ISSN 1439-6319, Volume 108, Number 3

Eur J Appl Physiol (2010) 108:589–597
DOI 10.1007/s00421-009-1246-8

ORIGINAL ARTICLE

Effects of age and glutathione levels on oxidative stress in rats after chronic exposure to stretch-shortening contractions

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Accepted: 1 October 2009 / Published online: 31 October 2009

Abstract We investigated effects of age and glutathione synthesis inhibition on the oxidative stress status of tibialis anterior muscles from young and old Fisher 344 × Brown Norway male rats after chronic administration of stretch-shortening contractions. Oral supplementation of L-buthionine-(*S*,*R*)-sulfoximine (BSO) inhibited glutathione synthesis. Dorsiflexor muscles in the hindlimb were exposed to 80 maximal stretch-shortening contractions (SSCs) three times per week for 4.5 weeks. We measured malondialdehyde, hydrogen peroxide (H_2O_2), and free isoprostanes to determine oxidative stress. Glutathione peroxidase activity was measured as an indicator of H_2O_2 scavenging. Glutathione measurements confirmed the effectiveness of BSO treatment. In young rats, the SSC exposure protocol prevented oxidative stress and enhanced H_2O_2 scavenging. In old rats, malondialdehyde was increased in the exposed muscle and a BSO-induced increase in H_2O_2 was not alleviated with SSC exposure as seen in young rats. In addition, glutathione peroxidase activity and total glutathione were increased in old rats relative to their young counterparts. All comparisons were significant at the 0.05 level. Overall, BSO administration was effective in decreasing total glutathione levels and increasing H_2O_2 levels in old and young rats exposed to SSCs. In addition, effects of chronic exposure to high-force resistive loading SSCs in active muscle from old animals are: (1) antioxidant capacity is enhanced similar to what is seen with endurance training and (2) oxidative stress is increased, probably as a consequence of the enhanced vulnerability due to aging.

Keywords Aging · In vivo · L-Buthionine-(*S*,*R*)-sulfoximine · Muscle · Oxidative stress · Stretch-shortening contraction

Communicated by Roberto Bottinelli.

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Introduction

During the process of aging, skeletal muscle performance decreases, and aged muscle recovers more slowly following injury (Brooks and Faulkner 1990; Brooks and Faulkner 1996; Koh et al. 2003; McBride et al. 1995; Sacco and Jones 1992; Lavender and Nosaka 2006a; Lavender and Nosaka 2006b; Manfredi et al. 1991; Zerba et al. 1990). As the work force in the United States continues to age, it is imperative to understand the effects of aging on the susceptibility to work-related musculoskeletal disorders (MSDs) (NRC 2001). The magnitude, cost and burden of work-related musculoskeletal disorders (WMSDs) are enormous. Work-related musculoskeletal disorders account for approximately 38% of cases involving days away from work in the United States (United States Department of Labor, Bureau of Labor Statistics 2007). These facts suggest that there is a need to determine if aging increases muscle injury susceptibility and how it can be avoided.

We use an in vivo animal model to study repetitive high intensity mechanical loading of muscle that models repetitive occupational exposures in humans by chronically exposing the lower limb of rats to stretch-shortening contractions (SSCs). In a prior study, older rats dorsi flexor muscles could not adapt to repetitive SSCs while young

animals did adapt and showed dramatic performance increases (Cutlip et al. 2006). Natural movement of humans and animals includes SSCs (i.e., reciprocal concentric and eccentric muscle actions), making SSCs an effective way to administer resistive contractile activity in skeletal muscle (Komi 2000; Avela and Komi 1998). We developed the SSC exposure protocol as a high repetition 'exposure' protocol, not an injury-inducing protocol, to enhance our understanding of appropriate doses of skeletal muscle contractile activity for older adults in the work place. Both resistance and endurance conditioning protocols that include eccentric contractions protect young and old animals (Brooks and Faulkner 2001; Brooks and Faulkner 1990), and humans (Lavender and Nosaka 2008; Nosaka et al. 2005) from eccentric contraction-induced injury and oxidative stress.

