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Effect of lifelong overexpression of HSP70 in skeletal muscle on age-related oxidative stress and adaptation after nondamaging contractile activity

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ABSTRACT **Skeletal muscle aging is characterized by atrophy, a deficit in specific force generation, increased susceptibility to injury, and incomplete recovery after severe injury. The ability of muscles of old mice to produce heat shock proteins (HSPs) in response to stress is severely diminished. Studies in our laboratory using HSP70 overexpressor mice demonstrated that lifelong overexpression of HSP70 in skeletal muscle provided protection against damage and facilitated successful recovery after damage in muscles of old mice. The mechanisms by which HSP70 provides this protection are unclear. Aging is associated with the accumulation of oxidation products, and it has been proposed that this may play a major role in age-related muscle dysfunction. Muscles of old wild-type (WT) mice demonstrated increased lipid peroxidation, decreased glutathione content, increased catalase and superoxide dismutase (SOD) activities, and an inability to activate nuclear factor (NF)-κB after contractions in comparison with adult WT mice. In contrast, levels of lipid peroxidation, glutathione content, and the activities of catalase and SOD in muscles of old HSP70 overexpressor mice were similar to adult mice and these muscles also maintained the ability to activate NF-κB after contractions. These data provide an explanation for the preservation of muscle function in old HSP70 overexpressor mice.—Broome, C. S., Kayani, A. C., Palomero, J., Dillmann, W. H., Mestril, R., Jackson, M. J., McArdle, A. Effect of lifelong overexpression of HSP70 in skeletal muscle on age-related oxidative stress and adaptation after nondamaging contractile activity. *FASEB J.* 20, E855–E860 (2006)**

Key Words: aging · exercise

The ability of muscles of old rodents to produce HSPs after a period of nondamaging exercise is severely diminished (1, 2). Studies in our laboratory using transgenic mice have provided evidence that this diminished production of HSP70 in muscles of old mice plays a major role in the development of age-related functional deficits (3).

Normal aging is associated with a progressive decline in skeletal muscle mass, ability to generate force, an enhanced susceptibility to injury, and a poor ability to repair (4–7). Muscle atrophy and weakness are the major causes of loss of mobility for the elderly (8). The cellular mechanisms underlying this age-related decline are unclear, although considerable support exists for a role of reactive oxygen species (ROS) in modulating the aging process (9–11). Changes in markers of ROS production in skeletal muscle during aging have received some attention (12–15), although the functional effect of these changes has not been clearly examined.

Skeletal muscle contractions result in an increased ROS generation (16–19), which can be potentially damaging. However, muscle cells have defense systems that provide protection against an increase in the production of ROS. The two major endogenous defense systems involved in this adaptation in muscle are the antioxidant defense enzymes [including superoxide dismutase (SOD), catalase, and glutathione peroxidase] and a highly conserved family of proteins known as stress or heat shock proteins (HSPs; ref 18). Up-regulation of these systems occurs in muscle in response to increased ROS production via activation of redox-responsive transcription factors. Nuclear factor-κB (NF-κB) and activator protein-1 (AP1) transcription factors are involved in the up-regulation of antioxidant enzymes such as SOD and catalase in response to oxidative stress (20–22), whereas HSP expression in response to acute stress in eukaryotic cells is primarily regulated by the transcription factor heat shock factor 1 (HSF1; ref. 23).

The mechanisms by which lifelong overexpression of HSP70 may preserve muscle function in old mice are unclear. Others (9–15) have suggested that dysfunction occurs in muscle and other cells as a consequence of the accumulation of oxidative damage to cellular components. HSPs are known to provide protection against

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acute ROS-mediated cell damage (3, 24, 25), although the effects of increased HSP expression on changes in markers of ROS production have not been examined.

We hypothesized that the inability of muscles of old mice to produce HSPs after stress results in the accumulation of cellular oxidation products and that overexpression of HSP70 protects against the age-associated increase in ROS-mediated damage to cellular components and preserves the ability of muscle cells to activate redox-responsive transcription after stress, which results in protection against the development of age-related functional deficits.

MATERIALS AND METHODS

The study used adult (10–12 mo) and old (26–28 mo) male and female wild-type (WT) B6XSJL and transgenic mice that had a chimeric transgene that consisted of an inducible HSP70 gene of a rat under a β-actin promoter (HSP70 transgenic mice; 24). The mice were bred from heterozygous breeding pairs that produced homozygous, heterozygous, and WT mice. The mice were fed a standard laboratory diet and exposed to a 12 h dark-12 h light cycle.

