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Heather K. Vincent, Scott K. Powers, Darby J. Stewart, Haydar A. Demirel, **R. Andrew Shanely** , Hisashi Naito

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Abstract: These experiments tested the hypothesis that short-term endurance exercise training would rapidly improve (within 5 days) the diaphragm oxidative/anti- oxidant capacity and protect the diaphragm against contraction-induced oxidative stress. To test this postulate, male Sprague-Dawley rats (6 weeks old) ran on a motorized treadmill for 5 consecutive days (40-60 min · day^{-1}) at approximately 65% maximal oxygen uptake. Costal diaphragm strips were excised from both sedentary control (CON, $n = 14$) and trained (TR, $n = 13$) animals 24 h after the last exercise session, for measurement of in vitro contraction properties and selected biochemical parameters of oxidative/antioxidant capacity. Training did not alter diaphragm force-frequency characteristics over a full range of submaximal and maximal stimulation frequencies ($P > 0.05$). In contrast, training improved diaphragm resistance to fatigue as contraction forces were better-maintained by the diaphragms of the TR animals during a submaximal 60- min fatigue protocol ($P < 0.05$). Following the fatigue protocol, diaphragm strips from the TR animals contained 30% lower concentrations of lipid hydroper-oxides compared to CON ($P < 0.05$). Biochemical analysis revealed that exercise training increased diaphragm oxidative and antioxidant capacity (citrate synthase activity +18%, catalase activity +24%, total superoxide dismutase activity +20%, glutathione concentration +10%) ($P < 0.05$). These data indicate that short-term exercise training can rapidly elevate oxidative capacity as well as enzymatic and non-enzymatic antioxidant defenses in the diaphragm. Further- more, this up-regulation in antioxidant defenses would be accompanied by a reduction in contraction-induced lipid peroxidation and an increased fatigue resistance.

Key words Diaphragm · Oxidative stress · Fatigue · Lipid peroxidation · Antioxidants

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Introduction

It has been reported that free radicals and other reactive oxygen species (ROS) are produced during muscle contractions in the diaphragm (Davies et al. 1982; Reid et al. 1992a; Diaz et al. 1993). Furthermore, production of ROS during repeated diaphragm contractions in vitro or resistive breathing in vivo has been shown to contribute to diaphragm oxidative injury, such as lipid and protein oxidation (Anzueto et al. 1992, 1993; Vincent et al. 1999). It has been found that extensive lipid peroxidation in cells can result in a loss of cellular homeostasis resulting from impaired function of membrane bound receptors, ion channels, and reduced enzyme activity (Halliwell and Gutteridge 1990; Kukreja and Hess 1992). This has been considered to be physiologically significant because oxidative damage to myocytes is associated with contraction dysfunction, including reduced maximal tension, reduced rate of force development and early onset of fatigue (Shindoh et al. 1990; Reid et al. 1993; Anzueto et al. 1994; Jacinto et al. 1996; Supinski 1998).

Since ROS are a product of normal metabolism, cells are equipped with an endogenous antioxidant defense system to counteract ROS-induced oxidative stress (reviewed in Yu 1994; Ji 1995). The primary components of this defense include both antioxidant enzymes (superoxide dismutase, SOD, catalase, CAT, and glutathione peroxidase, GPX) and non-enzymatic antioxidants such as glutathione. The enzyme SOD is responsible for dismutation of superoxide radicals resulting in the formation of hydrogen peroxide. Hydrogen peroxide can be removed by CAT and/or GPX. Furthermore, GPX can degrade not only hydrogen peroxide but can remove

other organic hydroperoxides as well. Glutathione is a non-protein thiol that serves as a co-substrate for GPX and performs numerous other antioxidant functions.

Numerous in vitro experiments have indicated that increasing components of the antioxidant defense system protect the contracting myocyte against ROS-mediated injury and improves muscle endurance (Shindoh et al. 1990; Ji and Fu 1992; Reid et al. 1992a; Jenkins 1993; Suspinski et al. 1997). Furthermore, recent in vivo studies have indicated that regular endurance exercise increases diaphragm antioxidant capacity and reduces contraction-induced oxidative damage (Lawler et al. 1993; Powers et al. 1994; Oh-Ishi et al. 1997; Vincent et al. 1999). For example, 12 weeks of endurance exercise training have been shown to reduce lipid peroxidation following fatiguing contractions in vitro (Vincent et al. 1999). In this regard, it has commonly been believed that these training-induced changes in the diaphragm are the cumulative result of successive periods of exercise over several weeks or months. Collectively, these changes could lead to improved diaphragm protection from oxidative injury.

