Prolonged treadmill training increases HSP70 in skeletal muscle but does not affect age-related functional deficits

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Submitted 9 August 2007; accepted in final form 6 November 2007

Kayani AC, Close GL, Jackson MJ, McArdle A. Prolonged treadmill training increases HSP70 in skeletal muscle but does not affect age-related functional deficits. Am J Physiol Regul Integr Comp Physiol 294: R568–R576, 2008. First published November 7, 2007; doi:10.1152/ajpregu.00575.2007.—Skeletal muscle atrophy and weakness are major causes of frailty in the elderly. Functional deficits in muscles of old humans and rodents are associated with attenuated production of heat shock proteins (HSPs) after exercise, and transgenic overexpression of HSP70 reverses this functional decline. We hypothesized that training would increase HSP70 content of muscle in adult and old wild-type mice and that this would protect against the development of age-related functional deficits. A 10-wk treadmill training protocol at 15 m/min, for 15 min, 3 days/wk resulted in a significant increase in HSP70 content of muscles of adult mice. Muscles of old untrained mice demonstrated a significant increase in HSP70 protein content and a reduction in HSP70 mRNA content compared with adult untrained mice. Training for 12 mo starting at age 12–14 mo old or for 10 wk starting from age 24 mo old resulted in modification of HSP70 protein and mRNA content to levels of adult mice. Training did not change force generation of extensor digitorum longus muscles of old mice or improve recovery after damaging contractions. The twofold increase in HSP70 content in muscles of adult mice after training may have not been sufficient to provide protection in this instance.

mRNA; exercise; aging; heat shock protein

SKELETAL MUSCLE ATROPHY AND weakness are major causes of loss of mobility, increased frailty, and increased incidence of falls in the elderly. The deficits seen in muscles of old humans and rodents include a reduction in maximum tetanic force generation, which is associated with a reduction in muscle mass and cross-sectional area (CSA), and a reduction in specific force generation (13, 18, 20). In addition, muscles of old mice demonstrate an increased susceptibility to damage and diminished ability to regenerate successfully after damage (4, 22). The mechanisms underlying these age-related deficits remain unclear, but reduced physical activity and the attenuated ability of skeletal muscles to adapt by changes in expression of protective proteins following exercise have been proposed to play a role in this functional decline (8).

One of the major adaptive responses in skeletal muscle cells after exercise is the rapid production of stress or heat shock proteins (HSPs). Increased skeletal muscle content of HSPs has been demonstrated in humans and rodents after both acute exercise (23, 27, 37, 38) and longer-term training (11, 33). In contrast, muscles of old rodents fail to produce HSPs after acute exercise (37, 38). A lack of change in mRNA levels and protein levels for HSPs in muscles of old mice suggests that this defect in HSP production in muscles of old mice may have occurred at the transcriptional level (38).

Transgenic studies have demonstrated that the inability to produce HSPs in response to exercise plays a major role in the age-related decline in muscle function. We have demonstrated that lifelong overexpression of HSP70 in extensor digitorum longus (EDL) muscles of transgenic mice prevented the loss of specific force observed in muscles of old wild-type mice but did not prevent the loss of muscle mass and age-related reduction in tetanic force (22). These mice also demonstrated a substantially improved recovery after lengthening contraction-induced damage compared with muscles of old wild-type mice, which demonstrated a sustained deficit (4, 22).

HSP70 overexpression is associated with a reduced accumulation of markers of oxidation in muscles of aged mice and maintenance of activation of reactive oxygen species-mediated transcription factors (6). NF-κB is activated in muscles of adult wild-type mice immediately after an acute nondamaging contraction protocol (38), and this leads to increased muscle activity of cytoprotective proteins such as superoxide dismutase and catalase (23). The ability of muscles of old wild-type mice to activate NF-κB following the same contraction protocol was abolished (38), whereas NF-κB activation in response to this contraction protocol was maintained in muscles of old HSP70-overexpressing mice (6). Thus it appears that the mechanism by which HSP70 overexpression provides protection from age-related functional deficits may occur, at least in part, via reduced accumulation of oxidation products and in maintenance of the ability of muscles to increase the production of cytoprotective proteins after acute exercise stress via activation of reactive oxygen species-responsive transcription factors (6).