Contraction protocol

Experiments were carried out in accordance with UK Government Home Office guidelines. Adult and old mice were anesthetized with sodium pentobarbital (10 mg/100 g of body wt) via an intraperitoneal injection. Supplemental doses were administered as required to maintain a depth of anesthesia sufficient to prevent response to tactile stimuli. Limbs were fixed and the hind-limb musculature was stimulated to contract via surface electrodes placed around the upper limb and ankle to induce isometric contractions (18). Muscles were electrically stimulated to contract for 15 min with square wave pulses of 0.1 millisecond duration at 100 Hz and 70 V for 0.5 s every 5 s (18). Mice were killed by overdose of sodium pentobarbital. *Anterior tibialis* (AT) muscles were rapidly removed, frozen in liquid nitrogen, and stored at −70°C for further analysis. Control AT muscle samples were obtained from quiescent mice that were killed by overdose of anesthesia. Muscles were ground to powder under liquid nitrogen and divided equally for the following analyses.

Analysis of HSP70 content of muscles by Western blotting

Samples were homogenized in a 1% solution of sodium dodecyl sulfate (SDS) containing protease inhibitors (18). Each sample was centrifuged at 4°C, and the total protein content of the supernatant was measured using bicinchoninic acid (Sigma, Dorset, UK). Total protein was separated by SDS-PAGE using a 12% polyacrylamide gel (National Diagnostics). Proteins were transferred onto a nitrocellulose membrane as described previously (18). The muscle content of HSP70 was analyzed using a monoclonal antibody (Stressgen, Victoria, Canada). Bands were visualized using an enhanced chemiluminescence (ECL) detection system (Amersham Lifesciences, Amersham) and Chemidoc image capture system with Quantity One software (Bio-Rad).

Total SOD and catalase activity of skeletal muscles

Muscle samples were homogenized in 100 mM phosphate buffer, pH 7.0, and the supernatant was analyzed for total SOD activity according to the method of Crapo et al. (26). Catalase activity was measured by following the kinetic decomposition of hydrogen peroxide spectrophotometrically at 240 nM using the method described by Claiborne (27).

Total glutathione and thiol content of skeletal muscles

The protein thiol content was analyzed by the method of Di Monte et al. (28) adapted for use on a 96-well plate reader. The automated glutathione recycling method described by Anderson (29) was used to assess the total glutathione content of samples, using a 96-well plate reader (Benchmark, Bio-Rad).

Malonaldehyde content of skeletal muscles

Muscle samples were homogenized in 0.9% saline, and the malonaldehyde (MDA) content of the homogenate was measured as an index of lipid peroxidation using the HPLC-based method of Chirico (30).

Determination of protein carbonyl content of muscle samples

Muscle samples were homogenized in 20 mM HEPES buffer, pH 7.4 containing 150 mM NaCl, 1 mM EDTA, 5% β-mercaptoethanol, and protease inhibitors (1 mM iodoacetimide, 1 mM benzithonium chloride, and 5.7 mM PMSF). To detect oxidized proteins, the Oxyblot detection kit (Intergen Company Purchase) was used. The assay is based on derivitisation of carbonyl groups in the presence of 2,4-dinitrophenylhydrazine (DNPH). Forty micrograms of protein were denatured with the addition of 12% SDS, and the sample was derivitized by incubating with DNPH for 15 min at room temperature. Samples were neutralized and loaded onto a 12% polyacrylamide gel. Proteins were transferred onto a PVDF membrane. The muscle content of protein carbonyls was analyzed using a primary antibody against the dinitrophenylhydrozone moieties. Bands were visualized using an ECL detection system (Amersham Lifesciences, Amersham) and Chemidoc image capture system with Quantity One software (Bio-Rad). Densitometric quantification of three independent experiments was carried out.

NF-κB EMSA

Activity of NF-κB was determined in nuclear extracts of muscle using a modification of the method of Kumar et al. (31). Frozen AT muscles were ground to powder under liquid nitrogen and suspended in low-salt lysis buffer (18 μl buffer/mg of muscle tissue) containing 10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothretiol, and protease inhibitors (1 mM iodoacetimide, 1 mM benzithonium chloride, and 5.7 mM PMSF). The suspension was incubated on ice for 10 min and then vortexed for 10 s. The sample was centrifuged for 10 s at 4°C, and the supernatant (cytoplasmic extract) was removed. The nuclear pellet was resuspended (5 μl/mg of original tissue wt) in ice-cold nuclear extraction buffer (20 mM HEPES, pH 7.9, 420 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10 mM $MgCl_2$, 20% glycerol, 1 mM dithiothreitol, 1 mM iodoacetimide, 1 mM benzithonium chloride, and 5.7 mM PMSF) and incubated on ice for 30 min with intermittent vortexing. Samples were centrifuged for 5 min at 4°C, and the nuclear extract was stored at −70°C. The NF-κB EMSA was performed by incubation of 10 μg of nuclear extract with 1.75 pmol of the ^{32}P-end-labeled NF-κB consensus oligonucleotide and 1 μg of poly dI · dC in binding buffer (10 mM Tris-HCl, pH 7.5, 0.5

mM EDTA, 0.5 mM dithiothreitol, 4% glycerol, 1 mM $MgCl_2$, and 50 mM NaCl) in a final volume of 20 μl for 20 min at 25°C. The DNA-protein complex was separated from free oligonucleotides in a 4% native polyacrylamide gel. Electrophoresis was carried out at room temperature for 1 h at 350V (Bio-Rad DCode system, Bio-Rad). Competition experiments were performed with 100-fold molar excess of unlabeled NF-κB consensus oligonucleotide. Densitometry values for NF-κB DNA binding were obtained from two EMSAs. Values are mean and SE of five mice per time point and are percentage of the adult WT control value.