The minimal duration of exercise training required to protect the diaphragm from oxidant-induced lipid peroxidation is unknown. However, evidence exists that even a single period of endurance exercise promotes expression of numerous proteins including cytoprotective stress proteins (e.g. 60 kD, 70 kD) in both skeletal muscle and the heart (Salo et al. 1991; Locke et al. 1995). In addition, Ji (1995) has recently reported that GPX activity is elevated in skeletal muscle following a single period of endurance exercise, indicating rapid alterations in transcription rates of antioxidant enzymes. Therefore, it seems reasonable to speculate that exercise training-induced changes in the antioxidant capacity of skeletal muscle could rapidly occur following several repeated periods of exercise. This adaptation could ultimately provide cytoprotection against a subsequent oxidative challenge. Hence, the purpose of this study was to de- termine if short-term endurance exercise training (5 consecutive days) results in enhanced diaphragm protection from contraction-induced oxidative stress. Considering data that has demonstrated enhanced cytoprotection within 1 to 5 days of endurance training (elevated heat shock protein contents and antioxidant enzyme mRNA; Salo et al. 1991; Locke et al. 1995; Ji et al. 1995) we hypothesized that 5 consecutive days of exercise training would enhance the diaphragm primary antioxidant defense and reduce oxidant-mediated injury (lipid peroxidation) following fatiguing contractile activity in vitro.

Methods

Animals

The invasive nature of these experiments on the diaphragm precluded the use of humans. However, the morphological characteristics (i.e. fiber type and anatomical structure) of the rat diaphragm are very similar to that of humans, and, therefore, the rat has been widely used as a model for studying diaphragm adaptation. These experiments were approved by the University of Florida's Committee for the Use of Animals in Research and followed the guidelines established by the American Physiological Society. Male Sprague-Dawley rats (6 weeks old) were housed in the laboratory animal vivarium, were fed purina rat chow and water ad libitum and were maintained on a 12:12-h light-dark photoperiod at 21°C during the experiment.

Experiment design and exercise training protocol

The animals were randomly assigned to one of two groups: (1) sedentary control (n = 14); or (2) exercise training (n = 14). Both control and exercise trained animals were housed in the same room during the period of the experiment.

The exercise trained animals exercised for 5 days consecutively on the treadmill. On day 1, the animals ran at 25 m · min^{-1} for 40 min (0% gradient). For untrained young adult animals, this running intensity has been shown to correspond to approximately 65% maximal oxygen uptake (Lawler et al. 1993). Mild electrical shocks were sparingly used to encourage the animals to run. On days 2 and 3 of training, the exercise duration was increased by 10 min a day until the animals were running for 60-min · day^{-1}. This training duration was maintained on days 4 and 5 of the training period. During each training session, if an animal could not keep pace with the treadmill, they were given a 3-min rest prior to resuming exercise.

In vitro contraction properties

The trained animals were administered an intraperitoneal injection of sodium pentobarbital (100 mg · kg^{-1}) 24 h following the last training session. After reaching a surgical plane of anesthesia, the diaphragm was surgically excised and placed in a cooled (21°C) dissecting chamber containing Krebs-Hensleit solution equilibrated with a 95% O_2 and 5% CO_2 gas mixture. Samples of diaphragm muscle were then collected for both contraction measurements and biochemical analysis. Approximately 300 mg of the left ventral costal region of the diaphragm was removed and immediately frozen in liquid nitrogen and stored at −80°C for subsequent biochemical analysis. For contraction measurements, two muscle strips, including the tendinous attachments at the central tendon and rib cage (dimensions rv20 x 2 mm), were dissected from the right ventral costal region. These strips were then each suspended vertically be- tween two light weight plexiglass clamps connected to force transducers (Grass, model FT10) in a jacketed tissue bath. Transducer output was amplified and differentiated by operational amplifiers and underwent A/D conversion for analysis using a computer based data acquisition system (Superscope II, GW Instruments).

The dual jacketed tissue baths were aerated with gas (95% O_2 and 5% CO_2), pH was maintained at 7.4 ± 0.05, and the osmolality of the bath was approximately 290 mmol · kg^{-1}. Temperature in the organ bath was maintained at approximately 37 ± 0.5°C, and was monitored using a digital thermometer.

In vitro contraction measurements commenced with determination of the optimal stimulation length (L_o) of the muscle for development of isometric tetanic tension (resting tension −1.5 g). The muscle field was stimulated at 140 V (modified Grass Instruments S48 stimulator) along its entire length with platinum elec- trodes using monophasic pulses of 2-ms duration; length was progressively increased until maximal isometric twitch tension was obtained. Once the highest twitch force was achieved, all contraction properties were measured isometrically at L_o. Maximal twitch tension was determined from a series of single twitches. The following isometric characteristics were then determined using the stored analogue trace from the twitches: time to peak tension (TPT), half-relaxation time (2RT), and maximal rate of tension development (+dP/dt). The 2RT was computed using computer-based algorithms and +dP/dt was determined by analysis of the temporal pattern of the decline in force following a maximal twitch.

