



Adaptation of Upper Airway Muscles to Chronic Endurance Exercise

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We tested the hypothesis that chronic endurance exercise is associated with the recruitment of four major upper airway muscles (genioglossus, digastric, sternohyoid, and omohyoid) and results in an increased oxidative capacity and a fast-toward-slow shift in myosin heavy chain (MHC) isoforms of these muscles. Female Sprague–Dawley rats (n = 8; 60 days old) performed treadmill exercises for 12 weeks (4 days/week; 90 minutes/day). Age-matched sedentary female rats (n = 10) served as control animals. Training was associated with an increase (p < 0.05) in the activities of both citrate synthase and superoxide dismutase in the digastric and sternohyoid muscles, as well as in the costal diaphragm. Compared with the control animals, Type I MHC content increased (p < 0.05) and Type IIb MHC content decreased (p < 0.05) in the digastric, sternohyoid, and diaphragm muscles of exercised animals. Training did not alter (p > 0.05) MHC phenotype, oxidative capacity, or antioxidant enzyme activity in the omohyoid or genioglossus muscle. These data indicate that endurance exercise training is associated with a fast-to-slow shift in MHC phenotype together with an increase in both oxidative and antioxidant capacity in selected upper airway muscles. It seems possible that this exercise-mediated adaptation is related to the recruitment of these muscles as stabilizers of the upper airway.

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We tested the hypothesis that chronic endurance exercise is associated with the recruitment of four major upper airway muscles (genioglossus, digastric, sternohyoid, and omohyoid) and results in an increased oxidative capacity and a fast-toward-slow shift in myosin heavy chain (MHC) isoforms of these muscles. Female Sprague–Dawley rats ($n = 8$; 60 days old) performed treadmill exercises for 12 weeks (4 days/week; 90 minutes/day). Age-matched sedentary female rats ($n = 10$) served as control animals. Training was associated with an increase ($p < 0.05$) in the activities of both citrate synthase and superoxide dismutase in the digastric and sternohyoid muscles, as well as in the costal diaphragm. Compared with the control animals, Type I MHC content increased ($p < 0.05$) and Type IIb MHC content decreased ($p < 0.05$) in the digastric, sternohyoid, and diaphragm muscles of exercised animals. Training did not alter ($p > 0.05$) MHC phenotype, oxidative capacity, or antioxidant enzyme activity in the omohyoid or genioglossus muscle. These data indicate that endurance exercise training is associated with a fast-to-slow shift in MHC phenotype together with an increase in both oxidative and antioxidant capacity in selected upper airway muscles. It seems possible that this exercise-mediated adaptation is related to the recruitment of these muscles as stabilizers of the upper airway.

Keywords: respiratory muscles; oxidative; antioxidant; myosin; upper airway muscles

Maintenance of pharyngeal patency during breathing requires the coordinated activity of upper airway and thoracic respiratory muscles (1, 2). During inspiration, subatmospheric pressures are produced in the upper airway as a result of inspiratory muscle contraction. The tendency for the pharyngeal lumen to collapse is opposed by the activation and contraction of the upper airway muscles including dilators, such as the sternohyoid (SH) and the omohyoid (OH), and pharyngeal lumen regulators, such as the genioglossus (GG) and digastric (DG) muscles (2).

Ventilatory muscles, particularly the diaphragm, work at higher work rates during exercise training (3). In response to this overload stimulus, metabolic adaptations occur in the diaphragm that improve fatigue resistance (4). These adaptations include improvements in muscle oxidative and antioxidant capacity (5, 6) and a shift in the myosin heavy chain (MHC) phenotype to that of a greater expression of slow isoforms (Type IIb \rightarrow Type II/d/x \rightarrow Type IIa \rightarrow Type I) (4). Currently, it is

unknown as to whether chronic endurance exercise can promote cellular adaptations in respiratory upper airway muscles similar to those observed in the diaphragm; this forms the rationale for the current experiments.

On the basis of the knowledge that exercise increases ventilatory requirements, it seems plausible that endurance exercise will result in the recruitment of respiratory upper airway muscles such as the SH, OH, GG, and DG to increase upper airway diameter and reduce airway resistance. Hence, we hypothesize that exercise training will elevate the recruitment of these muscles and therefore will improve the oxidative capacity of these muscles and promote a shift in fast-toward-slow MHC isoforms similar to the exercise-induced adaptations that occur in the diaphragm.