Statistical analyses

Data are presented as mean ± SE of values from six to eight mice for each experimental condition. Multiple comparisons were carried out using a one-way ANOVA, with Bonferroni's modified *t* test where appropriate. Individual comparisons were carried out using a Student's *t* test.

RESULTS

HSP70 content of quiescent AT muscles from adult and old WT and HSP70 transgenic mice

HSP70 was undetectable in AT muscles of WT mice (**Fig. 1**). Titration experiments demonstrated that the HSP70 content of AT muscles of adult HSP70 overexpressor mice was increased by ≈10- to 20-fold compared with muscles of adult WT mice. The HSP70 content was further elevated by ≈30% in muscle of old HSP70 overexpressor mice (Fig. 1).

Changes in markers of oxidation in AT muscles from quiescent adult and old WT and HSP70 transgenic mice

Catalase and total SOD activity was significantly increased in quiescent muscles from old WT mice. This age-associated increase was not evident in muscles from HSP70 transgenic mice (**Fig. 2*A*** and ***B***).

The total glutathione content of quiescent muscles of old WT mice was significantly lower than that of adult WT mice (Fig. 2*C*). In contrast, this age-related decrease in total glutathione content was not evident in muscles from old HSP70 transgenic mice. There was an approximate doubling in the mean total protein thiol content of quiescent muscles of old WT mice compared with muscles of adult WT mice; however, this did not reach statistical significance. The total protein thiol content of adult and old muscles from HSP70 transgenic mice did not differ (Fig. 2*D*).

Figure 1. Western blot of HSP70 protein content of AT muscles of adult and old WT and HSP70 overexpressor mice. Lanes 1–3: adult WT; lanes 4–6: adult HSP70 overexpressor mice; lanes 7–10: old WT; and lanes 11–14: old HSP70 overexpressor mice.

The MDA content of muscles of WT mice increased significantly with age. This age-related increase in muscle MDA content was not evident in the muscles of old HSP70 overexpressor mice (Fig. 2*E*).

The total protein carbonyl content increased with age in muscles of WT mice. Electrophoresis and Western blot analysis of protein carbonyls resulted in the identification of several distinct proteins of different molecular masses (**Fig. 3*A***), the intensity of which was further analyzed by densitometric quantification of three independent experiments (Fig. 3*B*). Of the proteins identified, five proteins with molecular masses of ≈75, 50, 35, 32, and 25 kDa were increased in muscles of old WT mice compared with muscles of adult WT mice, although this increase was more variable in samples from old mice (Fig. 3). This age-related increase in protein carbonyl content was not evident in muscles from the HSP70 transgenic mice (Fig. 3).

NF-κB activation in muscles of adult and old WT and HSP70 overexpressor mice

Figure 4*A* shows a representative EMSA of NF-κB in nuclear extracts from quiescent muscles of adult and old WT and HSP70 overexpressor mice and muscles after the isometric contraction protocol, and quantification is shown in Fig. 4*C*. The DNA binding activity for NF-κB was elevated after contractile activity in muscles of the adult WT and adult HSP70 overexpressor mice. The DNA binding activity was increased by ≈50% in quiescent muscles of old WT mice compared with adult mice, although this did not reach statistical significance. Muscles of old WT mice showed no change in NF-κB binding activity in response to the isometric contraction protocol. However, NF-κB binding activity of nuclear extracts from old HSP70 overexpressor mice was significantly elevated after contractile activity to a comparable concentration as adult mice. The specificity of the DNA binding of the NF-κB complex was demonstrated using a 100-fold excess of an unlabeled oligonucleotide (Fig. 4*B*).

DISCUSSION

These data are novel and provide a fundamental insight into the mechanisms responsible for the well-documented age-related failure in muscle function and recovery from damage. Overexpression of HSP70 in skeletal muscles of transgenic mice throughout life prevented the specific force deficit observed in muscles of old WT mice. The complete recovery of muscles of old HSP70 overexpressors by 14 days after contraction-induced damage was in contrast to the severe force deficit that remained in muscles of old WT mice (3). The current study has demonstrated that the dramatic improvement in the functional capacity of muscles of old HSP70 overexpressor mice compared with old WT