Force-frequency relationship

The force-frequency relationship was determined as follows. Isometric tetanic contractions were produced using a supramaximal stimulus train of 500-ms duration (160 Hz). Force was monitored by computerized ergometry as previously described. Peak isometric tension was determined from a series of three contractions with a 2-min period for recovery between measurements. Maxi- mal isometric force was then determined at 15, 30, 40, 50, 80, 120,
160 and 200 Hz using the stimulation parameters previously described. A 2-min period for recovery separated each contraction.

Assessment of diaphragm endurance (fatigue protocol)

To determine whether 5 days of treadmill exercise training was a sufficient stimulus to confer protection against fatigue in vitro, diaphragm strips were subjected to a 60-min fatigue protocol. Strips were stimulated every 2 s at 30 Hz with stimulation trains lasting 330 m. A fatigue index was calculated using the following equation: (peak tension at 60 min divided by tension at first contraction of protocol) x 100 (Anzueto et al. 1992).

Determination of muscle cross-sectional area

Following each of the contraction protocols, the total muscle cross-sectional area (CSA) of the in vitro strips was calculated by the algorithm: CSA (cm^2) [wet mass/(muscle bundle length x 1.056)) where wet mass is the mass of the diaphragm strip in grams, 1.056 is the density of muscle in grams per centimetre cubed and muscle bundle length is expressed in centimetres and measured at L_o (Gollnick et al. 1981).

Oxidative and antioxidant enzyme activities

Approximately 50 mg of non-contracted diaphragm was homogenized (on ice) in 5 ml of 100 mmol $\cdot$ l^{-1} potassium buffer, using a tissue homogenizer (Ultra-Turrax T25 IKA Works, Cincinnati, Ohio). Each sample was then centrifuged (3°C) at 400 *g* for 10 min to remove large debris. The supernatant was decanted and kept on ice until analysis. Enzyme activities were determined spectropho- tometrically immediately following homogenization. Citrate synthase [CS Enzyme Commission no. (EC) 4.1.4.7) activity was used as a marker of oxidative capacity and was determined using the
procedure previously described by Srere (1969). The enzyme CAT (E.C. 1.11.1.6) activity was determined by the method of Aebi (1984), that of GPX (E.C. 1.11.1.9) activity was determined using the technique described by Flohe and Gunzler (1984), and that of SOD (E.C. 1.15.1.1) was assessed using the methods described by Oyanagui (1984). Enzyme activities were normalized to the protein concentrations of the homogenate as described by Bradford (1976).

Lipid peroxidation

Lipid peroxidation is a form of cellular oxidative injury that has been shown to result from the formation of lipid hydroperoxides derived from unsaturated phospholipids (Halliwell and Gutteridge 1990). To assess the magnitude of lipid peroxidation in the diaphragm strips subjected to the prolonged stimulation protocol, we measured the hydroperoxide content of the muscle using a ferrous oxidation technique described by Hermes-Lima et al. (1996). Briefly, diaphragm muscle samples were homogenized in ice cold methanol (1:20, w/vol), centrifuged at 400 *g* for 10 min and the supernatants removed for subsequent analysis. All samples were assessed in triplicate, and cumene hydroperoxide was used as the assay standard. All samples were assayed on the same day and expressed in cumene hydroperoxide equivalents per milligram protein.

Cellular thiol content

Since tissue thiols (molecules containing sulfhydryl groups) are important in the regulation of both cellular redox status and antioxidant capacity, we assayed total, protein and non-protein thiols in the diaphragms of control and trained animals. The non-protein fraction is a representative measure of glutathione, as 90% of the non-protein thiols have been shown to constitute glutathione (Ji 1995). Thiols were determined spectrophotometrically using the dithionitrobenzene-based technique described by Jocelyn (1989).

Data analysis

Group comparisons were made by a one-way analysis of variance. Bivariate Pearson correlations were performed between antioxidant enzyme activities, lipid peroxidation and fatigue index values. For all statistical analysis, significance was established at $P < 0.05$.

Results

Of 14 animals 13 successfully completed 5 consecutive days of exercise training. There was 1 animal eliminated from the study due to an exercise-related limb injury. At the time of sacrifice, the mean body mass for the controls was 303.8 (SEM 7.1) g and 290.4 (SEM 7.4) g for the trained animals.

Contractile properties and force-frequency curve

The results for the contraction properties are shown in Table 1. Significant differences existed between groups for TPT and slope (+dP/dt; $P < 0.05$). In contrast, no differences existed between groups in maximal tetanic specific force, peak twitch force, or 2RT. Further, training did not alter ($P > 0.05$) diaphragm force production at any frequency of stimulation (data not shown).

