of old animals, they still retain the ability to adapt to long-term challenges. In support of this notion, Vasilaki et al. (2003, 2002) reported unchanged HSP72 levels in muscle of old rats and unaltered HSP25 expression in muscles of aged mice following acute isometric contractions, while long-term exercise training increased HSP levels both in the liver and in the skeletal muscle of old rats (Kregel and Moseley, 1996; Naito et al., 2001). Although increases in HSP expression have been reported in muscles of young rats after overload (Locke et al., 1994) and in muscles from both young and old animals after endurance exercise (Naito et al., 2001), the effect of resistance training on HSP induction in old animals has never been investigated.

A direct role of HSP72 in protection of skeletal muscle was demonstrated both in cultured myotubes (Maglara et al., 2003) and in transgenic animals (McArdle et al., 2004). For example, animals that overexpress HSP72 by ~20 times had a 63% lower force deficit as compared to wild type animals 3 days after a session of lengthening contractions, followed by complete force recovery by day 14 (McArdle et al., 2004). Overexpression of HSP72 attenuates force deficit in response to injury in both adult and old transgenic mice (McArdle et al., 2004). These data provide strong evidence for the protective role of HSP72 in preventing damage and force loss in skeletal muscle. In contrast to injury protocols, it is not clear if aging reduces the level of HSP expression in response to a given resistance protocol that is not designed to induce widespread injury.

In this study, we tested the hypothesis that aging would attenuate basal HSP levels in fast contracting skeletal muscles exposed to long-term resistance exercise. We speculated that this would be associated with reduced hypertrophy in muscles of old animals as compared to muscles of young animals as a result of lower levels of chaperone function and lower cytosolic protein protection occurring from lower HSP levels in the muscles from old rats. We studied the tibialis anterior because this muscle (i) hypertrophies in response to resistance training in young animals (Cutlip et al., 2006), (ii) has attenuated hypertrophy in old animals in response to resistance loading (Cutlip et al., 2006), which is consistent with a reduced hypertrophy observed with aging in other models of overload (Carson et al., 1995; Carson et al., 1995; Degens and Alway, 2003), and (iii) primarily contains fast type II myosin isoforms (Isfort et al., 2002) and therefore may have lower levels of HSP in response to exercise with aging relative to more oxidative muscles of young animals (Naito et al., 2001). We examined the protein levels of HSP72, HSC70 and HSP25 in skeletal muscles of young and old rats that were exposed to non-injurious chronic heavy resistance exercise via stretch-shortening contractions. Moreover, we were interested in whether mRNA levels of various *HSP72* genes (*Hsp70*-1, *Hsp70*-2, *Hsp70*-3) were differentially induced in response to the training intervention both in muscles of young and old animals. In this study, we show that muscles of old animals are capable of substantial increases in HSP levels in response to high intensity contractile stress. The data are consistent with the hypothesis that higher levels of oxidative stress in muscles of old animals limit HSP levels and/or function in response to high intensity contractile stress.

2. Methods

2.1. Animals

Male ($n=19$) male Fisher$_{344}\times$BN F1 rats were obtained from the National Institute of Aging colony. Young (3 months) ($n=10$) and old (30 months) ($n=9$) rats were housed in an AAALAC accredited animal quarters. Temperature and light/-dark cycles were held constant for all animals and food and water were provided ad libitum. After 1 week of acclimatization, all animals were subjected to a standardized experimental protocol with a prior approval of the Animal Care and Use Committee.

2.2. Electrically evoked resistance training

Rats were anesthetized with 2% isoflurane and placed supine on the heated $x-y$ positioning table of a custom-built rodent dynamometer with improved software and data acquisition system (Cutlip et al., 1997). Position was controlled by a Unidex 100 motion controller (Aerotech Inc., Pittsburgh, PA) for precise control of a brushless DC servomotor (1410 DC, Aerotech Inc., Pittsburgh, PA). Indirect muscle stimulation was achieved by electrically activating the dorsiflexors. Fine needle electrodes were positioned adjacent to the common peroneal nerve. Square wave electrical impulses were generated from a muscle stimulator (Model SD9, Grass Medical Instruments, Quincy, MA) and delivered at 120 Hz stimulation frequency, 0.2 ms pulse duration, and 4 V.