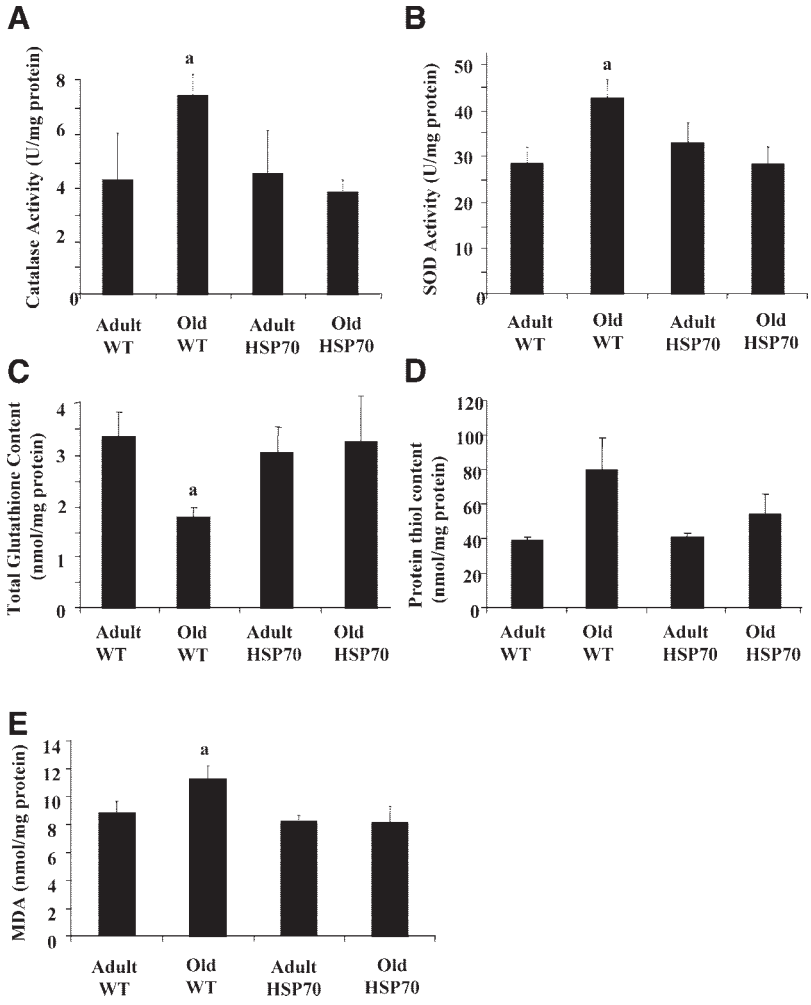

Figure 2. Catalase (*A*) and total SOD (*B*) activity, total GSH (*C*), protein thiol (*D*), and MDA (*E*) content of AT muscles of adult and old WT and HSP70 overexpressor mice. [a]$P < 0.05$ cf. adult WT.

mice (3) may have occurred as a consequence of protection against age-related accumulation of products of oxidative damage.

Muscles of old WT mice demonstrated a chronic up-regulation of SOD and catalase activity in a similar manner to other studies (32–35). Despite this increased antioxidant defense enzyme activity, muscles of old WT mice also showed a fall in total glutathione content and increased MDA and protein carbonyl content, suggesting that oxidative stress remained elevated in these muscles. In addition, the ability of muscles of old WT mice to activate NF-kB-mediated transcription after a moderate and nondamaging exercise stress was severely blunted in comparison with muscles of adult WT mice. The mechanisms responsible for the reported changes in antioxidant defenses in skeletal muscle with increasing age remain unclear, although several authors suggest that this occurs in response to increased oxidative challenge with age (32, 33). This suggests that chronic adaptation of antioxidant defense enzymes to age-related increased ROS generation occurs, although this is not sufficient to prevent further oxidative damage. Evidence of ongoing oxidative damage may also explain the inability of muscles of old WT mice to activate NFkB-mediated transcription. Activation of NF-kB is dependant on the increased production of ROS (20), which is known to occur during the nondamaging contraction protocol used in this study (18). Lack of activation of NF-kB in muscles of old mice suggests that the signal for activation does not occur or that NF-kB may be oxidatively damaged. However, examination of these mechanisms was beyond the scope of the current study.

Lifelong overexpression of HSP70 provided protection against the age-associated accumulation of oxidative damage. Several studies have demonstrated that increased HSP content of different tissues provides protection against damage, particularly ROS-mediated damage (e.g., 3, 24, 25), and preservation of skeletal muscle during the aging process may occur via the same protective mechanism. The HSP70 content of skeletal muscle decreases with age (34, 35), and the ability of muscles to increase the production of HSPs after a variety of stresses, including oxidative stress, is also severely attenuated (34). Previous work from our laboratory has demonstrated that lifelong overexpression of HSP70 in skeletal muscle of mice significantly preserved muscle function (3), and data presented in the current study suggest that this preservation of muscle function occurs by preventing accumulation of oxidation products and preserving the ability of the muscle to activate ROS-mediated transcription after exercise stress. However, the causative link between