Diaphragm endurance

The results of the 60-min in vitro fatigue protocol are given in Fig. 1. Compared to control, diaphragm strips from the trained animals maintained a higher relative

Table 1 In vitro contractile properties of costal diaphragm strips from control and 5-day treadmill trained Sprague-Dawley rats. P_o isometeric tetanic specific force, P_t isometric twitch specific force, *TPT* time to maximal peak twitch isometric force, *1/iRT* half-relaxation time following a twitch contraction, *+dP/dt* maximal rate of specific force development during a twitch contraction

	Control		Trained	
	Mean	SEM	Mean	SEM
P_o (N $\cdot$ cm^{-2})	24.8	0.92	24.3	0.51
P_t (N $\cdot$ cm^{-2})	9.1	0.45	9.7	0.34
P_t/P_o	0.366	0.02	0.379	0.01
TPT (ms)	39.0	0.32	55.0	1.03*
2RT (ms)	37.0	0.08	35.0	0.06
+dP/dt (N $\cdot$ ms^{-1} $\cdot$ cm^{-2})	211.2	12.9	167.6	10.03*

*Significance between groups at the $P < 0.05$ level

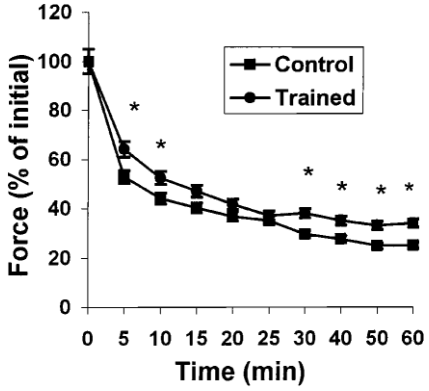

Fig. 1 Comparison of diaphragm contractile responses to a 60-min submaximal fatigue protocol in vitro. Values are means and SEM. * Significance at the $P < 0.05$ level

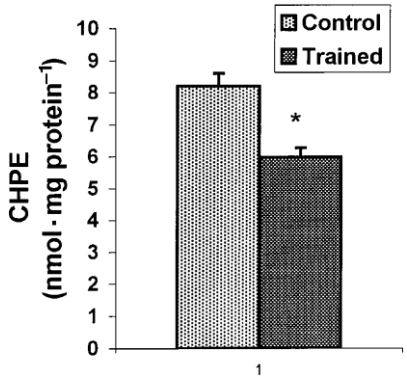

Fig. 2 Lipid hydroperoxide concentrations, defined as cumene hydroperoxide equivalents (*CHPE*), in diaphragms from control and trained diaphragms following a 60-min fatigue protocol. Values are means and SEM. * Significant at $P < 0.05$

force production at 5, 10, 30, 40, 50, and 60 min ($P < 0.05$).

Oxidative and antioxidant enzyme activities

The results for the oxidative and antioxidant enzymes are shown in Table 2. Compared to the controls, diaphragm CS and CAT activities were significantly in- creased ($P < 0.05$) in the trained animals. Furthermore, training resulted in a significant increase ($P < 0.05$) in total SOD activity in the diaphragm. Finally, training did not alter ($P > 0.05$) diaphragm GPX activity.

Lipid peroxidation

Figure 2 contains the lipid hydroperoxide concentrations from contracted diaphragms from both control and trained animals. Note that the control group showed a significantly higher ($P < 0.05$) level of peroxidation following the in vitro fatigue protocol compared to the trained group. Specifically, the trained group exhibited 30% lower lipid peroxides compared to the control group, suggesting a training-induced protective effect.

Thiol content

The diaphragm thiol content is shown in Fig. 3. Trained diaphragms contained a significantly greater content of total and non-protein bound thiols compared to the

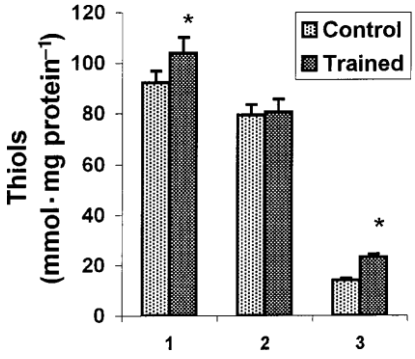

Fig. 3 Cellular thiol content in diaphragms of control and endurance trained animals, where *1* = total thiols, *2* = protein-bound thiols, and *3* = non-protein thiols. Values are means and SEM. * Significant at $P < 0.05$

control diaphragms ($P < 0.05$). We interpreted the training-induced increase in non-protein thiols as an indication that short-term training elevated diaphragm reduced glutathione (GSH) concentrations. No differences ($P > 0.05$) existed between the groups in the protein bound thiol content.

Correlations between antioxidant enzyme activities and diaphragm fatigue

Because previous experiments have shown that increasing the antioxidant capacity of the diaphragm improves

Table 2 Oxidative and anti-oxidant enzyme activities of control and trained Sprague-Dawley rats following 5 con-secutive days of endurance training. *CS* Citrate synthase, *GPX* glutathione peroxidase, *CAT* catalase, *SOD* superoxide dismutase

	Control		Trained		*P* value
	Mean	SEM	Mean	SEM	
CS (μmol · mg protein^{-1} · min^{-1})	0.51	0.02	0.60	0.03	0.02*
GPX (μmol · mg protein^{-1} · min^{-1})	0.10	0.004	0.11	0.006	0.76
CAT (U · mg protein^{-1})	0.62	0.02	0.77	0.03	0.002*
Total SOD (U · mg protein^{-1})	49.3	1.8	59.3	3.3	0.01*