In this current study, we use our unique exposure model to look at its effects on oxidative stress in the exercised muscle. If age affects the ability to buffer oxidative stress in response to repetitive mechanical loading, this may compromise the ability of aged workers to tolerate repetitive occupational exposures. Cellular oxidative stress is determined collectively by reactive oxygen species (ROS) generation and the defense capacity of the antioxidant systems (Ji 2001). Numerous published studies report changes in free radical (i.e., reactive oxygen and nitrogen species) production and oxidative damage (Bejma and Ji 1999; Davies et al. 1982; O'Neill et al. 1996), as well as changes in oxidant status (Hollander et al. 1999; Ji et al. 1992; Leeuwenburgh et al. 1994, 1997; Leeuwenburgh and Ji 1995; Molnar et al. 2006; Radak et al. 1999, 2002; Vasilaki et al. 2006; Venditti and Di Meo 1996; Parise et al. 2005b; Vincent et al. 2002, 2006a) due to contractile activity of skeletal muscle in both young and old animals. Many of these studies have been conducted to investigate the effects of both acute and chronic exercise on free radical generation and antioxidant activities. Long-term resistance exercise has been shown to decrease serum lipids in older adults and enhances cellular antioxidant capacity in skeletal muscle (Vincent et al. 2002, 2006a; Parise et al. 2005b). Also, chronic endurance training produces protective effects against oxidative stress in skeletal muscle of animals, such as increases in superoxide dismutase (SOD) (Hollander et al. 1999; Leeuwenburgh et al. 1994, 1997, Molnar et al. 2006), glutathione peroxidase (GPX) (Hollander et al. 1999; Ji 1993; Leeuwenburgh et al. 1994, 1997; Venditti and Di Meo 1996), catalase (Hollander et al. 1999) and glutathione (Leeuwenburgh et al. 1997). Furthermore, chronic endurance exercise decreases H_2O_2 (MOLNAR et al., 2006), protein carbonyls (Radak et al. 2002), and 8-hydroxy-2′-deoxyguanosine (8-OHdG) (Radak et al. 1999, 2002).

Despite all these findings with exercise, there has not been a study focusing on muscle activity that would be representative of natural movements encountered in the work place, and the effects of those exposures on oxidative stress. Therefore, we investigated the effects of a chronic exposure to SSCs on oxidative stress in the TA muscles of young and old rats. To address this, we exposed young and old rats to a SSC exposure alone, or a SSC exposure in the presence of a glutathione inhibitor. We chose to focus on glutathione because it is an important nonenzymatic antioxidant in cells and it plays a critical role in protection against oxidative stress. Glutathione has previously been shown to increase in certain muscle fiber types in response to exercise-induced increases in ROS (Leeuwenburgh et al. 1997) and to be enhanced in older adults after long term resistive exercise (Vincent et al. 2002).

Materials and methods

Animal handling

We obtained young ($N = 32$; 3 months old; 309.1 ± 27.9 g SD) and old ($N = 30$; 30 months old; 587.7 ± 42.2 g SD) male Fischer 344 × Brown Norway hybrid rats (F344 x BN F1) from the National Institutes on Aging colony. We housed all rats one per cage in an AAALAC accredited animal facility in which the rooms were temperature and humidity controlled and the light/dark cycle was reversed so that exposures were conducted during their most active period (dark cycle was from 7:00 a.m. to 7:00 p.m.). We provided food and water ad libitum. After 1 week of acclimatization, we subjected the rats to a chronic muscle exposure protocol (Cutlip et al. 2006) approved by the National Institute for Occupational Safety and Health (NIOSH) Animal Care and Use Committee.

SSC protocol

Exposure

Cutlip et al. (2006) previously described the SSC exposure protocol used in this study. Briefly, we exposed dorsiflexor muscles of the left hindlimbs of rats to 80 total SSCs administered in eight sets of ten repetitions each, with 2 min rest intervals between each set, on a custom—built rodent dynamometer (Cutlip et al. 1997, 2004). Within each set, there was a 2 s rest between each SSC. For each repetition, an electrical stimulator fully activated the dorsiflexor muscles for 100 ms, and then movement of the load cell fixture (60°/s) over a range of motion of 90–140° ankle angle initiated the eccentric contraction phase. The load cell fixture was immediately returned in the concentric phase at 60°/s to 90° ankle angle and the dorsiflexor muscles were deactivated 300 ms later. Total stimulation time per repetition was 2.06 s.

Performance tests

We measured a pre-test isometric contraction prior to the SSC protocol and a post-test isometric contraction following the SSC protocol at an ankle angle of 90° using a 300 ms stimulation duration (Davis et al. 2003; Willems and Stauber 2001). We also measured single SSCs 2 min preceding and following the SSC exposure protocol to evaluate the ability of the muscle to generate dynamic forces and to perform work during dynamic stretch-shortening (Cutlip et al. 2004, 2006). The single SSC 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 and immediately returning to 70° at 500°/s. Activation was continued for 300 ms after cessation of the movement.