Several studies have shown that exercise training improves some aspects of the age-related functional decline of muscles (5, 12, 19). For example, Gosselin (12) examined the effects of uphill (15% grade) treadmill training on the susceptibility of soleus muscles of adult and old rats to damage induced by a protocol of lengthening contractions in vitro. Ten weeks of treadmill training (45 min/day) resulted in a ~13% reduction in force deficit in the soleus muscle after the lengthening contraction protocol in both adult and old trained rats compared with that shown in age-matched control rats.

The aims of this study were 1) to develop a treadmill training protocol that resulted in increased HSP70 content of skeletal muscle of adult mice, 2) to examine the effects of prolonged...
treadmill training in adult and old mice on HSP70 mRNA and protein content, and 3) to examine the effects of training on age-related changes in muscle function.

We hypothesized that training would increase the HSP70 content of skeletal muscle in adult and old wild-type mice. Furthermore, this increased content of HSP70 would protect against age-related loss of muscle-specific force generation and improve the recovery of muscles of old mice from lengthening contraction-induced muscle damage in a manner similar to a previous study that used transgenic overexpression of HSP70 (22).

METHODS

Mice and treadmill training protocols. Adult (12–14 mo old at the start of training) and old (24 mo old at the start of training) male C57BL/6J mice were used in this study. Mice were housed singly in a specific pathogen-free environment with a 12:12-h light-dark cycle. Experiments were performed in accordance with UK Home Office Guidelines under the UK Animals (Scientific Procedures) Act 1986, and received ethical approval from the University of Liverpool Animal Welfare Committee.

Mice were block randomized into five groups. The trained groups of mice consisted of adult (12–14 mo) mice subjected to a 10-wk training protocol, old (24 mo) mice subjected to a 10-wk training protocol, and adult (12–14 mo) mice subjected to a 12-mo training protocol to the age of 24–26 mo; this was termed the 12-mo trained group. Two additional groups of adult and old mice were housed individually, and these served as age-matched untrained controls. For the cohort of adult and old mice that underwent the 10-wk training protocol, groups of six mice were treadmill trained and a further six mice served as untrained controls. In the 12-mo training study, 72 mice were used; 36 were treadmill-trained, and 36 served as age-matched untrained controls. After we allowed for 50% death in the untrained control group, lengthening contraction studies were undertaken at 24–26 mo old. Maximum tetanic force was measured in EDL muscles of eight mice from both the 12-mo trained and age-matched control group before the lengthening contractions. After lengthening contractions were completed, four mice from both the trained and age-matched control group were allowed to recover for 3 h before remeasurement of maximum tetanic force. Similarly, four mice from each group were allowed to recover for 28 days before remeasurement of tetanic force generation.

Mice were weighed monthly, and any deaths were recorded daily. Training consisted of treadmill running on a motorized treadmill (Columbus Instruments) at 0% gradient at 15 m/min for 15 min on 3 days/wk. Mice were acclimatized to the treadmill at speeds of 9–14 m/min during the first 2 min of the 15-min training session. Twenty-four hours after the final treadmill run, a cohort of mice from each of the five groups was killed by cervical dislocation, and hindlimb muscles were removed for analysis of HSP protein and mRNA content and succinate dehydrogenase (SDH) activity.

The effect of treadmill training on force generation by EDL muscles of adult and old mice. Mice were anesthetized with 50–100 mg/kg body wt pentobarbital sodium by intraperitoneal injection. Additional intraperitoneal doses were given as required to maintain a sufficient depth of anesthesia for the complete procedure such that mice did not respond to tactile stimuli. Maximum tetanic force and specific force of EDL muscles were measured in situ in cohorts of mice from adult 10-wk trained and 12-mo trained mice together with age-matched control groups (25). To do this, the knee of the right hindlimb was fixed. The distal tendon of the EDL muscle was exposed, maintained intact, and attached to the lever arm of a servomotor (Cambridge Technology). The peroneal nerve was exposed, and stainless steel needle electrodes were placed across the nerve. Stimulation voltage and muscle length were adjusted to produce a maximum twitch force. The optimum muscle length that produced the maximum twitch force is also the optimum muscle length (L0) for the production of maximum tetanic force (25). Maximum tetanic force was determined by electrical stimulation of the muscle at L0. The muscle was electrically stimulated to contract at optimal stimulation voltage (8–10 V) every 2 min for a total of 500 ms with 0.1-ms pulse width. The frequency of stimulation was increased for each 500-ms stimulus from 10 to 50 Hz and subsequently in 50-Hz increments to a maximum of 400 Hz. Maximum tetanic force was identified when the maximum force reached a plateau despite increasing stimulation frequency, and stimulation was halted at this point. Mice were killed by cervical dislocation, and muscles were rapidly removed and frozen in liquid nitrogen for later analyses.