* Significant difference ($P < 0.05$) between trained and control animals

Table 3 Relationship between diaphragm oxidative and antioxidant enzyme activity, magnitude of lipid peroxidation, and the fatigue index following 60 min of contractile activity in vitro. *CS* Citrate synthase activity, *GPX* glutathione peroxidase activity, *CAT* catalase activity, *SOD* superoxide dismutase activity, *Lipox* tissue lipid peroxidation

	Fatigue index		*P* value
Lipox	*r*	−0.66	*0.0005
CS	*r*	0.48	*0.049
GPX	*r*	0.27	0.265
CAT	*r*	0.72	*0.001
SOD	*r*	0.70	*0.005

* $P < 0.05$

fatigue resistance (Shindoh et al. 1990; Reid et al. 1992a), we determined the correlations between the diaphragm fatigue index and the activities of antioxidant enzymes. In addition, we examined the relationship between the diaphragm fatigue index and the amount of lipid peroxidation in diaphragm strips exposed to the fatigue protocol obtained from both the control and trained animals. A significant negative correlation ($P < 0.05$) existed be- tween the diaphragm fatigue index and the amount of lipid peroxidation in the diaphragm following 60 min of contractile activity (Table 3). In contrast, a significant positive correlation existed between the diaphragm fatigue index and the activities of total SOD and CAT. Although these relationships did not prove cause and effect, they indicated that diaphragm endurance would seem to be associated with the antioxidant capacity of the muscle and fatigue with lipid peroxide formation.

Discussion

To our knowledge, this is the first investigation of the effects of short-term exercise training on diaphragm antioxidant capacity and protection against contraction-induced oxidative injury. We tested the hypothesis that short-term exercise training would enhance diaphragm antioxidant protection and reduce oxidant-mediated lipid injury following prolonged contractile activity. The data supported this postulate. Indeed, 5 consecutive days of endurance exercise training resulted in an increase in several components of the diaphragm antioxidant system and this increase was associated with protection from oxidative damage. In addition, short- term exercise training promoted an increase in oxidative capacity of the diaphragm and improved fatigue resistance of the diaphragm during prolonged contractions in vitro. A brief discussion of these issues follows.

Increased antioxidant defense and protection against lipid peroxidation

Our findings indicated that important components of the diaphragm antioxidant defense system are increased following short-term endurance exercise training. Specifically, total SOD and CAT activities were significantly increased by 20% and 24%, respectively. Furthermore, GSH concentrations in the diaphragm were elevated by 10%. Despite the relatively short period of training, the exercise-induced elevations in total SOD activity and GSH content are similar to those that have been observed following training of prolonged duration (8-12 weeks; Sen et al. 1992; Powers et al. 1992, 1994; Oh-Ishi et al. 1997; Vincent et al. 1999). This observation would suggest that the training-induced elevation in diaphragm antioxidant capacity can be achieved rapidly after the onset of endurance exercise training. Furthermore, data from our laboratory and others would suggest that the magnitude of adaptation of the antioxidant defense may be influenced in part by the metabolic properties of the muscle. Specifically, it appears that chronic training promotes elevations in antioxidant enzyme activities (i.e. SOD) in highly oxidative locomotor muscles such as the soleus and red gastrocnemius but not in glycolytic muscles such as the white gastrocnemius (reviewed in Powers et al. 1999). It is unclear whether this phenomenon is due to fiber type-regulated differences in adaptability or directly due to the ordered fiber type recruitment during exercise. To date, there have been no published reports with regard to the effect of short-term training on locomotor muscle antioxidant capacity.

Previous investigations on the effects of chronic exercise training on the antioxidant capacity of skeletal muscle have generally revealed a training-induced in- crease in skeletal muscle GPX activity with little or no change in CAT activity (Powers et al. 1994; Venditti and DiMeo 1996, 1997). However, in the present study, short-term exercise training resulted in an increase in diaphragm CAT activity with no change in GPX activity. This finding would suggest that increases in CAT activity in diaphragm muscle occur during the initial adaptation to exercise training. Hence, it would appear that in the early stages of an exercise training program, SOD works cooperatively with CAT to scavenge ROS (i.e. superoxide radicals and hydrogen peroxide) produced in the diaphragms of exercising animals.

It seems likely that following this initial phase of antioxidant adaptation, other antioxidant enzymes such as GPX are also expressed in the diaphragm further to improve antioxidant defenses. Up-regulation of SOD activity would assist in removing superoxide radicals generated either in the mitochondria or in the cytosol. Further, since the K*m* for GPX is much lower than catalase (rv1 $\mu mol \cdot l^{-1}$ compared to 1 $mmol \cdot l^{-1}$; Ji 1995), GPX is more effective at removing hydrogen peroxide than CAT at lower levels of substrate. Hence, an increase in muscle tissue GPX would be useful in removing even low levels of hydrogen peroxide with increased levels of respiration as has been suggested by Yu (1994). Alternatively, it has been suggested that short-term exercise training may alter the kinetics and

catalytic activity of the antioxidant enzymes in muscle tissue by allosteric or covalent modification (Ji 1995). For example, the maximal velocity and K*m* values for CAT and SOD may be altered to improve the scavenging activity of the enzymes during the initial adaptation period of training.