2.3. Exposure protocol

The stretch-shortening contractions were administered in eight sets of 10 repetitions each at 2-min intervals between each set. Within each set, there was 2 s rest between each stretch-shortening contraction to reduce the effect of fatigue. The dorsiflexor muscles were fully activated for 100 ms duration during each repetition, and the eccentric contraction phase was initiated with a movement velocity of the load cell fixture of 60° s^{-1} over the prescribed range of motion of 90–140° ankle angle The load cell fixture was then immediately returned in the concentric phase at 60° s^{-1} to the starting position of 90° ankle angle. The dorsiflexor muscles were then deactivated 300 ms later. Total stimulation time per repetition was 2.06 s. The total stimulation per set was 21.3 s.

2.4. Isometric force measurements

A pre-test isometric contraction (pre-test isometric force) was measured on the dorsiflexor muscle group at an ankle angle of 90° using 300-ms stimulation duration. An isometric

Table 1
Primer pairs used for real-time PCR analysis of heat shock protein genes

Gene	ACCN	Length (bp)
HSC70-sense 5′ CGA CAA TGC AGT GCC ACA AGC 3′	Y00054	122
HSC70-anti 5′ TGA ACC TTG GCG ACT ACT GAC TGC 3′		
HSP70-1-sense 5′ CGA GGG CAT CGA CTT CTA CAC G 3′	X77207	129
HSP70-1-anti 5′ ATC TGC GCC TTG TCC AGC TTG 3′		
HSP70-2-sense 5′ CTC GTC CAT GGT GCT GAC CAA G 3′	X77208	108
HSP70-2-anti 5′ CCG CTG CGA GTC GTT GAA GTA G 3′		
HSP70-3-sense 5′ AGG ACT CAA CGT GCT GCG AAT C 3′	X77209	137
HSP70-3-anti 5′ TCA GGA TGG ACA CGT CGA ACG 3′		

ACCN, GeneBank accession number. Length, the base pair (bp) length of the corresponding PCR product.

contraction (post-test isometric force) was also performed immediately following the exposure protocol that consisted of eight sets of 10 stretch-shortening contractions.

2.5. Dynamic force test

A single stretch-shortening contraction was measured on the dorsiflexor muscle group 2 min preceding and following treatment with the exposure protocol as previously described (Cutlip et al., 2004). This test was used to evaluate the ability of the dorsiflexors to generate dynamic forces and to perform work during dynamic stretch-shortening. The stretch-shortening contraction was performed by activating the dorsiflexor muscles for 300 ms then moving the load cell fixture from 70 to 140° at an angular velocity of 500° s^{-1}. The load cell fixture was immediately returned to 70°, also at 500° s^{-1}. Activation was continued for 300 ms after cessation of the movement.

2.6. Chronic exposure

The exposure protocol and performance tests were administered three times per week for a total of 14 exposures over a 4.5-week period.

2.7. Muscles examined

The rats were sacrificed with an overdose of pentobarbital sodium (0.5 mg/g) 24 h following the last stretch-shortening exposure. The tibialis anterior muscle from control and experimental muscles was removed, blotted, weighed, and frozen in liquid nitrogen. The muscles were stored at −80 °C until used for RT-PCR and Western blot analyses.

2.8. RT PCR

RNA was extracted from ~70 mg of muscle in 1 ml of TriReagent (Sigma Chemical Co., St Louis, MO). Two micrograms of RNA was reverse transcribed, using random primers (Invitrogen, Carlsbad, CA) and Superscript III enzyme (Invitrogen, CA). The cDNA was amplified by real-time PCR (ABI Prism, Applied Biosystems, Foster City, CA) using the QuantiTech SYBR Green PCR kit (Qiagen, Valencia, CA), using primers designed from published sequences in GeneBank (Table 1). These methods are conducted routinely in our laboratory (Alway et al., 2002).