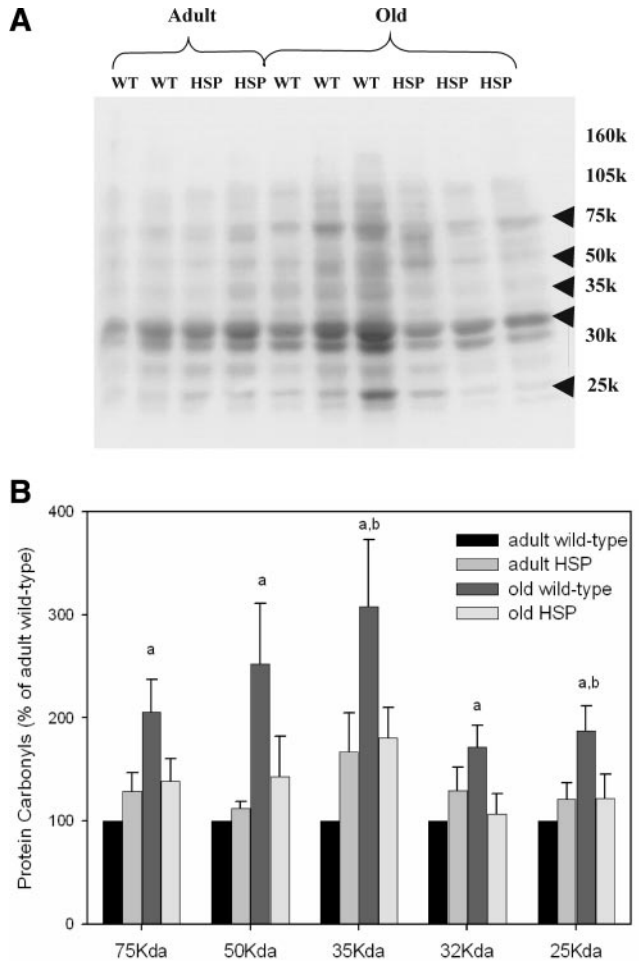

Figure 3. *A)* Typical Western blot of protein carbonyls in quiescent AT muscles of adult and old WT and HSP70 overexpressor mice. *B)* Densitometric quantification of 3 independent experiments. $^{a}P < 0.05$ cf. adult WT; $^{b}P < 0.05$ cf. old HSP70.

HSP70 expression and protection against oxidative damage is yet to be fully elucidated.

Age-related changes in markers of oxidative damage can be reduced or prevented by calorie restriction (CR), a process known to increase mean and maximum longevity and preserve skeletal muscle mass (36, 37, 38). The mechanisms by which CR increases longevity are unclear, but studies suggest that CR results in an attenuation of the increased ROS production associated with normal aging (39). Lifelong CR has been shown to increase HSPs, particularly HSP70, in skeletal muscle of old rats (35), and data presented in the current study suggest that increased HSP content of skeletal muscles of CR rats may provide a fundamental mechanism by which CR results in preservation of muscle antioxidant defenses and function with increasing age.

The present findings suggest that the skeletal muscles from HSP70 overexpressor mice are protected from the accumulation of protein carbonyls with age. No age-related changes were observed in muscles from the HSP70 transgenic mice in any of the five proteins with molecular masses of ≈75, 50, 35, 32, and 25 kDa that showed increased levels of carbonyl content in muscles of old WT mice. Such alterations to cellular proteins may result in the loss of structural or enzymatic activity of the individual proteins, which will contribute to muscle dysfunction. In tissues of younger individuals, oxidized cellular proteins appear to be recognized and efficiently degraded by the proteasome and recent data demonstrate that proteosome activity is reduced in a variety of tissues, including skeletal muscle, of old animals (35, 40). It has been further suggested by these authors that the maintenance of HSP content of muscles in CR rats plays a major, though indirect, role in maintaining proteosome activity by reducing protein aggregation (35). This may also explain the protective effects of HSP70 overexpression in the current study, although proteosome function was not examined.

In summary, this study has demonstrated that the dramatic improvement in the functional capacity of muscles of old HSP70 overexpressor mice compared with old WT mice (3) may have occurred as a consequence of protection against age-related accumulation of products of oxidative damage and preservation of the ability of muscles to activate NF-kB-mediated tran-