To assess the effects of long-term treadmill training on the susceptibility of muscles to contraction-induced damage and the ability to recover from damage, after determination of maximum tetanic force of EDL muscles, mice from the 12-mo trained group and age-matched control mice were maintained under anesthesia as described above. The EDL muscles at L0 were then subjected to a protocol of 450 lengthening contractions at 150 Hz through a strain of 20% of muscle fiber length at a velocity of 1.5 fiber length/s, as previously described (22). Half of the mice were maintained under anesthesia for 3 h before remeasurement of maximum tetanic force to determine susceptibility of muscles to contraction-induced damage. The remaining mice were removed from the platform, the wound was sutured, and the mice were allowed to recover for 28 days before remeasurement of maximum tetanic force under general anesthesia as described above, to examine the extent of recovery from contraction-induced damage. After the final determination of tetanic force generation, mice were killed by cervical dislocation and muscles were rapidly removed. Half of the EDL muscle was mounted on a cork disk and frozen in isopentane cooled in liquid nitrogen for histological analysis. The remaining muscle was rapidly frozen in liquid nitrogen for subsequent analysis. Blood was taken from mice after the remeasurement of maximum force and centrifuged at 20,000 g and 4°C for 10 min for analysis of serum creatine kinase (CK) activity as an additional index of muscle damage.

Analysis of muscle HSP70 protein and mRNA content. Whole quadriceps muscles were ground under liquid nitrogen in 1% SDS with protease inhibitors and analyzed for HSP70 content by SDS-PAGE and Western blotting as previously described (6), using a monoclonal antibody for HSP70 (SPA-810; Stressgen, Victoria, BC, Canada). Western blots were analyzed by densitometry with QuantityOne software (Bio-Rad, Hercules, CA); data from Western blots are presented as a percentage of the mean adult control value. Statistical analysis was carried out on raw densitometric data.

Total RNA was isolated from gastrocnemius muscles using TRI-reagent (Sigma-Aldrich, Dorset, UK) and quantified as previously described (38). Total RNA (1.5 μg) was reverse transcribed with the iScript cDNA synthesis kit (Bio-Rad), which utilizes both random hexamer and oligo(dT) primers. Reverse transcription reactions were as follows: 1.5 μg of RNA template, 1.5 μl of reverse transcriptase, 6 μl of reaction mix, and nuclease-free water to a final volume of 30 μl (5 min at 25°C, 30 min at 42°C, and 5 min at 85°C). Real-time PCR reactions were performed in triplicate for each sample using iQ SYBR green Supermix (Bio-Rad) as follows: 12.5 μl of iQ SYBR green Supermix, 2 μl (final concentration 10 μM) of each forward and reverse primers for HSP70 (forward: 5′-GGCTGACAAAGAAG-GTGCC-3′; reverse: 5′-CTTTGACACGCCCCTGATGA-3′; amplicon length of 145 bp), and 8.5 μl of cDNA diluted 1:100. HSP70 mRNA expression levels were normalized to levels of the S29 ribosomal protein (forward: 5′-ATGGGTCACACAGCTCTA-3′; reverse: 5′-GTATTGCGGATCAGCGGT-3′; amplicon length of 102 bp). Real-time PCR was performed on a Bio-Rad iCycler with an iCycler iQ Multicolor real-time PCR detection system (Bio-Rad) with the following conditions: 95°C for 3 min, 95°C for 30 s, 62°C for 15 s.
Analysis of skeletal muscle SDH activity. Quadriceps muscles were ground under liquid nitrogen. Muscle powder was homogenized (model K43; TRI-R Instruments, Rockville Center, NY) in 100 mM potassium-sodium phosphate buffer (pH 7.2) containing 2 mM EDTA. Whole homogenate was used to determine SDH activity. Solutions were made in 50 mM potassium phosphate buffer assay buffer (pH 7.6). Homogenate (10 μl) was added to 210 μl of assay buffer, and 30 μl of 40 mM sodium azide and 20 μl of 0.5 mM 2,6-dichloroindophenol were then added to homogenates. The mixture was vortexed and placed in a 96-well microplate. Thirty microliters of 0.2 M succinic acid were added to initiate the reaction. The decrease in absorbance at 600 nm was measured for 20 min at 37°C using a microplate reader (Powerwave X340; Bio-Tech Instruments). SDH activity was calculated with the molar extinction coefficient of 2.6-dichloroindophenol of 2,100 M⁻¹·cm⁻¹ and corrected for protein concentration.