2.9. Western blot protocol

Approximately 50 mg of each tibialis anterior muscle was homogenized in 50 mM Tris–HCl (pH 7.8), containing a protease inhibitor coctail (1:200), (Sigma Chemical Company, St Louis, MO, USA, P8340). The protein content of the homogenate was measured using the BioRad DC assay (BioRad, Hercules, CA, 500–0112). Twenty-five micrograms of protein was loaded on a 12% SDS-polyacrylamide gel, and after 1 h of electrophoresis at 120 V, the proteins were transferred to a nitrocellulose membrane for 1.5 h at 200 mA. Equal loading of the lanes was verified by staining the membranes with ponceau red (BioRad, CA). Following a 2-h blocking step in 5% milk in TBS, the membrane was incubated in anti-HSP70 (Stressgen, Victoria, Canada, SPA-812) and anti-HSP25 (Stressgen, Victoria, Canada, SPA-801), (1:2500 in 2% milk in TBST) overnight at 4 °C and after incubation in goat anti-rabbit secondary antibody (1:3000) for 1 h the proteins were detected using a chemiluminescent detection method (ECL Western blotting kit, Amersham, Piscataway, NJ USA, RPN2106). Immediately, after this procedure the HSP70 antibody was stripped from the membrane and then the membrane was incubated with an AP-conjugated anti-HSC70 antibody (Stressgen, SPA-815AP) and detected using a different chemiluminescent method (BioRad, CA). The SOD-1 antibody (Santa Cruz, CA) was used in 1:200 dilutions. The signals were visualized by exposing the membranes to X-ray films (BioMax MS-1, Eastman Kodak, Rochester, NY) and digital records were captured with a Kodak 290 camera. The resulting bands were quantified as optical density (OD)×band area by a one-dimensional image analysis system (Eastman Kodak, Rochester, NY) and recorded in arbitrary units (Siu and Alway, 2005; Siu et al., 2004).

2.10. Statistics

ANOVA analyses were conducted to examine training, age and interaction affects for muscle wet weight, force and protein levels. The comparative C_T method was used to quantify gene expression. The C_T for 18S C_T was subtracted from the corresponding value of the gene of interest for that sample

to give a ΔC_T value. ΔC_T values were determined by subtracting the control muscle ΔC_T value from the trained muscle ΔC_T values. Descriptive data are presented as means ± SD. Pearson product correlations were performed between HSP, muscle force and SOD-1 levels. Statistical analyses were performed using the statistical software SPSS version 10.0 and significance levels were set at $p < 0.05$.

3. Results

3.1. Bodyweight, muscle weight and force

The bodyweight in the young rats was 331.7±4 g before training and 342±5 g after training. This represented a 3.3% increase in the bodyweight of the young rats over the course of the study. Before exercise, the bodyweight of the old rats was 522±5 g and after the 4.5 exercise period, their bodyweights averaged 482±5 g. This represented a ~8% loss in bodyweight in the old rats over the experimental period. All rats survived the training period. We did not observe any marked side effects to repeated anesthesia in old or young rats (marked loss of appetite, etc.). Nevertheless, because the old animals lost some body weight over the course of the study, we cannot fully rule out this possibility.

Muscle wet weight of the left tibialis anterior increased by 15.6% in young animals compared to the untrained right side, while the aged rats demonstrated no hypertrophy based on muscle wet weight. Relative to isometric force before the study, there was a 32.7% increase in isometric force for the young group after 4.5 weeks, while the old group had a decrement of 20.2%. A significant training by age interaction was found for tibialis anterior wet weight ($p < 0.05$) and for isometric force ($p < 0.01$). The complete set of pre- and post-training functional measurements, such as force and work data were reported elsewhere (Cutlip et al., 2005). These data show that muscle function increased in response to adaptations to stretch-shortening contractions in young animals but the same protocol failed to increase work or force parameters in muscles from old animals. In fact, the data show an aging-associated maladaptation to repetitive loading in muscles of old animals.

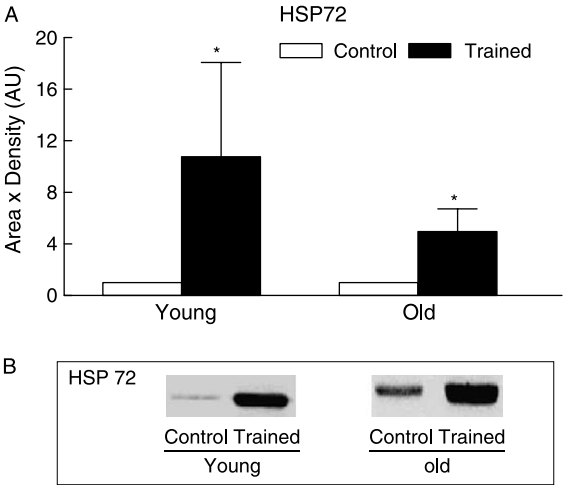

Fig. 1. (A) HSP72 protein expression in control and hypertrophied muscles from young ($n=10$) and old ($n=9$) rats, expressed relative to control values. Data are presented as arbitrary units (AU) of muscle protein area×optical density (OD). Control (non-exercised) muscles were set to 1.0. Indicates significant training effect ($p<0.01$); (B) representative Western blot from control and hypertrophied muscle samples in young and old rats.