We administered the SSC exposure and performance tests three times per week for a total of 14 exposures over a 4.5 week period as previously described (Cutlip et al. 2006). We anesthetized rats during the exposure with 2% isoflurane gas, which has been shown to have no effect on in vivo force production (Ingalls et al. 1996), using a small animal anesthetic system (Surgivet Anesco Inc., Waukesha, WI, USA).

Experimental groups

We randomly assigned rats in each age group to one of four treatment groups ($N = 8$ young and $N = 8$ old per treatment group); L-buthionine-(*S,R*)-sulfoximine supplemented (BSO + SSC), unsupplemented control (SSC), anesthesia control (ANES) and sedentary control (SED). One old rat in the SSC group and one old rat in the ANES group died during the study. The rats in the BSO + SSC group were given drinking water supplemented with 10 mM BSO, beginning 3 days before the first exposure (Watanabe et al. 2003).

We subjected rats in the BSO + SSC group to the chronic SSC exposure and performance tests. The rats in the SSC group received regular drinking water and were also subjected to the chronic SSC exposure and performance tests. The rats in the ANES group received regular drinking water but were subjected only to the performance tests. Age-matched SED rats received regular drinking water and were not exposed to anesthesia or any functional testing. We monitored water consumption throughout the study for the BSO + SSC and SSC groups to ensure there were no differences in water consumption based on supplementation.

Oxidative stress measurements

Twenty-four hours after the last exposure, we weighed, anesthetized with sodium pentobarbital (ip, 10 mg/100 g BW), and euthanized the rats by exsanguination. We dissected and weighed the left (EXP) and right (CON) tibialis anterior (TA) muscles. We allocated a portion from the midbelly of the TA for analyses of oxidative stress. For measurements of glutathione, we immediately homogenized a portion of the TA in phosphate buffered saline (PBS), transferred it to a cryotube and submerged the tube in liquid nitrogen. We pulled approximately 5 mL of blood directly from the left ventricle into Vacutainer containing EDTA (Becton–Dickinson, Franklin Lakes, NJ, USA) for free isoprostane measurements from plasma. We stored all frozen tissue, plasma and homogenate in a −80°C freezer.

Measurement of gluathione

We estimated glutathione in the TA muscle that had been homogenized in PBS using the Bioxytech GSH/GSSG-412 assay (Oxis International, Inc., Portland, OR, USA). Briefly, samples were extracted with 5% metaphosphoric acid (MPA) and then incubated with glutathione reductase, chromogen (5,5′-dithiobis-(2-nitrobenzoic acid)), and NADPH. We recorded the change in absorbance over 3 min at 412 nm.

Measurement of lipid peroxidation

We homogenized frozen tissue in PBS in the presence of butylated hydroxytoluene (BHT) and estimated lipid peroxidation in the hydrolyzed sample by measuring malondialdehyde (MDA) using the Bioxytech MDA-586 spectrophotometric assay (Oxis International, Inc., Portland, OR, USA).

Measurement of GPX activity

We measured GPX activity in TA muscle total protein homogenate by the method of Flohe and Gunzler (1984) with modifications (Hollander et al. 1999).

Measurement of H_2O_2

We measured the level of H_2O_2 in total muscle homogenate using a fluorometric H_2O_2 detection assay (Cell Technology, Inc., Mountain View, CA, USA), where fluorescence was measured at an excitation of 570 nm and an emission of 600 nm.

Measurement of free 8-isoprostanes

We measured the concentration of free 8-isoprostanes in plasma, using the 8-Isoprostane EIA assay (Cayman Chemical, MI, USA) following the standard micro plate protocol. Absorbance was measured at 410 nm.

Protein quantification

We quantified protein using a standard colorimetric bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, USA) with bovine serum albumin (BSA) as a standard.

Data analysis

We analyzed data using SAS/STAT software, Version 9.1 of the SAS System for Windows (SAS Institute, Cary, NC, USA). A mixed model three-factor within-subject analysis of variance (ANOVA) was used (Proc Mixed) to conduct the statistical analysis for all variables in which measures were taken in both limbs. The design factors included age, treatment, and limb. We included animal as a random effect to appropriately model the within-subject covariance structure. For measures from the circulation, we utilized two-way ANOVAs with age and treatment as factors. Post hoc comparisons were carried out using Fishers LSD. We considered all differences as significant at $P < 0.05$.