Histological analysis of skeletal muscles and determination of serum CK activity. Transverse sections (10 μm) were cut through the midpoint of the EDL muscle using a cryostat (CM1850; Leica Microsystems), transferred onto glass slides, and stained with hematoxylin and eosin (Merck, Dorset, UK) as previously described (22). EDL muscles from both control and 12-mo trained mice were examined histologically at 28 days after the lengthening contractions were performed. EDL muscles that had undergone the lengthening contractions were compared with the contralateral undamaged EDL. Regenerating fibers were identified by the presence of centrally located nuclei. The total number of fibers and the number of regenerating fibers were counted for three full sections of the damaged EDL for each mouse. These data were averaged per muscle, and the number of regenerating fibers was expressed as a percentage of the total number of fibers. Serum CK was analyzed as previously described (21).

Statistical analysis. Statistical analysis was carried out with Statistical Package for Social Sciences software version 13. Student’s t-tests were carried out to analyze differences between two groups. Where multiple comparisons were made, data were analyzed with ANOVA and accounted for repeated measures when necessary. When a significant F-value was observed, Tukey’s post hoc analysis was performed to identify where differences occurred. Survival analysis was carried out with Kaplan-Meier estimates using the “log-rank” test statistic, taking into account censored observations. Significance was set at the α-level of ≤0.05. Data are presented as means ± SE.

RESULTS

HSP70 protein and mRNA content and SDH activity in muscles of adult mice after 10 wk of treadmill training. A small but significant increase was seen in the HSP70 content of quadriceps muscles of adult mice after the 10-wk training protocol compared with that shown in muscles of untrained adult control mice (untrained adult control: 591.6 ± 24.1 arbitrary units; 10-wk treadmill trained adult: 783.8 ± 54.6 arbitrary units; P < 0.05, Fig. 1A). The 33% rise in HSP70 protein content in muscles of adult mice after the 10-wk training protocol was associated with a 28% rise in mean HSP70 mRNA content in gastrocnemius muscles, although this did not reach statistical significance (2.7 ± 0.6 and 3.8 ± 0.8 relative expression units, respectively; Fig. 1B). SDH activity was significantly increased in quadriceps muscles of adult mice after the 10-wk treadmill training, compared with muscles of adult control mice (4.96 ± 0.38 and 2.69 ± 0.15 μmol·min⁻¹·mg protein⁻¹, respectively; Fig. 1C).

Effect of age and 10 wk or 12 mo of treadmill training on HSP70 protein and mRNA content of muscles of old mice. A significant increase was observed in the HSP70 content of quadriceps muscles of untrained old control mice compared with muscles of untrained adult control mice (374 ± 70% of untrained adult control; Fig. 2A). In contrast, this significant difference was not observed in muscles of old mice after either 10 wk or 12 mo of treadmill training compared with results shown in muscles of untrained adult control mice (221.5 ± 56% and 200.9 ± 69% of adult control, respectively; Fig. 2A). The elevated HSP70 protein content of muscles of untrained old control mice was associated with a significantly reduced HSP70 mRNA content compared with adult control muscles (0.83 ± 0.6 relative expression units; Fig. 2B). This significant reduction of HSP70 mRNA in muscles of old mice was not evident after the 10-wk or 12-mo treadmill training protocol.
EFFECT OF INCREASED HSP70 WITH TRAINING ON AGING MUSCLES

Fig. 2. A: HSP70 content of quadriceps muscles from adult and old control mice and old mice after 10 wk or 12 mo of treadmill training. *P < 0.05 compared with muscles of adult control mice. B: HSP70 mRNA expression relative to S29 expression in gastrocnemius muscles from adult and old control mice and old mice after 10 wk or 12 mo of treadmill training. *P < 0.05 compared with muscles of adult control mice.

Effect of age and treadmill training on force characteristics of EDL muscles of adult and old mice. Contractile characteristics of EDL muscles of adult control, adult 10-wk treadmill-trained, old control, and 12-mo treadmill-trained mice are shown in Table 1.

A significant decrease was observed in maximum tetanic force of EDL muscles of old control mice compared with that shown in muscles of adult control mice. Although no significant effect of age was observed in the peak twitch forces of EDL muscles of old control mice, the time to reach peak twitch force and the half relaxation time were significantly greater in EDL muscles of old control mice than in adult control mice. The CSA of EDL muscles of old control mice was significantly reduced compared with that of adult control mice. Thus no evidence of altered specific force generation was seen in this cohort of old control mice.