What is the physiological significance of this training-induced elevation of the diaphragm antioxidant capacity? The biological importance of the elevated diaphragm antioxidant capacity was revealed by our finding that compared to untrained animals, lipid peroxidation was significantly reduced in diaphragms from trained animals following 60 min of muscle contractions in vitro (Fig. 2). Considering that extensive lipid peroxidation has been associated with a reduction in membrane integrity, myocyte dysfunction, and even cell death (Girotti 1998), prevention of this damage in cells is of great biological importance. For example, chronic obstructive pulmonary diseases (i.e. emphysema) increases airway resistance and diaphragm work, conditions which could lead to diaphragm fatigue or failure. It has been proposed that the increased formation of free- radicals contribute to diaphragm fatigue (Anzueto et al. 1992; Morales et al. 1993; Supinski 1998). Hence, any strategy that can counteract oxidant stress in the diaphragm may better maintain diaphragm function and reduce cellular injury in clinical situations.

Improved oxidative capacity

A period of 5 consecutive days of endurance training resulted in an increase (+18%) in the oxidative capacity of the diaphragm as evidenced by the increase in the activity of the mitochondrial enzyme, CS. Al- though it has been well established that endurance exercise training improves the oxidative capacity of the diaphragm, all of the previous studies have employed either acute exercise protocols or long-term exercise training as the stimulus (Grinton et al. 1992; Powers et al. 1992, 1994; Lawler et al. 1993). The current study is the first to report that training-induced increases in diaphragm oxidative capacity can occur rapidly after beginning a training program. It is interesting that following only 5 days of training, diaphragm CS activity increased by 18% which has been shown to be near the maximal increase in the diaphragm CS activity following 8-10 weeks of endurance training (reviewed in Powers and Criswell 1996). Nonetheless, the notion that activity-induced elevation in mitochondrial volume occurs quickly in skeletal muscles is not new. Indeed, rapid mitochondrial growth has been documented following chronic electrical stimulation of skeletal muscle in as few as 48 h (Lieber 1988, Pette 1990). The present data extended the findings of previous experiments using electrical stimulation by confirming that training-induced in- creases in diaphragm oxidative capacity can occur rapidly in a physiological environment.

Short-term training improved diaphragm endurance

An important functional outcome of this experiment was that short-term exercise training was associated with improved diaphragm endurance. Numerous factors are thought to contribute to muscle fatigue resulting from prolonged contractile activity and, based upon the current data alone, it is impossible to determine which training-induced biochemical changes in the diaphragm were responsible for the improvement in muscle performance. Nonetheless, it seems likely that training-induced metabolic adaptations, such as increased antioxidant and oxidative capacity along with fast- to-slow shifts in myosin isoforms, are potential mechanisms. Specifically, it has been suggested that the training-induced improvement in diaphragm fatigue resistance may be related to the training-induced elevation in the tissue antioxidant capacity (Powers et al. 1999). Indeed, the training-induced increase in diaphragm antioxidant capacity reduces oxidative stress caused by the contraction-induced increase in mitochondrial respiration. This could be of particular importance given the recent and growing evidence that ROS formation may promote muscle fatigue (Reid et al. 1992a; Anzueto et al. 1992, 1993; Morales et al. 1993; Supinski 1998). In this regard, numerous studies have shown that the addition of either in vitro or in vivo antioxidants reduces the rate of diaphragm fatigue during prolonged contractile activity (Shindoh et al. 1990; Reid et al. 1992a; Supinski et al. 1997). Specifically, Shattock and Had- dock (1991) have argued that free radicals can have a negative effect on muscle contractility by directly interfering with ion channels and membrane-bound protein gates, processes that can affect muscle tension development. Enzymatic or non-enzymatic antioxidants have been shown to assist in removing radicals from these sites of ion regulation, thus improving the muscle contractile efficiency by altering +dP/dt and improving resistance to fatigue (Shattock and Haddock 1991; Reid 1996). While correlational relationships do not prove cause and effect, our data indicate that the training-induced improvements in diaphragm antioxidant capacity were significantly correlated with diaphragm endurance (Table 3).

From a theoretical standpoint, the training-induced improvement in diaphragm oxidative capacity could also lead to improved contraction performance during prolonged activity. It is well known that an increase in muscle oxidative capacity would enhance the tissue's ability to metabolize fat and reduce the rate of muscle glycogen depletion. Furthermore, an increase in mitochondrial volume in the diaphragm could decrease the rate of lactate production during high intensity con- tractions (Holloszy and Coyle 1984; Gollnick et al. 1985). Therefore, an improved oxidative capacity has been shown to be complemented by an improved anti- oxidant defense to remove the ROS formed during in- creased respiration (Khawli and Reid 1994). It has been thought that collectively these changes might delay

fatigue during prolonged activity (Shindoh et al. 1990; Reid et al. 1992a).