Results

After the 4.5 week SSC exposure protocol, body weights of all old rats exposed to repeated anesthesia (BSO + SSC, SSC, and ANES groups) decreased by 11.5% compared to a 4% decrease in old SED rats ($P < 0.001$) (Fig. 1a). Body weights of young rats exposed to repeated anesthesia (BSO + SSC, SSC, and ANES groups) and young SED rats increased by 3 and 11%, respectively (Fig. 1b). However, we noted a trend for the young SED rats to gain body weight at a faster rate than those exposed to repeated anesthesia, but this was not statistically significant. Body weights of all old rats were significantly higher than young rats.

BSO decreased glutathione levels in the TA muscle of the old and young rats compared to the SSC group (Fig. 2). The SSC exposure protocol alone led to a 65% increase in glutathione in the EXP TA compared to the SED old rats ($P < 0.0001$), however, BSO treatment attenuated this response (Fig. 2). We noted no effects of the SSC exposure on glutathione levels in the young rats (Fig. 2).

Malondialdehyde (MDA) content, an indicator of lipid peroxidation, was increased by 28% in the EXP TA of BSO + SSC old rats compared to the SED old rats ($P < 0.05$) (Fig. 3). This trend was also seen in old SSC rats but was not statistically significant (Fig. 3). After SSC exposure in old rats, MDA levels in the old SSC and BSO + SSC groups were significantly higher than young rats by approximately 40% ($P < 0.05$) (Fig. 3).

BSO treatment led to a significant increase in H_2O_2 levels in the BSO + SSC groups of old rats ($P < 0.0001$)

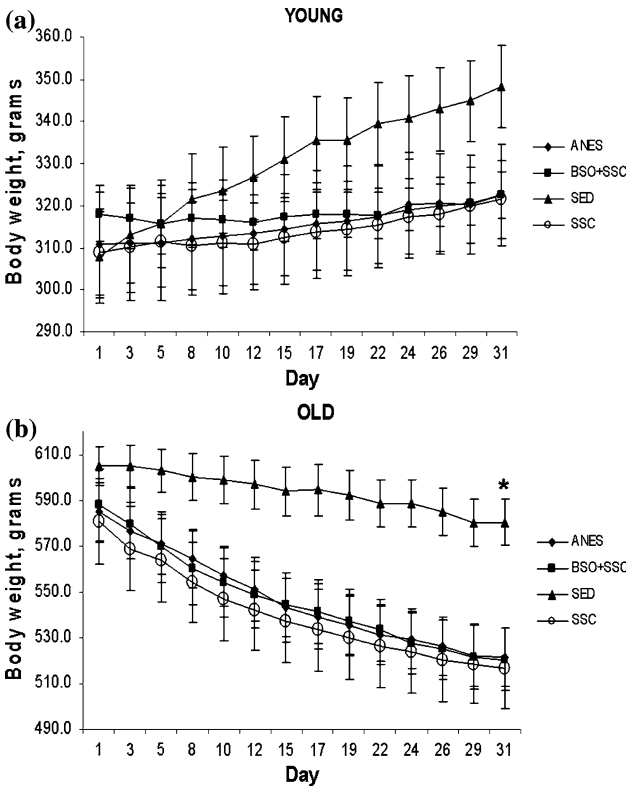

Fig. 1 Body weight changes in young (**a**) and old (**b**) rats from all groups during 4.5 weeks of the SSC exposure protocol. Day 31 is the day of the 14th (last) exposure. *$P < 0.001$, SED versus BSO + SSC/SSC/ANES

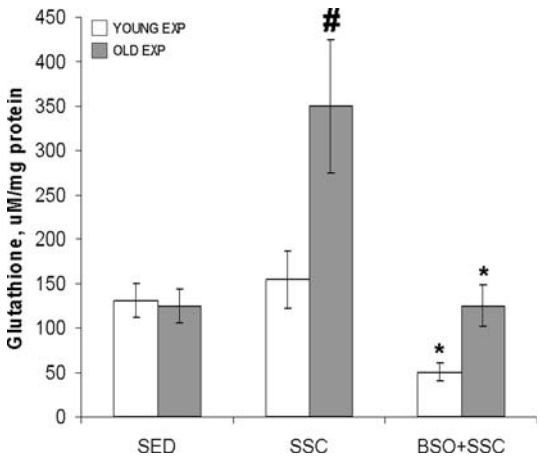

Fig. 2 Total glutathione levels in the EXP TA of old and young rats from BSO + SSC, SSC and SED groups. All values are group mean values ± SE. *$P < 0.05$, young BSO + SSC versus young SSC and old BSO + SSC versus old SSC. #$P < 0.0001$, old SSC versus old SED