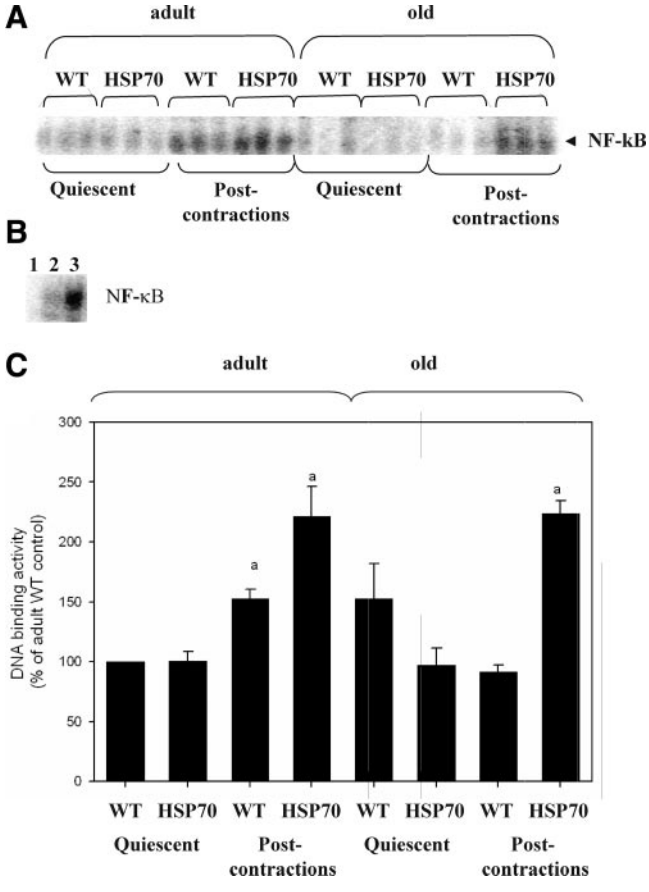

Figure 4. Activation of NF-κB in AT muscles from adult and old WT and HSP70 overexpressor mice after a 15 min period of nondamaging contractile activity. *A)* Representative EMSA. *B)* Specific NF-κB-DNA complex induced is indicated by an arrow. Lane 1: free probe; lane 2: specific competitor; lane 3: nonspecific competitor. *C)* Densitometry values for NF-κB binding were obtained from 2 EMSAs. Values are mean and SE of 5 mice per time point and are % of adult WT control value. $^{a}P < 0.05$ cf. quiescent adult WT.

scription after stress. Data suggest that maintenance of HSP levels in muscles of CR rodents may contribute to functional preservation during CR. The fundamental mechanism by which HSP70 overexpression prevents accumulation of oxidation products is unclear, although further studies examining the role of HSPs in preservation of proteosome activity are warranted. FJ

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SPECIFIC AIMS

Skeletal muscle aging is characterized by atrophy, a deficit in specific force generation, increased susceptibility to injury, and incomplete recovery after severe injury. It has been proposed that the accumulation of oxidation products in muscle and the inability to activate redox-sensitive transcription factors play a major role in age-related muscle dysfunction. Transgenic overexpression of heat shock protein (HSP)70 throughout life protects against the age-related fall in maximum force and facilitates successful recovery in comparison with muscles of age-matched wild-type (WT) mice. The aim of this study was to examine muscles of quiescent adult and old WT and HSP70 overexpressor mice for markers of oxidative damage and to examine the ability of these muscles to activate nuclear factor-κB in response to nondamaging contractile activity.

PRINCIPAL FINDINGS

1. Markers of oxidative damage were elevated in *anterior tibialis* muscles of old WT mice compared with muscles of adult WT mice

The study used adult (10–12 mo) and old (26–28 mo) male and female WT B6XSJL and transgenic mice that had a chimeric transgene that consisted of an inducible HSP70 gene of a rat under a β-actin promoter. This resulted in a 10- to 20-fold increase in HSP70 content of *anterior tibialis* (AT) muscles in adult and old HSP70 overexpressors compared with that of age-matched WT mice. Catalase and total superoxide dismutase (SOD) activity and muscle glutathione (glutathione) and protein thiol content of muscles were measured spectrophotometrically. Protein carbonyl content was determined by electrophoresis and Western blotting. Data demonstrated that the total SOD and catalase activity was significantly increased in quiescent muscles from old WT mice (**Fig. 1**). The total glutathione content of quiescent muscles of old WT mice was significantly lower than that of adult WT mice (Fig. 1), and the malonaldehyde and protein carbonyl content of muscles of WT mice increased significantly with age (Fig. 1).

2. Age-related changes in markers of oxidation in muscles of old WT mice were not evident in muscles of old HSP70 overexpressor mice

No effect of age was seen in SOD and catalase activity, glutathione malonaldehyde, or carbonyl content of muscles of HSP70 overexpressor mice (Fig. 1).

3. A nondamaging but demanding isometric contraction protocol resulted in increased NF-kB DNA binding in muscles of adult WT mice, but this increase was abolished in muscles of old WT mice

Muscles of anesthetized mice were electrically stimulated to contract for 15 min with square wave pulses of 0.1 millisecond duration at 100 Hz and 70 V for 0.5 s every 5 s. Mice were then killed, and muscles were removed. DNA binding activity of NF-κB was determined in nuclear extracts of muscles using EMSA. The DNA binding activity for NF-κB was elevated after contractile activity in muscles of the adult WT mice (**Fig. 2**). In contrast, muscles of old WT mice showed no change in NF-κB binding activity in response to the isometric contraction protocol (**Fig. 2**).