The 10-wk training protocol resulted in a reduction in the mean CSA of EDL muscles of adult mice, although no significant effect was seen on muscle mass (Table 1). No effect of treadmill training was seen on twitch characteristics or maximum force generation by muscles of adult mice, even when the change in CSA was taken into account.

No significant differences were seen in maximum force generation, CSA, or twitch characteristics between EDL muscles of old control and 12-mo treadmill-trained mice.

Effect of 12 mo of treadmill training on the susceptibility to, and recovery from, contraction-induced damage in old mice. Figure 3A shows the maximum tetanic force generation of EDL muscles of old control mice and age-matched 12-mo trained mice before and at 3 h and 28 days after a period of lengthening contractions. The protocol of lengthening contractions resulted in a significant main effect of time. Tukey’s post hoc analysis confirmed that force was significantly reduced at both the 3-h and 28-day time points compared with that shown preexercise. There was no significant main effect of group or any time × group interactions. No significant difference was observed in the maximum force generation of EDL muscles of old control and 12-mo treadmill-trained mice at 28 days after the lengthening contraction protocol (164.2 ± 23.4 and 221.5 ± 16.0 mN, respectively), resulting in force deficits of 41 ± 10.3% and 30 ± 6.5% compared with preexercise forces, respectively. Similarly, no significant difference was observed in the maximum force generation of EDL muscles of old control and 12-mo treadmill-trained mice at 28 days after the lengthening contraction protocol resulting in a significant increase in serum CK activity 3 h after the contractions in both old control and 12-mo treadmill-trained mice (Fig. 3B). No significant difference was observed in the number of regenerating fibers present in the muscles at 28 days after the contraction protocol. Muscles of mice that had been subjected to the 12-mo treadmill training protocol had a mean of 32 ± 12% of regenerating fibers compared with 43 ± 6% for control age-matched mice at 28 days after the lengthening contraction protocol (Fig. 3C).

Effect of 12-mo treadmill training protocol on body mass and survival. No significant effect of age or treadmill training was seen on body mass in the cohort of 12-mo treadmill-trained or age-matched control mice (Fig. 4A). Although the initial aims of this study were not to study survival of the mice

Table 1. Contractile characteristics of EDL muscles of adult control, adult 10-wk treadmill-trained mice, old control, and 12-mo treadmill-trained mice

<table>
<thead>
<tr>
<th></th>
<th>Adult Control</th>
<th>Adult 10-wk Trained</th>
<th>Old Control</th>
<th>12-mo Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle mass, mg</td>
<td>12.8±0.4</td>
<td>11.9±0.4</td>
<td>12.1±0.6</td>
<td>11.4±0.5</td>
</tr>
<tr>
<td>Muscle CSA, mm²</td>
<td>2.82±0.12</td>
<td>2.39±0.08*</td>
<td>2.54±0.10*</td>
<td>2.37±0.07*</td>
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<tr>
<td>Peak twitch force, mN</td>
<td>141.0±14.0</td>
<td>124.3±10.6</td>
<td>118.9±9.1</td>
<td>114.1±11.5</td>
</tr>
<tr>
<td>Time to reach peak twitch force, ms</td>
<td>8.73±0.40</td>
<td>9.5±0.76</td>
<td>11.6±0.66*</td>
<td>12.2±0.53*</td>
</tr>
<tr>
<td>Twitch half-relaxation time, ms</td>
<td>7.59±0.47</td>
<td>8.16±0.83</td>
<td>11.6±0.74*</td>
<td>11.7±0.80*</td>
</tr>
<tr>
<td>Maximum tetanic force, mN</td>
<td>436.2±27.0</td>
<td>423.4±28.0</td>
<td>330.1±21.6*</td>
<td>349.4±18.8</td>
</tr>
<tr>
<td>Maximum specific force, mN/mm²</td>
<td>157.6±13.6</td>
<td>177.1±10.4</td>
<td>148.3±11.1</td>
<td>145.3±8.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. See text for further description of experimental groups. CSA, cross-sectional area; EDL, extensor digitorum longus. *P < 0.05 compared with value for adult control mice.
in this cohort and mice were removed randomly from the cohort for experimentation at 24–26 mo old, deaths due to natural causes were monitored. Data for the 12-mo treadmill-trained compared with the untrained control mice (Fig. 4B) were analyzed by Kaplan-Meier estimates, which takes into account censored observations and survival data. Survival analyses of these data began when the mice were 14 mo old and the mice were randomly allocated into two groups, one being treadmill trained and the other acting as untrained controls. It was observed that the treadmill group had a 63% chance of surviving to ~730 days, whereas the control group had only a 29% chance of surviving to this time point. This change in survival time was deemed statistically significant ($P = 0.018$) by log-rank tests.