(Fig. 4). BSO treatment also significantly increased H_2O_2 levels in the CON TA of young rats. The SSC exposure of the EXP TA in the young BSO + SSC rats prevented the increase in H_2O_2 and maintained H_2O_2 at levels similar to the SSC and SEC groups (Fig. 4). In old SSC rats, SSC

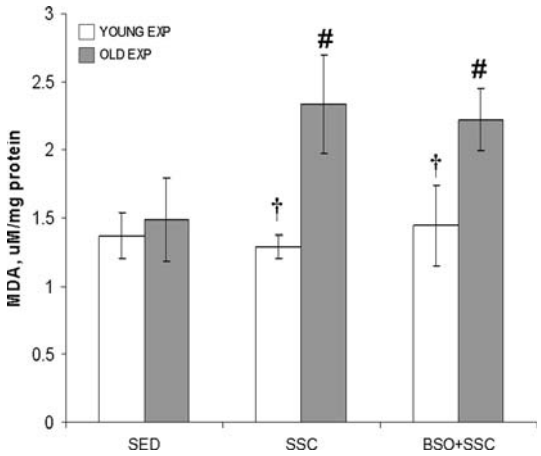

Fig. 3 MDA levels in the EXP TA of old and young rats from BSO + SSC, SSC and SED groups. All values are group mean values ± SE. #$P < 0.05$, old BSO + SSC versus old SED and old SSC versus old SED. †$P < 0.05$, young BSO + SSC versus old BSO + SSC and young SSC versus old SSC

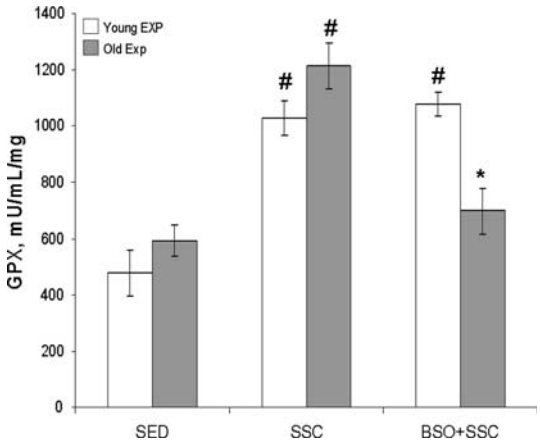

Fig. 5 GPX activity in the EXP TA of old and young rats from BSO, SSC and SED groups. All values are group mean values ± SE. #$P < 0.05$, old BSO + SSC versus old SED, young BSO + SSC versus young SED, young SSC versus young SED. *$P < 0.01$ old BSO + SSC versus old SSC

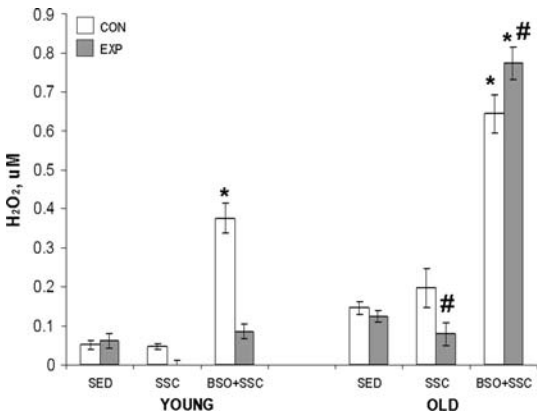

Fig. 4 Levels of H_2O_2 in the TA of old and young rats from BSO, SSC and SED groups. All values are group mean values ± SE. *$P < 0.0001$, old BSO + SSC EXP versus old SSC EXP, old BSO + SSC CON versus old SSC CON, young BSO + SSC CON versus young SSC CON. #$P < 0.05$, LTA versus RTA