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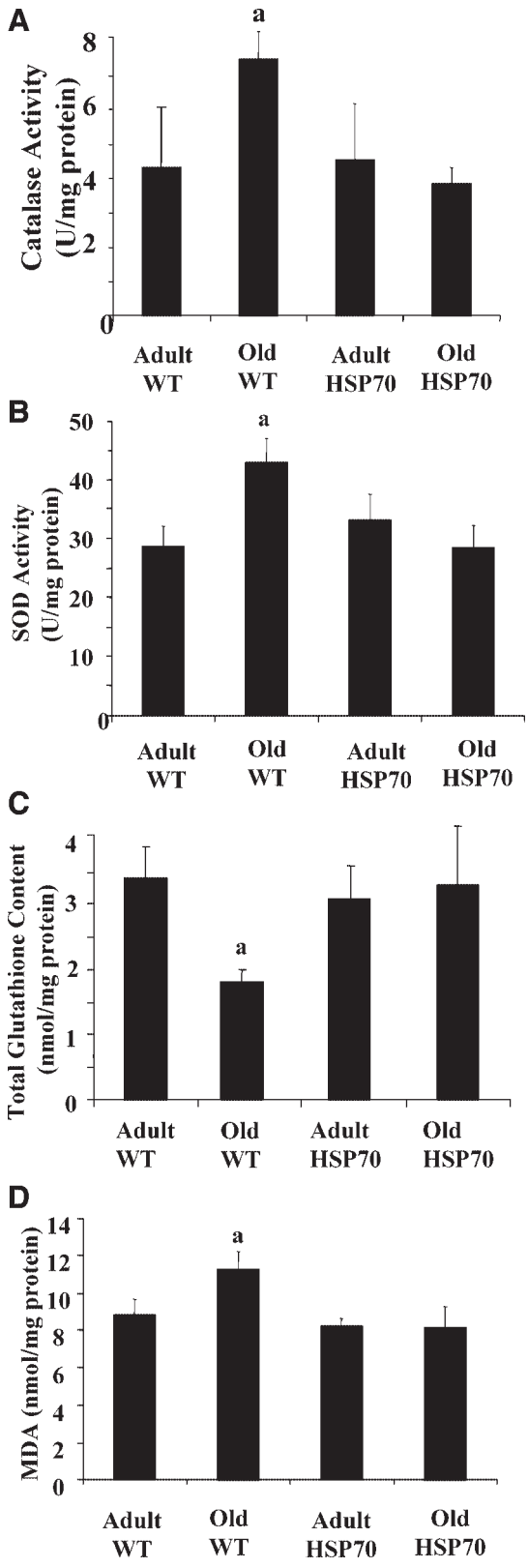

Figure 1. Catalase (*A*) and Total SOD (*B*) activity, glutathione (*C*), protein thiol (*D*), and MDA (*E*) content of AT muscles of adult and old WT and HSP70 overexpressor mice. $^{a}P < 0.05$ cf. adult WT.

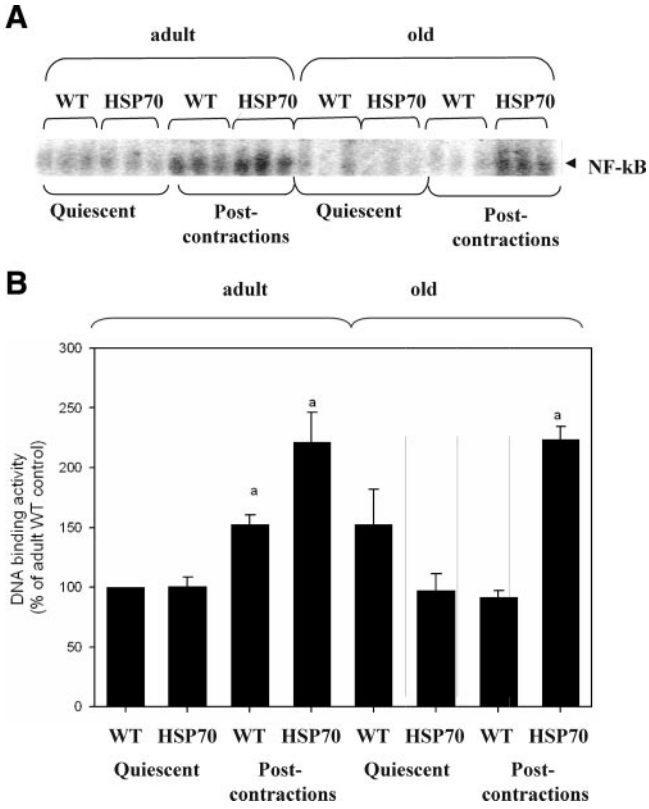

Figure 2. Activation of NF-κB in AT muscles from adult and old WT and HSP70 overexpressor mice after a 15 min period of nondamaging contractile activity. *A)* Representative EMSA. *B)* Densitometry values for NF-κB binding were obtained from 2 EMSAs. Values are mean and SE of 5 mice per time point and are % of adult WT control value. $^{a}P < 0.05$ cf. quiescent adult WT.

4. Overexpression of HSP70 in muscles resulted in a preservation of the activation of NF-kB binding in muscles of old HSP70 overexpressor mice

NF-κB DNA binding activity of nuclear extracts from old HSP70 overexpressor mice was significantly elevated after contractile activity to a comparable concentration as adult mice (Fig. 2).