It is also possible that a training-induced shift in fast-to-slow myosin isoforms could also have contributed to the observed improvement in diaphragm endurance performance. Indeed, it is well known that skeletal muscle fibers containing slow myosin are more fatigue resistant compared to fibers containing fast myosin isoforms. While we did not evaluate the training-induced changes in myosin isoforms, our data indicated that training resulted in a increase in TPT and a decrease in +dP/dt. Both of these changes are consistent with training-induced fast-to-slow shifts in the contractile proteins of skeletal muscle.

In summary, while our data clearly indicated that short-term exercise training improves diaphragm endurance, they did not provide a definitive answer as to what training-induced changes were responsible for the improved muscle performance.

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References

Aebi H (1984) Catalase in vitro. Meth Enzymol 105:121-126

Anzueto A, Andrade FH, Maxwell LC, Levine SM, Lawrence RA, Gibbons WJ, Jenkinson SG (1992) Resistive breathing activates the glutathione redox cycle and impairs performance of rat diaphragm. J Appl Physiol 72:529-534

Anzueto A, Andrade FH, Maxwell LC, Levine SM, Lawrence RA, Jenkinson SG (1993) Diaphragmatic function after resistive breathing in vitamin E-deficient rats. J Appl Physiol 74:267-271

Anzueto A, Brassard JM, Andrade FH, Lawrence RA, Maxwell LC, Levine SM, Jenkinson SG (1994) Effects of hyperoxia on rat diaphragm function. J Appl Physiol 779:63-68

Bradford M (1976) A refined sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248

Davies KJA, Quintanilha AT, Brooks GA, Packer L (1982) Free radicals and tissue damage produced by exercise. Biophys Res Commun 107:1198-1205

Diaz PT, Zhi-Wu She W, Davis B, Clanton TL (1993) Hydroxylation of salicylate by the in vitro diaphragm: evidence for hydroxyl radical production during fatigue. J Appl Physiol 75:540-545

Flohe L, Gunzler W (1984) Assays of glutathione peroxidase. Meth Enzymol 105:114-121

Girotti AW (1998) Lipid hydroperoxide generation, turnover, and effector action in biological systems. J Lipid Res 39:1529-1542

Gollnick PD, Timson B, Moore R, Reidy M (1981) Muscular enlargement and number of fibers in skeletal muscles of rats. J Appl Physiol 50:936-943

Gollnick P, Reidy M, Quintinskie J, Bertocci L (1985) Difference in metabolic potential of skeletal muscle fibres and their significance for metabolic control. J Exp Biol 115:191-199

Grinton S, Powers S, Lawler J, Criswell D, Dodd S, Edwards W (1992) Endurance training-induced increases in expiratory muscle oxidative capacity. Med Sci Sports Exerc 24:551-555

Halliwell B, Gutteridge JMC (1990) Role of free radicals and catalytic metal ions in human disease: an overview. Meth Enzymol 186:1-85

Hermes-Lima M, Wilmore W, Storey K (1995) Quantification of lipid peroxidation in tissue extracts based on Fe(III) xylenol orange formation. Free Rad Biol Med 19:271-280

Higuchi M, Cartier LJ, Chen M, Holloszy JO (1985) Superoxide dismutase and catalase in skeletal muscle: adaptive response to exercise. J Gerontol 40:281-286

Holloszy J, Coyle E (1984) Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol 56:831-838

Jacinto SM, Lokhandwala MF, Jandhyala BS (1996) Effect of pharmacological interventions in the prevention of lipid peroxidation and respiratory depression induced by oxygen free radicals in anesthetized rats. Free Rad Biol Med 21:791-799

Jenkins RR (1993) Exercise, oxidative stress and antioxidants: a review. Int J Sport Nutr 3:356-375

Ji LL (1995) Exercise and oxidative stress: role of the cellular antioxidant defense system. Exerc Sports Sci Rev 23:135-166

Ji LL, Fu RG (1992) Responses of glutathione system and antioxidant enzymes to exhaustive exercise and hydroperoxide. J Appl Physiol 72:549-554

Jocelyn P (1989) Spectrophotometric assay of thiols. Meth Enzymol 143:44-55

Khawli FA, Reid MB (1994) N-acetylcysteine depresses contractile function and inhibits fatigue of diaphragm in vitro. J Appl Phsyiol 77:317-324

Kukreja RC, Hess ML (1992) The oxygen free radical system: from equations through membrane-protein interactions to cardiovascular injury and protection. Cardiovasc Res 26:641-655

Lawler JM, Powers SK, Criswell D (1993) Inducibility of NADP-specific isocitrate dehydrogenase with endurance training in skeletal muscle. Acta Physiol Scand 149:177-181