Further, we postulate that endurance training will promote an increase in the antioxidant capacity of upper airway muscles that will result in lower resting levels of lipid peroxidation in the trained muscles. The rationale for this hypothesis is as follows. As aerobic metabolism is increased in contracting locomotor or respiratory muscles during exercise, the production of radicals and other reactive oxygen species (ROS) is also increased (7). Further, high rates of ROS production during intense muscular activity can promote oxidative damage in muscles (e.g., lipid peroxidation) (8, 9). In an effort to reduce this type of oxidative injury, cells increase their antioxidant defenses in response to repeated exposure to oxidative stress (8). Indeed, regular endurance exercise training has been shown to elevate the antioxidant capacity of the diaphragm and locomotor muscles (10). It follows that chronic endurance exercise could enhance antioxidant enzyme activities in contracting respiratory upper airway muscles as an adaptive response to the oxidative stress associated with exercise.

Therefore, to test the aforementioned hypotheses, these experiments were designed to determine whether (1) endurance training-induced increases in the oxidative capacity and MHC phenotype of the respiratory upper airway musculature (i.e., SH, OH, DG, and GG muscles) are similar to those of the diaphragm; and (2) endurance exercise training promotes an increase in the activity of primary antioxidant enzymes and reduces resting levels of lipid peroxidation in respiratory upper airway muscles.

METHODS

These experiments followed the guidelines established by the American Physiological Society and were approved by the Animal Care and Use Committee of both the University of Florida and the University at Buffalo. Sprague–Dawley rats were randomly divided into three experimental groups: (1) control, sedentary ($n = 10$); (2) endurance exercise-trained ($n = 10$); and (3) acute exercise with measurement of electromyographic (EMG) activity of upper airway muscles ($n = 12$).

The animals assigned to the exercise-training group were exercised during a 12-week, progressive treadmill protocol. Animals exercised 4 days/week (90 minutes/day) at approximately 75% maximal oxygen consumption. The protocol began with animals running at 30 m/minute at

0% grade. Increasing the treadmill grade by 2.5% every 2 weeks increased the exercise work rate over time.

Biochemical Measurements

Twenty-four hours after the last training session, animals were anesthetized by intraperitoneal injection of sodium pentobarbital (65 mg/kg). After reaching a surgical plane of anesthesia, the diaphragm and selected airway muscles were rapidly excised, frozen in liquid nitrogen, and stored at -80°C until analysis. SH, OH, DG, and GG muscle samples were chosen for investigation on the basis of their role in regulating the airway aperture diameter in the rat. As a comparison, anterior sections of the costal diaphragm samples were selected for analysis.

Samples were homogenized and centrifuged, and supernatants were assayed for the oxidative enzyme citrate synthase (CS, E.C. 4.1.3.7) and antioxidant enzymes glutathione peroxidase (GPX, E.C. 1.11.1.9) and superoxide dismutase (SOD, E.C. 1.15.1.1). The activities of the manganese dependent isoform of SOD (Mn-SOD) and the copper-zinc dependent cytosolic isoform (CuZn-SOD) were also determined. CS, SOD, and GPX were measured using the methods described by Srere (11), Flohe and Gunzler (12), and Oyanagui (13).

To determine if a training-induced upregulation of the antioxidant enzyme activities in upper airway muscles was associated with protection against ROS-mediated lipid damage, lipid hydroperoxide contents of all muscles were measured as described by Hermes-Lima and coworkers (14).

Protein concentrations of all homogenates were determined using the Bradford technique (15). All enzyme activities and lipid peroxide concentrations were normalized to the protein concentration of the homogenate.

Electrophoretic separation to determine MHC composition was achieved using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis procedure described by Talmadge and Roy (16). The relative concentrations of MHC isoforms were determined by scanning the gels using a computerized image analysis system.