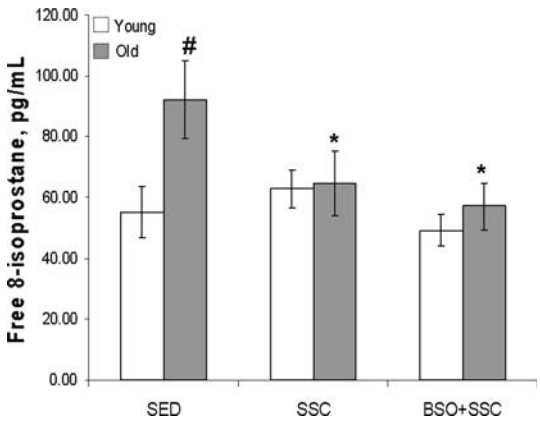

Fig. 6 Free 8-isoprostanes levels in plasma of old and young rats from BSO + SSC, SSC and SED groups. *$P < 0.05$, old BSO + SSC versus old SED and old SSC versus old SED. #$P < 0.001$, old SED versus young SED

exposure of the EXP TA decreased H_2O_2 levels compared to the CON TA ($P < 0.05$). However, treatment with BSO + SSC led to higher levels of H_2O_2 in the EXP TA compared to the CON TA ($P < 0.05$) (Fig. 4).

GPX activity was increased in the EXP TA compared to the CON TA in both old and young rats regardless of treatment (Fig. 5). In old rats, however, BSO + SSC treatment attenuated this SSC exposure-induced increase in GPX activity by approximately 37% compared to the old SSC group ($P < 0.01$) (Fig. 5).

Basal levels of free 8-isoprostanes in the plasma of old SED rats were higher than in young SED rats ($P < 0.001$) and there were no differences within the young groups (Fig. 6). In old rats, the SSC exposure in the BSO + SSC and SSC groups decreased the free 8-isoprostane levels to that of young rats (Fig. 6). BSO treatment did not have any effect on the plasma levels of free 8-isoprostanes in either age group.

In measurements of GSH_t, MDA, H_2O_2, and GPX activity, no significant differences were seen in basal levels between old SED rats and young SED rats (Fig. 2, 3, 4, 5). Additionally, we looked at whether effects of the repeated anesthesia influenced any oxidative stress measures and found that anesthesia alone had no significant effect on any of the measurements compared to the SED groups in either age group (Table 1).

We monitored water consumption for the BSO + SSC and SSC groups and noted no significant differences. Young rats from the BSO + SSC and SSC groups drank an average of 109.07 ± 5.6 SD and 123.07 ± 15.6 SD mL/week,

Table 1 Effects of anesthesia on total glutathione and oxidative stress measures in EXP TA, CON TA and plasma from young and old animals in the ANES and SED groups

	GSH_t (μM/mg)	GPX activity (mU/mL/mg)	H_2O_2 (μM)	MDA (μM/mg)	Free 8-isoprostanes (pg/mL)
Old SED EXP TA	125.7 ± 19.3	593.1 ± 53.1	0.119 ± 0.02	1.49 ± 0.3	92.12 ± 12.6
CON TA Plasma	166.7 ± 22.4	590.9 ± 45.6	0.140 ± 0.02	1.70 ± 0.3	
Old ANES EXP TA	233.1 ± 38.5	735.4 ± 90.7	0.099 ± 0.02	2.12 ± 0.3	76.06 ± 13.2
CON TA Plasma	178.9 ± 42.2	539.4 ± 70.6	0.176 ± 0.03	1.56 ± 0.1	
Old SED EXP TA	1,313.3 ± 18.8	479.3 ± 82.9	0.055 ± 0.02	1.37 ± 0.2	55.22 ± 8.3
CON TA Plasma	95.2 ± 30.5	603.0 ± 47.7	0.044 ± 0.01	1.35 ± 0.3	
Old ANES EXP TA	145.5 ± 26.9	665.3 ± 53.0	0.075 ± 0.02	1.35 ± 0.2	62.69 ± 8.7
CON TA Plasma	116.0 ± 21.3	586.8 ± 37.1	0.046 ± 0.01	1.07 ± 0.1	

All values are group mean values ± SE. No significant differences between ANSE and SED groups were noted

respectively, and the old rats from the BSO + SSC and SSC groups drank an average of 106.18 ± 10.2 SD and 114.72 ± 13.9 SD mL/week, respectively.