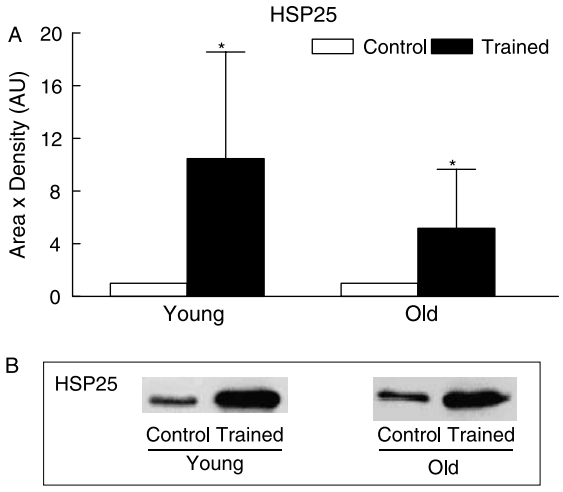

Fig. 2. (A) HSP25 protein expression in control and hypertrophied muscles from young ($n=10$) and old ($n=9$) rats, expressed relative to control values. Data are presented as arbitrary units (AU) of muscle protein area×optical density (OD)±SD. Control (non-exercised) muscles were set to 1.0. ***Indicates significant training, age and interaction effects ($p<0.01$); (B) representative Western blot from control and hypertrophied muscle samples in young and old rats.

3.2. mRNA and protein levels

There were no differences in mRNA expression between controls and experimental muscles in either the old or the young animals for any of the four genes examined (data not shown). Similarly, there was no change in HSC70 protein

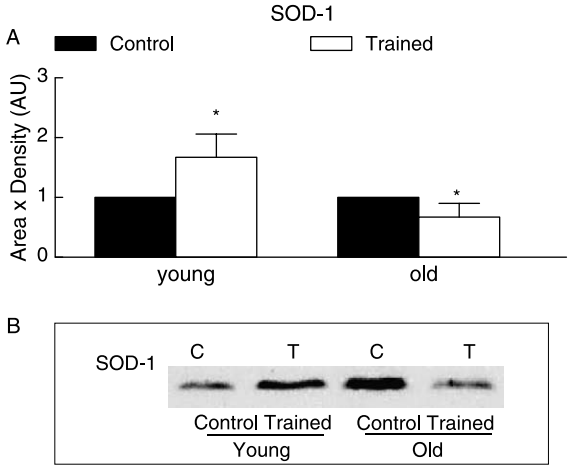

Fig. 3. (A) SOD-1 protein expression in the young ($n=10$) and old ($n=9$) control and hypertrophied muscles, are expressed relative to control values. Data are presented as arbitrary units (AU) of muscle protein area×optical density (OD)±SD. Control (non-exercised) muscle data were set to 1.0. **Indicates significant training by age effect ($p<0.05$); (B) a representative Western blot from control and hypertrophied muscle samples in young and old rats. C, control; T, trained.

expression in response to the training intervention in either age group (data not shown). On the other hand, Western blot analysis showed a significant training effect ($p<0.01$) for HSP72 demonstrating 968.8% increase in young rats (Fig. 1). A representative Western blot for HSP72 is shown in Fig. 1B.

Trained muscles in old rats showed a 409.1% increase in HSP72 protein expression. No age interactions, or training by age interactions were found.

Training increased HSP25 protein expression by 943.1% in young animals and by 420.3% over the control levels in old rats (Fig. 2) with significant ($p<0.01$) age, training, and interaction effects. A representative Western blot for HSP25 is shown in Fig. 2B.

The antioxidant SOD-1 enzyme level increased by 66.6% in the trained muscles of the young rats, while the old animals responded with a suppressed enzyme level that was 32.9% lower compared to the untrained control side (Fig. 3). Moreover, a significant ($p<0.05$) training by age interaction was found for SOD-1 enzyme levels.