Measurement of Upper Airway EMG Activity during Exercise

We performed EMG measurements to provide insight into the pattern of upper airway muscle recruitment during running. An overview of these measurements follows. Animals were anesthetized using an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (10 mg/kg); additional doses were used as necessary to maintain a surgical plane of anesthesia. Under aseptic conditions, three fine-wire multistranded Teflon-coated stainless steel wire electrodes, bared at the tip (0.5 mm), were chronically implanted in the upper airway muscles (i.e., SH, OH, DG, and GG) and the parasternal intercostal (PS) muscle in the 12 rats. Due to the high risk of pneumothorax with EMG electrode placement in the diaphragm, the EMG activity of the PS muscle was taken as an index of phasic ventilatory muscle recruitment because its recruitment mirrors that of the diaphragm (unpublished observations). Due to limitations in the recording system, only three muscles were studied per animal. That is, electrodes were placed in the PS of all animals and in two of the upper airway muscles studied. The ends of the electrodes were tunneled under the skin to the back of the neck, where a patch of skin over the dorsum of the rat's skull was removed. The bony calvarium was scraped clean of soft tissue and dried; the electrical socket was then fixed to the skull with dental cement. All incisions were closed with 4-0 silk sutures. Analgesics included 0.05 mg/kg Buprenex administered intramuscularly immediately post-surgery and 0.025 mg/kg Buprenex administered intramuscularly the following day. Antibiotic treatment included 10 mg/kg Enrofloxacin administered intramuscularly on the day of surgery and topical treatment of all surgical sites with antibiotic cream.

The EMG activity pattern of upper airway muscles was recorded 15 to 27 days postsurgery. Before testing, all animals were habituated to the treadmill on three to four occasions. On the day of recording, the animal was connected to three channels of an eight-channel polygraph (MT-950 chart recorder/Grass P511 preamplifiers; Astro-Med, West Warwick, RI). Raw EMG signals were band-pass filtered between 10 and 30 Hz. The animal was then placed on the treadmill and allowed to move freely within the treadmill chamber for 20–30 minutes. The gains on the amplifiers, as well as the electrode pairings used for recording, were set during this initial quiet period and, once set,

remained unchanged for the duration of the study. Muscle recruitment during nonexercise was then recorded for 10 minutes. The treadmill was then turned on, and the speed was progressively increased to 19–22 m/minute over a 2-minute period. Muscle recruitment was recorded continuously during the run test for 10 minutes. At the end of 10 minutes, the treadmill was abruptly turned off, whereas EMG recording continued for 2–5 minutes postexercise. This transition period between hyperpnea during locomotion to hyperpnea without locomotion (immediate postexercise events) was used to distinguish respiratory from locomotory activation.

Statistical Analysis

Comparisons between the control and trained groups for each biochemical-dependent variable were made by a *t* test. Significance was established at $p < 0.05$.

RESULTS

Animal Body Weights

Eight animals successfully completed the 12-week training program. Both sedentary and trained groups showed a similar increase in body weight during the 12-week experimental period, indicating that exercise training did not affect weight gain throughout the 12-week study. The initial and final body weights of the animals used in the training study were (mean \pm SEM): (1) control animals— 257 ± 5 g (initial), 296 ± 5 g (final); and (2) trained animals— 259 ± 6 g (initial), 283 ± 7 g (final).

Antioxidant and Oxidative Enzyme Activities

The results for the oxidative and antioxidant enzymes are contained in Table 1. Compared with control animals, CS activity in the trained animals was significantly higher ($p < 0.05$) in the diaphragm (DIA), SH, and DG muscles. Further, training was associated with an increase ($p < 0.05$) in GPX activity in the DG muscle. Similarly, Mn-SOD activities in the DG, SH, and DIA of trained animals were significantly ($p < 0.05$) greater than in control animals. In addition, training elevated ($p < 0.05$) CuZn-SOD activity in the DG muscle.

Lipid peroxidation values for all airway muscles are given in Table 2. The levels of lipid hydroperoxides were higher ($p < 0.05$) in the SH, DG, and DIA of the control group compared with their trained counterparts.

MHC Analysis

MHC profiles for the costal diaphragm and the upper airway muscles are illustrated in Figure 1. Note that training was associated with a reduction ($p < 0.05$) in Type IIB MHC content in the SH and DIA muscles with a corresponding increase in the content of Types IIa and I MHC. Similarly, the content of Type IIB MHC was reduced in the DG of trained animals with a corresponding increase in Type IId/x and I MHC isoforms. No significant differences existed between experimental groups ($p > 0.05$) in the MHC content in either the GG or the OH muscle.