Leeuwenburgh C, Hollander J, Fiebig R, Leichtweis S, Griffith M, Ji LL (1997) Adaptations of glutathione antioxidant system to endurance training are tissue and muscle fiber specific. Am J Physiol 272:R363-R369

Lieber RL (1988) Time course and cellular control of muscle fiber type transformation following chronic stimulation. ISI Atlas Animal Plant Sci 1:189-194

Locke M, Noble EG, Tanguay RM, Feild MR, Ianuzzo SE, Ianuzzo DE (1995) Activation of heat-shock transcription factor in rat heart after heat shock and exercise. Am J Physiol 268:C1387-C1394

Morales CF, Anzueto A, Andrade F, Levine SM, Maxwell LC, Lawrence RA, Jenkinson SG (1993) Diethylmaleate produces diaphragmatic impairment after resistive breathing. J Apppl Physiol 75:2406-2411

Oh-Ishi S, Takato K, Ookawara T, Sakurai T, Izawa T, Nagata N, Ohno H (1997) Endurance training improves the resistance of rat diaphragm to exercise-induced oxidative stress. Am J Respir Crit Care Med 156:1579-1585

Oyanagui Y (1984) Reevaluation of assay methods and establishment of kit superoxide dismutase activity. Anal Biochem 142:290-296

Pereira D, Dusterhoft S (1992) Altered gene expression in fast-twitch muscle induced by chronic low-frequency stimulation. Am J Physiol 262:R333-R338

Pette D (1990) The dynamic state of muscle fibers. DeGruyter, Berlin

Powers S, Criswell D (1996) Adaptive strategies of respiratory muscles in response to endurance training. Med Sci Sports Exerc 28:1115-1122

Powers SK, Grinton S, Lawler J, Criswell D, Dodd S (1992) High intensity exercise training-induced metabolic alterations in respiratory muscles. Respir Physiol 89:169-177

Powers SK, Criswell D, Lawler J, Martin D, Ji LL, Herb RA, Dudley G (1994) Regional training-induced alterations in diaphragmatic oxidative and antioxidant enzymes. Respir Physiol 95:227-237

Powers SK, Ji LL, Leeuwenburgh C (1999) Exercise training-induced alterations in skeletal muscle antioxidant capacity: a brief review. Med Sci Sports Exerc 31:987-997

Reid MB (1996) Reactive oxygen and nitric oxide in skeletal muscle. News Physiol Sci 11:114-119

Reid MB, Haack KE, Francheck KM, Valberg PA, Kobzik L, West MS (1992a) Reactive oxygen in skeletal muscle I. Intracellular oxidant kinetics and fatigue in vitro. J Appl Physiol 73:1797-1804

Reid MB, Shoji T, Moody MR, Entman ML (1992b) Reactive oxygen in skeletal muscle II. Extracellular release of free radicals. J Appl Physiol 73:1805-1809

Reid MB, Khawli FA, Moody MR (1993) Reactive oxygen in skeletal muscle III. Contractility of unfatigued muscle. J Appl Physiol 73:1081-1087

Salo D, Donovan CM, Davies KJA (1991) HSP70 and other possible heat-shock or oxidative stress proteins are induced in skeletal muscle, heart and liver during exercise. Free Rad Biol Med 11:239-246

Sen CK, Marin E, Kretzschmar M, Hanninen O (1992) Skeletal muscle and liver glutathione homesostasis in response to training, exercise and immobilization. J Appl Physiol 73:1265-1272

Shattock MJ, Haddock PS (1991) Oxidant stress and the heart: modulation of ion transport mechanisms during ischemia and reperfusion. In: Immunopharmacology of free radical species. Academic Press, Baltimore, md., pp 53-72

Shindoh C, DiMarco A, Thomas A, Manubay P, Supinski G (1990) Effect of N-acetylcysteine on diaphragm fatigue. J Appl Physiol 68:2107-2113

Srere P (1969) Citrate synthase. Meth Enzymol 13:3-5

Supinski G (1998) Free radical induced respiratory muscle function. Mol Cell Biochem 79:99-110

Supinski GS, Stofan D, Ciufo R, DiMarco A (1997) N-acetylcysteine administration alters the response to inspiratory loading in oxygen-supplemented rats. J Appl Physiol 82:1119-1125

Venditti P, DiMeo S (1996) Antioxidants, tissue damage, and endurance in trained and untrained young male rats. Arch Biochem Biophys 331:63-68

Venditti P, DiMeo S (1997) Effect of training on antioxidant capacity, tissue damage and endurance of adult male rats. Int J Sports Med 18:497-502

Vincent HK, Powers SK, Demirel H, Coombes JS, Naito H (1999) Exercise training protects against contraction-induced lipid peroxidation in the diaphragm. Eur J Appl Physiol 79:268-273

Yu BP (1994) Cellular defenses against damage from reactive oxygen species. Phys Rev 74:139-162