Discussion

In the current study, we investigated the effects of our chronic SSC exposure protocol on oxidative stress in muscle from young and old rats. Oxidative stress has historically been shown to increase with age, especially in combination with exercise (Bejma and Ji 1999; Fulle et al. 2004; Hammeren et al. 1992; Ji 2001; Ji et al. 1998; Leeuwenburgh et al. 1994; Parise et al. 2005a; Radak et al. 2002). However, few published studies specifically investigate oxidative stress in response to an exposure protocol modeling loaded high repetition contractile activity in skeletal muscle in combination with aging. The free radical theory of aging suggests that "reactive oxygen species are produced as a normal by-product of aerobic life and that accumulation of oxidative damage caused by reactive oxygen species underlies the fundamental changes found in senescence" (Harman 1956; Ji et al. 1998). Senescent muscle has higher levels of lipid peroxidation and ROS production as well as increases in antioxidant activities suggesting that it is under higher amounts of oxidative stress during aging, and appears to be more susceptible to exercise-induced oxidative damage (Ji et al. 1990, 1998). During exercise, senescent muscle is more likely to endure mechanical injury which activates an acute phase response of the immune system, thus leading to more ROS formation (Ji et al. 1998). Levels of ROS generation and oxidative stress can increase following an acute bout of exercise (Ji et al. 1992; Liu et al. 2000; Molnar et al. 2006; Vasilaki et al. 2006; Venditti and Di Meo 1997), especially in aged muscle. However, this vulnerability can be alleviated with chronic endurance (Hollander et al. 1999; Ji 1993; Leeuwenburgh et al. 1994, 1997; Molnar et al. 2006; Radak et al. 1999; Radak et al. 2002; Venditti and Di Meo 1996, 1997) and resistance (Parise et al. 2005a, b; Vincent et al. 2002, 2006a) training by lowering the levels of ROS and increasing the antioxidant defense systems.

In this study, we also investigated whether changes in the antioxidant system, specifically the glutathione system, compromises the ability of TA muscle from young and old animals to withstand rigors of chronic resistive exposures. Oxidant status and antioxidant defense capacity were both measured in muscle from male rats following chronic SSC exposure to determine overall oxidative stress. BSO, a well-established irreversible inhibitor of γ-glutamylcysteine synthetase (GCS) (Meister 1991), decreased levels of glutathione, however, previously published reports from other chronic glutathione depletion studies using BSO showed larger decreases of muscle GSH than in our study (Griffith and Meister 1979; Leeuwenburgh and Ji 1995; Martensson and Meister 1989). The differences are likely due to the amount of BSO supplemented in the drinking water (10 vs. 20 mM), administration of BSO (i.p. injections versus water supplementation) and/or animal species (rats vs. mice).

SSC exposure increased glutathione levels in TA muscle from old rats by about 65% compared to old SED rats, but did not affect glutathione levels in young rats. A study done by Leeuwenburgh et al. (1997) observed an increase of the glutathione content in type IIa muscle following chronic endurance training via treadmill running in young female rats. The differences in age effects between our study and the study by Leeuwenburgh et. al. (1997) may be due to alternative training protocols and/or the gender of the animals. Unlike old rats in our study, young rats may adequately tolerate increases in free radical production during SSC exposure without the need to increase glutathione. The fact that glutathione increases after chronic exposures to SSCs is not surprising based on previous data from senescent muscle.

One of the most consistent findings in the oxidative stress and exercise literature is the increase in GPX activity

after chronic exercise training in hind limb muscles from both young and old animals (Hollander et al. 1999; Ji 1993; Leeuwenburgh et al. 1994, 1997; Venditti and Di Meo 1996). This upregulation appears to be fiber-type specific, with the most prominent increases found in highly oxidative muscle (e.g., soleus, type I, and DVL, type IIa) (Hollander et al. 1999; Ji et al. 1998; Leeuwenburgh et al. 1994; Oh-Ishi et al. 1995; Ji et al. 1992), and the magnitude of upregulation is influenced by both intensity and duration of exercise (Powers and Lennon 1999). After exposure to our chronic SSC protocol, we detected increases in GPX activity in the TA muscle, which is a fast, oxidative-type muscle, similar to what has been reported with endurance training (Hollander et al. 1999; Leeuwenburgh et al. 1997). However, we observed this in both young and old rats. The differences in age effects between the Leeuwenburgh study and our study is likely due to different exposure protocols (endurance vs. eccentric/resistive).