**DISCUSSION**

The aims of this study were 1) to develop a treadmill training protocol that resulted in increased HSP70 content of skeletal muscle of adult mice, 2) to examine the effect of prolonged treadmill training of adult mice to old age on HSP70 mRNA and protein content, and 3) to examine the effects of training on age-related changes in muscle function. Previous data from our laboratory have demonstrated that a transgenic increase in HSP70 in old mice preserved the ability to adapt to a nondamaging contraction protocol (6), maintained specific force, and resulted in an improved ability to recover force after lengthening contractions compared with old wild-type mice (22).

The large number of analyses undertaken and the need for different preparative steps for these analyses necessitated the use of three muscle groups in this study. The EDL muscle is appropriate for the determination of the effect of aging and treadmill running on force characteristics and susceptibility to damaging contractions (3, 4, 22). This muscle was also used for histological examination for evidence of regenerating muscle fibers following contraction-induced damage compared with undamaged contralateral muscles. The gastrocnemius muscle was used for analysis of HSP70 mRNA content, and the quadriceps muscle was used for analysis of SDH activity and HSP70 protein content. It is acknowledged that the use of...
different muscles may be a limitation to the study if the fiber-type composition was notably different or if one or more of these muscles were not activated during treadmill running. However, unlike muscles of rats, the muscles of mice used in this study are all predominantly composed of type 2B muscle fibers (1, 15). Moreover, EMG studies have shown that the EDL, gastrocnemius, and quadriceps muscles are all activated during normal locomotion in rodents (29). Therefore, we determined that it was appropriate to compare data across the three muscle groups.

The treadmill training protocol was established at a level of intensity that resulted in increased HSP content of muscles of adult mice but at an intensity to allow use of the protocol with old mice. Thus the intensity of the training protocol was relatively mild. SDH activity was significantly increased in the quadriceps muscles of adult mice after the 10-wk training protocol (Fig. 1C), suggesting that the training protocol was successful in increasing oxidative capacity of these muscles. This was associated with an increase in HSP70 content of these muscles (Fig. 1A). This protocol had therefore resulted in an increase in muscle HSP70 protein content and was used to train old mice for 10 wk or 12- to 14-mo-old mice for 12 mo.

Effect of age on HSP70 protein and mRNA content of skeletal muscles. A significant increase in the HSP70 content of quadriceps muscles of old untrained control mice was observed compared with that shown in muscles of adult control mice (Fig. 2A). This age-related increase in HSP70 has been observed by others (7, 36, 38). In contrast, a significant decrease in resting HSP70 mRNA was observed in muscles of untrained old control mice compared with adult control mice (Fig. 2B). Several other studies have demonstrated significantly lower HSP70 mRNA levels in tissues of aged animals, including muscle (36), heart (2), and lymphocytes (30). The mechanism by which this differential effect on protein and mRNA content occurs is unclear.

One explanation for high HSP70 protein level together with low HSP70 mRNA level in muscles of old control mice may be reduced protein turnover. Our group (6) has demonstrated that oxidized proteins accumulate within muscle of aged mammals. This accumulation may result from increased oxidation of proteins per se or decreased clearance of oxidized proteins due to a malfunctioning of the proteasome system. The proteasome is one of the major pathways for degradation proteins; the 20S proteasome has been identified as being important for the degradation of oxidized proteins (14). However, oxidized proteins are poor substrates for degradation and may inhibit proteasome activity (35). There is considerable evidence demonstrating changes in the functioning of the proteasomes during aging. In skeletal muscle, Husom et al. (17) demonstrated a significant decrease in 20S proteasome catalytic activity together with alterations in the subunit composition in muscles of old rats (29– 40 mo old) compared with young rats (5–12 mo old). Ferrington et al. (10) demonstrated reduced ability to degrade oxidized calmodulin by the 20S proteasome in muscles of old rats (30–37 mo old) compared with young rats (5–14 mo old). The accumulation of HSPs in the muscles of old control mice may therefore be due to the direct oxidation of HSPs, due to the increased levels of other oxidized proteins within the muscle cell resulting in malfunctioning of the proteasome, which reduces the turnover of HSPs, or due to the requirement for higher levels of HSPs, which are known to associate with oxidized proteins. It may be that all or part of the increased HSP70 detected in muscles of the old control mice in the present study is associated with oxidized proteins. If this is the case, the HSP70 may not be “functionally” available to chaperone other proteins. HSP production is regulated by a negative feedback mechanism whereby high HSP70 protein levels reduce further production by silencing the transcription factor heat shock factor 1, preventing activation and further transcription of the HSP70 gene (9, 34), and this may result in the lower HSP70 mRNA levels seen in muscles of old mice. Data for other housekeeping genes including GAPDH and β2-microglobulin do not demonstrate a decrease in mRNA in muscles of old control mice compared with adult mice, suggesting that this change is specific for HSP70 mRNA and not a general reduction in transcription (data not shown).