3.3. Relationship between HSP72, HSP25, SOD-1 and force

Pearson product correlations were conducted between force and HSP72 (Fig. 4A and D), and HSP25 (Fig. 4B and E). Although moderately strong positive linear correlations were observed between these two proteins and force in young animals, there was a large amount of variability, especially

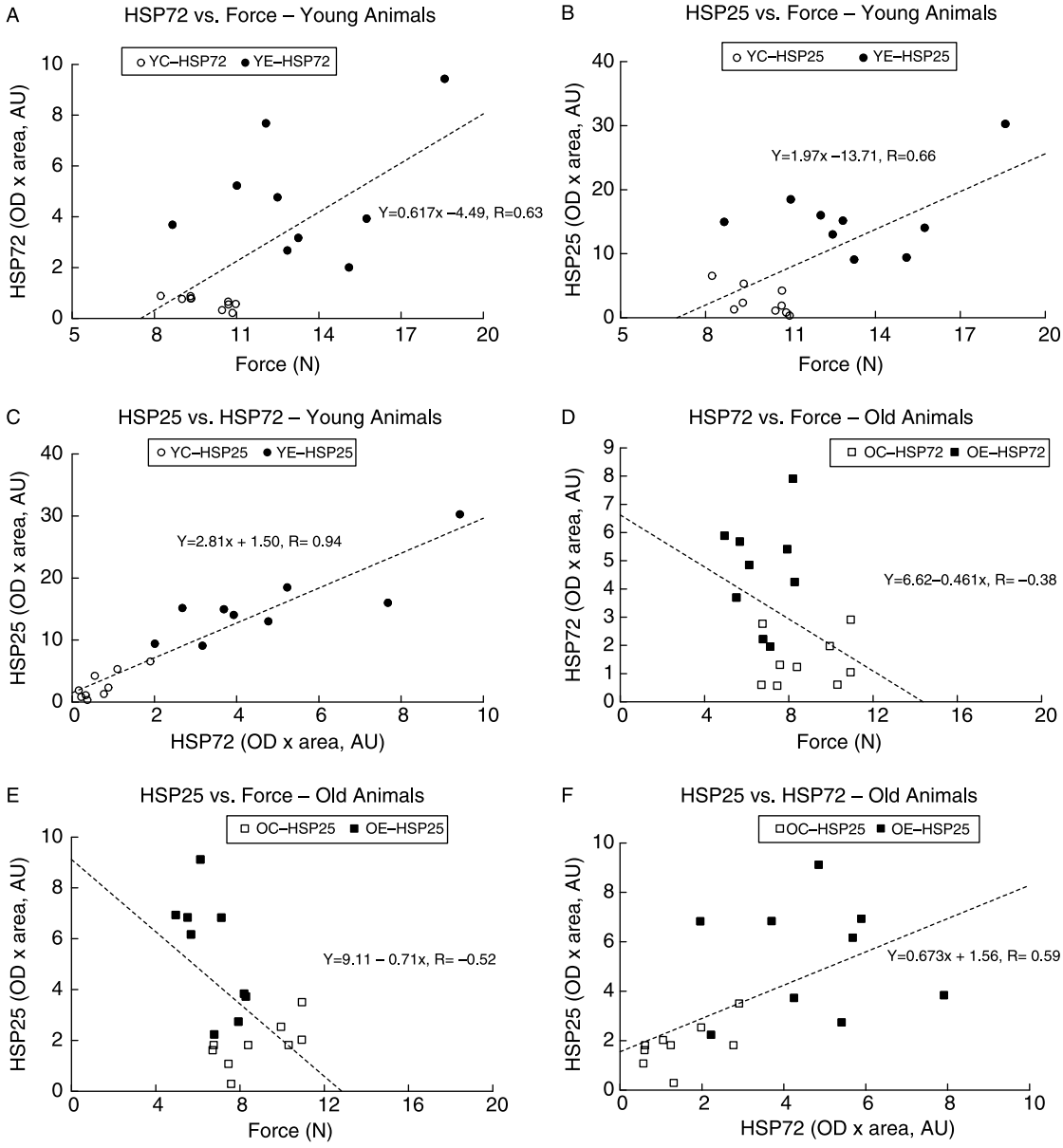

Fig. 4. Pearson product correlations between force and HSP72 (A and D), and HSP25 (B and E). Correlations between HSP25 and HSP72 were established for muscles in young (C) and old (F) animals. Data are shown for control non-exercised muscles (open symbols) and contralateral exercised muscles (closed symbols) for young (A,B,C; $n=10$) and old (D,E,F; $n=9$) rats. The equation describing each correlation was calculated by combining control and exercised data for a variable within each age group. Abbreviations: YC, young control; YE, young exercised; OC, old control; OE, old exercised.