Markers of oxidative stress after chronic exercise training have also been studied in addition to the antioxidant profiles. MDA is a common indicator of lipid peroxidation. In the old animals from our study, SSC exposure led to an increase in MDA levels, a trend not seen in young rats. These data suggest that our SSC protocol leads to lipid peroxidation in old rats but not their young counterparts. Others have reported similar increases in MDA levels in fast-twitch muscle from rats after eight weeks of eccentric training (Liu et al. 2000; Molnar et al. 2006). Both of these observations indicate that lipid peroxidation is increased after training. In human adults, lipid peroxidation increased after 8 weeks of cycle training, and antioxidant supplementation alleviated this increase and brought values down below baseline levels (Vincent et al. 2006b). When we supplemented our rats with the glutathione antagonist, the lipid peroxidation observed in the old rats was not greater than that seen in old unsupplemented rats after SSC exposure. However, supplementation with antioxidants, possibly a glutathione agonist, may be beneficial to these old rats such as seen in the human subjects in the study by Vincent et al. (2006b).

Under controlled, unsupplemented conditions, our SSC exposure protocol decreased H_2O_2 in the exercised left tibialis anterior compared to the contra-lateral control right tibialis anterior in the old animals. This trend was also observed in the young rats but was not statistically significant. Decreases in H_2O_2 have also been reported in the literature as an effect of chronic endurance training (Molnar et al. 2006).

BSO supplementation alone led to a significant increase in H_2O_2 levels in the TA muscle of young and old rats in our study as seen in the unexposed CON TA data. This increase was prevented with SSC exposure in young rats but not old rats, suggesting that the chronic SSC exposure had a positive effect on the oxidant status in young animals, even in a glutathione-depleted state. In the old rats, the higher levels of H_2O_2 suggest an increase in oxidative stress and this oxidative stress was exacerbated with the SSC exposure. The fact that the SSC exposure did not have the same positive impact in the old rats treated with BSO may suggest that the old rats are more sensitive to depletions of the glutathione defense system.

Free isoprostane levels are a common biomarker of lipid peroxidation and oxidative stress (Roberts and Morrow 2000; Ward et al. 2005). We found that basal levels of free isoprostanes in the circulation were higher in old animals compared to young animals, which is supportive of earlier studies (Ward et al. 2005). After the exposure to the SSC protocol, free isoprostanes decreased in the old animals to levels that were comparable to the young animals suggesting a systemic adaptive response of the old animals to the chronic resistance exposure. BSO had no effect on free isoprostane levels suggesting that the glutathione system alone may not play a significant role in regulating circulating isoprostanes. In 2002, Vincent et al. measured serum levels of lipid peroxidation using TBARS and the ferrous oxidation/xylenol orange technique (Hermes-Lima et al. 1995). They found that resistance training produced a decrease in serum levels of lipid peroxidation after 6 months of low- or high-intensity training in their elderly ($\sim$68 years old) subjects (Vincent et al. 2002) which is in agreement with our findings.

It is reasonable to suggest that damage to lipid membranes via lipid peroxidation may contribute to a loss or decrease in muscle performance in old rats. However, in our current study, performance of the old rats did not decrease as observed previously (Cutlip et al. 2006). Instead, performance parameters (i.e., isometric force) remained mostly unchanged (unpublished results) in old rats from the beginning of the chronic SSC exposure protocol to the end of the protocol. The reason for the difference between this study and our previous study is unknown since the exposure and strain, source, gender and age of rats were all identical to the first study. Nevertheless, it is possible that more oxidative damage may have been evident had the performance decreased as in the previous study. It is also possible that if the SSC exposure protocol were titrated by increasing the rest cycles between exposures and/or repetitions, the muscle performance of old rats may improve similar to young rats. Further studies are needed to determine appropriate doses of resistance exposures to promote adaptation in old populations.

In the current study, we determined that young rats were capable of defending against skeletal muscle oxidative stress in response to our chronic high intensity mechanical loading exposure protocol, even with decreased levels of glutathione (i.e., BSO treatment). However, in old animals, chronic

exposure to SSCs led to an increase in lipid peroxidation (i.e., MDA) in the exposed TA muscle. We did not see a concurrent increase in hydrogen peroxide, which would be expected with the increase in lipid peroxidation. It is possible that the increase in GPX activity was enough to alleviate any increases in hydrogen peroxide that may have occurred during the chronic exposure. Our results are consistent with endurance training effects and indicate that chronic exposure to SSCs may elicit positive effects that are similar to those effects noted with endurance training in old rats.

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