Effect of training on HSP70 protein and mRNA content of skeletal muscle of old mice. The age-related changes in HSP70 protein and mRNA content observed in muscles of old control mice (Fig. 2) were not observed in muscles of old mice that had been treadmill trained for either 10 wk or 12 mo, suggesting that the training had modified the mRNA and protein levels in muscles of old trained mice to a ratio more like that shown in adult mice (Fig. 2). If the high HSP protein and low mRNA levels in muscles of old control mice were due to increased muscle content of oxidized proteins and/or modified proteosome function, these data suggest that the treadmill training had prevented or delayed these modifications. Some studies have demonstrated reduced oxidative damage to DNA and proteins in muscles of adult and old rodents as a result of exercise training. Radak et al. (31) demonstrated decreased DNA and protein oxidation in muscles of young and adult rats after 9 wk of swimming training together with an increased proteasome activity in these muscles. Radak et al. (32) subsequently demonstrated that 8 wk of treadmill running at 5% grade at 6–10 m/min for 5 days/wk resulted in increased proteasome activity in muscles of adult and old rats. These studies suggest that proteosomal activity can be increased in muscles of adult and old rodents by prolonged exercise and furthermore that this can reduce levels of oxidized proteins within muscles, thus providing an explanation for the normalization of HSP70 protein and mRNA levels toward adult levels in muscles of old trained mice.

Effect of age and treadmill training on contractile characteristics of EDL muscles. The 10-wk treadmill training protocol did not affect any of the contractile characteristics of EDL muscles of adult mice compared with those of adult control mice with the exception of muscle CSA (Table 1). The CSAs of trained mice were 15% lower than those of control mice. This may be because of loss of fat mass, connective tissue, or contractile material from the muscles of trained mice compared with untrained mice, although this was not examined in the present study. These data are similar to those of Gosselin (12) who demonstrated no changes in specific force, maximum force, time to peak, and half relaxation times of soleus muscles of adult rats after 10 wk of treadmill training (15% gradient, 45 min/day, 5 days/wk). This indicates that treadmill training with a moderate protocol at 0% gradient may not significantly affect these parameters of muscle function.