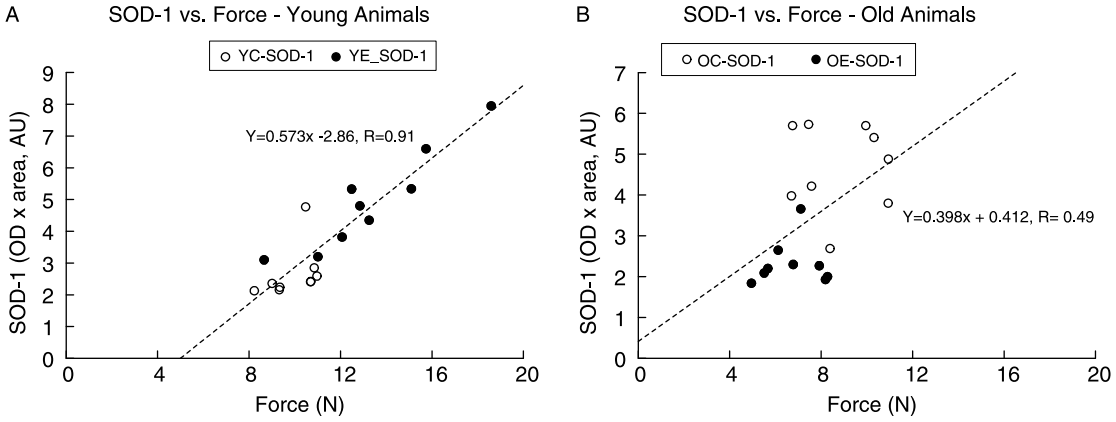

Fig. 5. Pearson product correlations between SOD-1 and muscle force production in young (A, $n=10$), and old (B, $n=9$) rats. The equation describing each correlation was calculated by combining control and exercised data for a variable within each age group. The data suggest that improvements in the muscle's antioxidant ability (SOD-1) is strongly related to improvement in muscle force in young animals, but old animals are unable to improve the muscle's SOD-1 ability or force production in response to exercise. Abbreviations: YC, young control; YE, young exercised; OC, old control; OE, old exercised.

in the exercised muscle (Fig. 4A and B). In contrast, there were negative correlations with the HSP72 and HSP25 and force in muscles of old animals (Fig. 4D and E). The negative correlations occurred because force declined in the exercised limb, although the HSP proteins both increased in muscles of old rats. Very strong positive linear correlations were found between HSP25 and HSP72 in muscles of young animals (Fig. 4C), but this relationship was not as strong in muscles of old animals (Fig. 4F).

Because oxidative stress might have a role in regulating HSP function and/or abundance we examined the relationship between SOD-1 and force. This relationship was strongly positive ($R=0.91$, Fig. 5A) in muscles of young animals, but was much weaker in muscles of old animals ($R=0.49$, Fig. 5B).

3.4. Vitamins C and E and repetitive loading

Because we found that oxidative stress appeared to be elevated in control muscles of old vs. young rats, and this coincided with a lower increase in HSP levels in muscles of old rats, we examined whether improving the animal's oxidant status rats might also improve HSP and/or SOD-1 levels in repetitively loaded muscles. In this study, old rats (30 mo, $n=3$) given a typical control diet containing 126 mg/kg of vitamin E, 0% vitamin C were compared to rats given an identical diet except for containing 30,000 mg/Kg of vitamin E and 2% vitamin C (30 mo, $n=3$). All rats were exposed to identical repetitive loading parameters for 4.5 weeks as described in the methods. The data show that old animals receiving the typical rat chow had a loss of force that did not recover throughout 4.5 weeks of repetitive loading (Fig. 6). However, antioxidant vitamin supplementation improved muscle force recovery, HSP levels and SOD-1 levels in trained muscles of old rats as compared to the non-supplemented rats (Fig. 6).