CONCLUSIONS AND SIGNIFICANCE

These data are novel and provide a fundamental insight into the mechanisms responsible for the well-documented age-related failure in muscle function and recovery from damage. This study has demonstrated that the dramatic improvement in the functional capacity of muscles of old HSP70 overexpressor mice compared with old WT mice may have occurred as a consequence of protection against age-related accumulation of products of oxidative damage and the preservation of the ability to activate redox-sensitive transcription factors such as NF-kB. Muscles of old WT mice demonstrated a chronic up-regulation of SOD and catalase activity. Despite this increased antioxidant de-

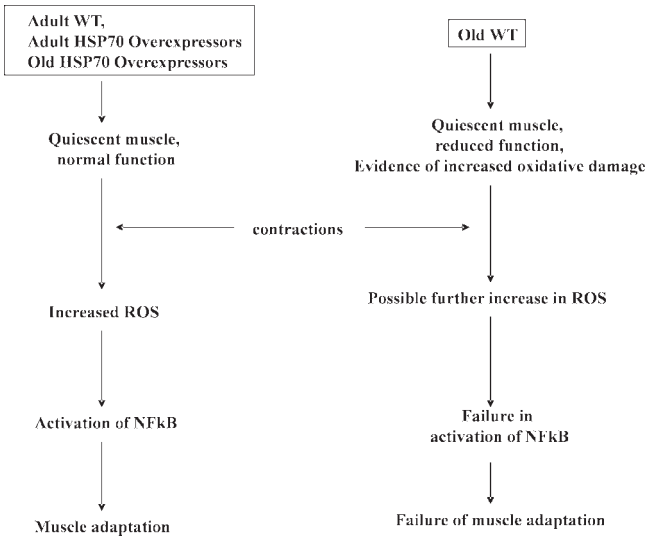

Figure 3. Schematic diagram showing effect of modifications of ROS production and oxidative damage at rest and after contractile activity on adaptations to skeletal muscle in adult and old WT mice and beneficial effect of increased muscle content of HSP70 in old mice.

fense enzyme activity, muscles of old WT mice also showed a fall in total glutathione content and increased MDA and protein carbonyl content, suggesting that oxidative stress remained elevated in these muscles. In addition, the ability of muscles of old WT mice to activate NF-kB-mediated transcription after a moderate and nondamaging exercise stress was severely blunted in comparison with muscles of adult WT mice. Evidence of ongoing oxidative damage may explain the inability of muscles of old WT mice to activate NF-kB-mediated transcription. Activation of NF-kB is dependant on a controlled increase in the production of reactive oxygen species (ROS). Lack of activation of NF-kB in muscles of old mice suggest that the signal for activation does not occur or that NF-kB may also be oxidatively damaged.

Lifelong overexpression of HSP70 provided protection against the age-associated accumulation of oxidative damage. Several studies have demonstrated increased HSP content of different tissues provides protection against ROS-mediated damage and that preservation of skeletal muscle during the aging process may occur via the same protective mechanism. The HSP70 content of skeletal muscle decreases with age and the ability of muscles to increase the production of HSPs after a variety of stresses, including oxidative stress, is also severely attenuated. Previous work from our laboratory has demonstrated that lifelong overexpression of HSP70 in skeletal muscle of mice significantly preserved muscle function, and data presented in the current study suggest that this preservation of muscle function occurs by preventing accumulation of oxidation products and preserving the ability of the muscle to activate ROS-mediated transcription after exercise stress. However, the causative link between HSP70 expression and protection against oxidative damage is yet to be fully elucidated.

The present findings suggest that the skeletal muscles from HSP70 overexpressor mice are protected from the accumulation of protein carbonyls with age. No age-related changes were observed in muscles from the HSP70 transgenic mice in any of the five proteins with molecular masses of ≈75, 50, 35, 32, and 25 kDa, which showed increased levels of carbonyl content in muscles of old WT mice. Such alterations to cellular proteins may result in the loss of structural or enzymatic activity of the individual proteins, which will contribute to muscle dysfunction. In tissues of younger individuals, oxidized cellular proteins appear to be recognized and efficiently degraded by the proteasome and recent data demonstrate that proteosome activity is reduced in a variety of tissues, including skeletal muscle, of old animals. One possibility is that maintenance of HSP content of plays a major, though indirect, role in maintaining proteosome activity by reducing protein aggregation.

In summary, this study demonstrated that the dramatic improvement in the functional capacity of muscles of old HSP70 overexpressor mice compared with old WT mice may have occurred as a consequence of protection against age-related accumulation of products of oxidative damage and preservation of the ability of muscles to activate NF-kB–mediated transcription after stress. The fundamental mechanism by which HSP70 overexpression prevents accumulation of oxidation products is unclear, although further studies examining the role of HSPs in preservation of proteosome activity are warranted. FJ