When contractile characteristics of EDL muscles from adult and old control mice were compared (Table 1), muscle CSA was significantly smaller (10%), but no significant differences
were observed in muscle mass or peak twitch force. However, these characteristics did tend to decrease in muscles of old mice. Muscle mass decreased by a mean of 5%, and peak twitch force decreased by 19% compared with muscles of adult mice, suggesting that the muscles of the old mice had begun to atrophy. Significant increases in the time to peak twitch and half relaxation time were observed in muscles of old mice compared with those of adult mice, suggesting a slowing of contractile characteristics in EDL muscles of old mice. This lack of effect of age on muscle mass does not allow any definitive conclusions of the effect of treadmill training or increased HSP content on maintenance of muscle mass in old mice. In contrast, a significant decrease in maximum tetanic force was observed in muscles of old mice compared with adult mice, but no significant loss of specific force was observed in the EDL of old mice compared with adult mice. Although a reduction in tetanic force is seen with age in the majority of studies, the lack of reduction in specific force with age is sometimes lacking. Thus McArdle et al. (22) demonstrated a significant decrease in both maximum tetanic force and specific force of EDL muscles of old BL6XSJL mice (26–28 mo old) compared with adult mice (10–12 mo old). However, data from Brooks and Faulkner (3) demonstrated a significant decrease in maximum tetanic force but not specific force of EDL muscles of 26- to 27-mo-old C57BL6/J mice compared with 9-to 10-mo-old mice. This suggests that C57BL6/J mice may not lose specific force until a later age than other strains of mice, making it difficult to compare force data between strains. After the 12-mo training protocol and unlike muscles of old control mice, no significant difference was seen in maximum force generation between EDL muscles of 12-mo trained mice compared with muscles of adult control mice. However, there was no significant difference in maximum force generation between EDL muscles of old control and old treadmill-trained mice. The 12-mo treadmill training did not significantly affect any of the contractile characteristics of the EDL muscle of trained mice compared with old control mice (Table 1). The 12-mo treadmill training had no significant effect on body mass, although the mass of the control group increased to 8% greater than the treadmill-trained group by the age of 17 mo (Fig. 4A). This suggests that the treadmill training may have prevented an increase in body mass observed in the control group caused by inactivity. The 12-mo treadmill training protocol resulted in a significant improvement in percent survival compared with that shown in untrained mice; however, this protection was observed after a protocol of only 20 lengthening contractions. McArdle et al. (22) demonstrated a 50–70% force deficit in the EDL muscles of both adult and old mice 3 h after the protocol of 450 lengthening contractions, demonstrating that this protocol damaged muscles of adult and old mice to the same extent. However, muscles of adult mice had fully recovered by 28 days after the contractions, whereas muscles of old mice had a force deficit of 44% at 28 days after the lengthening contraction protocol. This deficit was reversed in old transgenic mice that were overexpressing HSP70 in muscles throughout life (22). Another possible explanation for the lack of protective effect of the treadmill training protocol on age-related functional changes may be due to the absolute levels of HSPs achieved in the muscles. The level of HSP70 in the muscles of these transgenic mice was between 10- and 20-fold higher than that of control mice. In the present study, 10 wk of treadmill training in adult mice resulted in a less than twofold increase in HSP70 content of the muscles, which may not have been sufficient to prevent the onset of age-related functional deficits and protect against a severe damaging protocol in the 12-mo trained group. The dose-response effect of exercise on HSP production has yet to be determined. The level of production of HSPs depends on a number of factors, including the initial HSP levels within the tissue and the nature of the stress. Thus, if quiescent skeletal muscle has a relatively high content of HSPs, the HSP response to stress, particularly exercise stress, is blunted compared with muscles with lower HSP content before exercise (27). An additional factor is that the pattern and level of HSP production by skeletal muscles following exercise stress are different from those following heat treatment (28); therefore, the pattern of HSP response after a stress that has several active components (e.g., increased oxidation, heat, metabolic changes) may be complicated. A study by Milne and Noble (26) demonstrated that increased HSP70 content of...
EFFECT OF INCREASED HSP70 WITH TRAINING ON AGING MUSCLES

R575

muscles is intensity dependent. Comparison of the less than twofold response in muscles of treadmill-trained mice in the present study with the three- to fourfold increase in muscles after an acute period of maximal isometric contractions suggests that a graded response is also possible in muscles of rodents, but future studies examining the effect of graded exercise on muscle HSP content are warranted.

Perspectives and Significance

Aging results in a significant reduction in skeletal muscle force generation and an inability to recover completely after exercise-induced muscle damage. These changes in skeletal muscle function have major effects on quality of life. It was hypothesized that exercise training would result in increased HSP70 content of muscles of old mice and that this increase in HSP70 content would provide protection against age-associated loss in maximum tetanic force generation and improve muscle recovery after contraction-induced muscle damage. Data demonstrated age-related differences in skeletal muscle HSP70 protein and mRNA content, whereby muscles of old mice had high HSP70 protein content and decreased mRNA content compared with muscles of adult mice. These changes were modified by short-term (10 wk) and long-term (12 mo) treadmill training to a ratio more like that seen in muscles of adult mice. In contrast, the 12-mo training protocol had no effect on the age-related loss of muscle force generation and did not improve regeneration after a severe protocol of damaging lengthening contractions. We propose that this lack of improvement in skeletal muscle function may be related to the magnitude of increase in HSP70 content observed after exercise training compared with that previously seen in HSP70-overexpressing mice. Future studies should include the development of protocols that would increase muscle HSP70 content to the levels seen in transgenic mice, possibly using a more demanding exercise protocol or pharmacological interventions.

ACKNOWLEDGMENTS

The authors thank Research into Ageing for generous financial support and Drs. Claire Lee and Caroline Broome for help with the development of laboratory protocols.

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