4. Discussion

We had hypothesized that aging would: (i) attenuate basal HSP levels in fast contracting tibialis anterior muscles and (ii) because lower HSP levels would reduce both the protective effect on contractile proteins and diminish chaperone function during protein synthesis, this would reduce the extent of muscle hypertrophy in response to long-term resistance exercise. Our data are novel and show for the first time that high intensity resistance training induces stress proteins in muscles of aged rats. Both HSP72 and HSP25 proteins were increased in 30-month old animals in response to our chronic stretch-shortening cycle training protocol and although the average relative increase of HSP expression appeared to be larger in the young rats, these changes were statistically different only for HSP25 from those of the old group. The large individual variability both in the young and old groups may have contributed to the lack of significant age-associated effects for HSP72 in this study. Nevertheless, on average, muscles from old rats had ~40% of the increase in HSP72 as found in the muscles of young rats.

Muscles from old rats were unable to adapt to repetitive contractile activity in the same fashion that muscles in young animals responded. In addition, the loss of contractile function in old rats (and the inability to regain force development) suggests that the muscles in old rats were in a maladaptive state (Cutlip et al., 2006). Our data are consistent with the idea that the aged animals experienced greater oxidative stress as a result of the maximum-intensity contractile exercise protocol, whereas the young rats had lower levels of oxidative stress which therefore had a smaller effect on HSP72 levels, resulting in improved chaperone function and therefore increased protein synthesis. The strong linear relationship between SOD-1 and force in muscles of young animals (Fig. 5A) suggests that improvement in the muscles' antioxidant capacity might be important for a positive adaptation to exercise and the ability to improve muscle force. Because the muscles in old

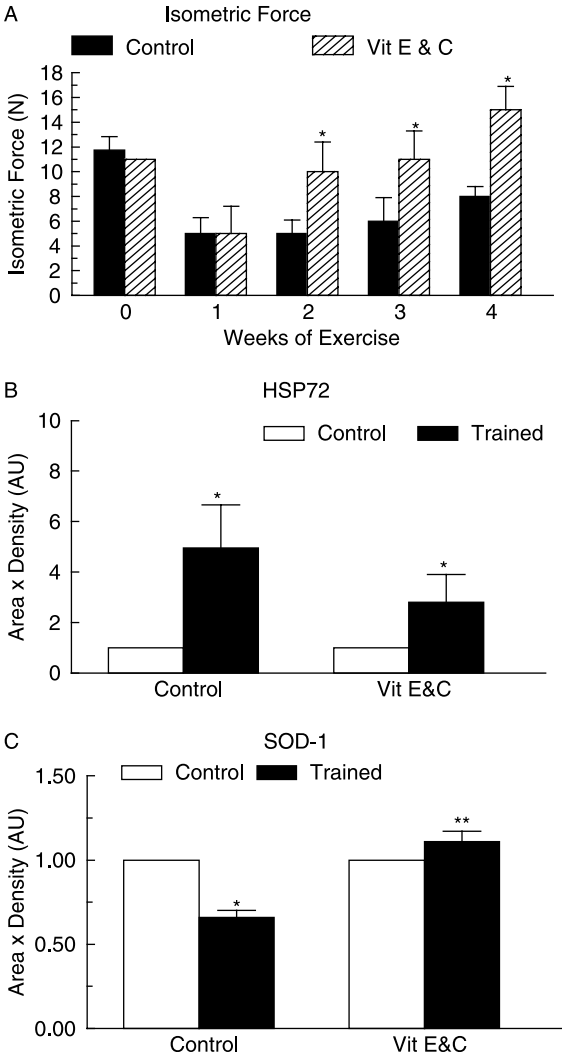

Fig. 6. Rats consumed either a control diet (126 mg/Kg of vitamin E; 0% vitamin C). E and C, $n=3$ or a control diet that was supplemented with vitamins E and C (vitamins E and C, vitamin E, 30,000 mg/kg; vitamin E, 2%, $n=3$): (A) isometric force of the dorsiflexors of old (30 mo of age) rats was assessed after 1–4 weeks of repetitive loading as described in the methods. Greater force was generated in the dorsiflexors of rats supplemented with vitamins E and C vs. rats fed the control diet. *$p<0.05$, control vs. antioxidant treated muscle; (B) HSP72 protein levels in the tibialis anterior muscle of rats fed the control or antioxidant supplemented diets; (C) SOD-1 protein expression protein levels in the tibialis anterior muscle of rats fed the control or supplemented diets. Data are presented in (B) and (C) as arbitrary units (AU) of muscle protein area × optical density (OD) ± SD. Control (non-exercised) muscle data were set to 1.0. *$p<0.05$, control vs. exercised. **$p<0.05$, trained supplemented vs. trained control diets.

animals had a decrease in force and a decrease in SOD-1, the loss of force in response to repeat exercise might be related to the inability to offset increased oxidative stress in muscles of old animals (Fig. 5B).

Our data suggest that maladaption to exercise in muscles of old animals was due in part to increased oxidative stress. This is important because increased oxidative stress may impair HSP abundance or activity. Smolka et al. (2000) reported that HSP72 is induced as a complementary protection against oxidative stress because they observed increased HSP72 levels after exercise only in the muscles of sedentary animals that also showed decreased antioxidant enzyme activity. In the present study, we found that the SOD-1 enzyme level decreased significantly only in the aged muscles and this was positively related to the decline in muscle force. Furthermore, our preliminary data show that old animals given antioxidant supplements had greater muscle levels of HSP72, HSP25, SOD-1 and force after 4.5 weeks of exercise than non-supplemented animals. We recognize that these data were only obtained on three old animals and are therefore not definitive. Nevertheless, together with these data our study suggest that the exercise training stresses may have overwhelmed the antioxidant defense in muscles of old animals preventing adequate HSP responses and contractile adaptations in response repetitive loading.

It is possible that the function of HSPs might be altered with aging, as a result of increased oxidative stresses in muscles of old rats. Carbonylation detection has been used to show that HSP70 protein oxidation increases during conditions of oxidative stress (Nystrom, 2002). Furthermore, HSC70, HSP70, and HSP90 form intermolecular disulfide bonds and HSP70 forms mixed disulfide bonds with several cytoplasmic proteins under conditions of increased oxidative stress (Cumming et al., 2004). Although the effect of the disulfide bond formations is not clear, it is possible that these could affect the function of HSP70. Exercise increases the level of oxidative stress in muscle, because SOD-1 is increased in the muscles of young rats. Since, SOD-1 is diminished in the muscles of the old rats, it is possible that even higher amounts of HSP70 may be unable to compensate for the compromised function of HSP70 that results from elevated conditions of oxidative stress in muscles of old rats.

Hsp70 genes can be induced differentially depending on the type of perturbation (Lee and Seo, 2002). Therefore, we were interested in determining whether resistance training elicits the expression of specific gene products in muscles of young and old rats. However, mRNA expression did not change in muscles of either age group following resistance exercise training intervention for any of the four genes (*Hsc70*, *Hsp70*-1, *Hsp70*-2, *Hsp70*-3) examined. In contrast to our investigation, other studies have reported elevated Hsp72 mRNA expression in response to exercise when measured up to 23 h after the intervention (Febbraio and Koukoulas, 2000; et al., 2002; Puntschart et al., 1996). The available data indicate that *Hsp* gene expression is a rapid response that returns to baseline level within a short time after the stimulus (Poso et al., 2002; Salo et al., 1991). Therefore, if *Hsp* gene expression increased transiently in our study, our experimental design did not permit the detection of elevated mRNA levels 24 h following the last exercise bout. Moreover, it has been shown that HSP72 protein expression is primarily regulated at the level of translation (Moseley et al., 1993); therefore as our results indicate, HSP72 protein level can be significantly elevated without any change in transcription, possibly due to increased translational efficiency and mRNA stability.

In summary, we show that tibialis anterior muscles in old animals have lower HSP72 protein expression and HSP25 levels are also attenuated in muscles aged rats compared to muscles in young rats in response to high intensity contractile activity. Because chaperone function in protein synthesis is a major function of heat shock proteins (Thomason and Menon, 2002), it is possible that the increased HSP levels in the young animals reflect this role. However, the underlying cellular mechanisms of stress protein induction might differ between these age groups. We speculate that HSP's may be induced by oxidative stress in old muscles, and greater protein synthesis may require greater HSP levels for chaperone functions in the hypertrophied muscles from young animals. Furthermore, oxidative stress may reduce HSP levels and function in muscles of old animals, thereby exacerbating the decreased chaperone function of this protein during attempts to improve protein accumulation in response to loading. Although more work is needed, together these data implicate increased levels of oxidative stress as having a role in reducing HSP70 levels and/or function in aging muscles. Nevertheless, because only young and old rats were examined, and we did not study a middle aged group we cannot conclusively determine the degree of differences in HSP expression and levels that were due to maturation and what extent might have been due to aging per se. Additional studies are needed to determine the importance of increased oxidative stress on regulating heat shock protein levels and function in response to a given loading stress in muscles from old, middle aged and young animals.

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