EMG Activity during Exercise

EMG studies were conducted on 12 male Sprague-Dawley rats weighing 362 ± 5.3 g. Our EMG measurements were not designed to provide quantitative data on upper airway muscle recruitment during exercise but rather were designed to provide qualitative insight into the pattern of upper airway muscle recruitment during treadmill running. Figure 2 illustrates the EMG activity at rest and during exercise in two experimental animals. During the pre-exercise period, the PS was phasically active in 10 of 12 rats, whereas it was tonically active in the remaining two animals. Treadmill running elicited increased phasic recruitment of the PS muscle in all 12 rats

TABLE 1. ANTIOXIDANT AND OXIDATIVE ENZYME ACTIVITIES IN THE UPPER AIRWAY MUSCLES OF CONTROL AND ENDURANCE TRAINED SPRAGUE-DAWLEY RATS

	Control (n = 10)	Trained (n = 8)	p-Value
Sternohyoid			
GPX*	20.1 ± 0.9	20.0 ± 1.0	NS
Total SOD [†]	2.7 ± 0.2	3.3 ± 0.2	<0.05*
Mn-SOD [†]	0.7 ± 0.1	1.1 ± 0.1	<0.05*
CuZn-SOD [†]	2.0 ± 0.1	2.3 ± 0.2	NS
CS*	30.6 ± 1.2	35.9 ± 1.3	<0.05*
Omohyoid			
GPX*	16 ± 1.1	14.6 ± 1.9	NS
Total SOD [†]	1.9 ± 0.2	2.3 ± 0.2	NS
Mn-SOD [†]	0.5 ± 0.1	0.6 ± 0.1	NS
CuZn-SOD [†]	1.4 ± 0.2	1.7 ± 0.2	NS
CS*	28.4 ± 2.1	30.8 ± 2.3	NS
Digastric			
GPX*	22.5 ± 2.8	29.7 ± 1.9	<0.05*
Total SOD [†]	2.9 ± 0.1	3.7 ± 0.3	<0.05*
Mn-SOD [†]	0.8 ± 0.1	1.1 ± 0.1	<0.05*
CuZn-SOD [†]	2.1 ± 0.1	2.7 ± 0.2	<0.05*
CS*	41.7 ± 1.1	46.1 ± 2.6	<0.05*
Genioglossus			
GPX*	32.9 ± 1.8	33.5 ± 1.6	NS
Total SOD [†]	3.1 ± 0.2	3.9 ± 0.1	<0.05*
Mn-SOD [†]	1.1 ± 0.1	1.0 ± 0.1	NS
CuZn-SOD [†]	2.1 ± 0.2	2.8 ± 0.4	NS
CS*	52.9 ± 3.7	57.5 ± 3.1	NS
Diaphragm			
GPX*	17.1 ± 1.8	24.4 ± 2.1	<0.05*
Total SOD [†]	3.1 ± 0.3	3.7 ± 0.2	<0.05*
Mn-SOD [†]	1.0 ± 0.1	1.4 ± 0.1	<0.05*
CuZn-SOD [†]	2.1 ± 0.1	2.3 ± 0.2	NS
CS*	49.3 ± 1.4	55.2 ± 1.9	<0.05*

Definition of abbreviations: CS = citrate synthase; GPX = glutathione peroxidase; NS = not significant; SOD = superoxide dismutase.

* μ, Mol substrate converted/minute/mg protein, mean ± SEM.

† U/mg protein, mean ± SEM.

studied (Table 3) and was also often associated with some tonic activation. Cessation of locomotion was associated with an abrupt reduction in PS activation in all rats studied.

During the pre-exercise period, the OH and SH muscles were usually silent and exhibited no phasic respiratory recruitment, although large activation of both infrahyoid muscles studied was noted during postural, grooming, or exploratory related movements before exercise. Neither treadmill running, nor stoppage of the treadmill produced any consistent activation of the OH compared to that noted during the non-exercise period. In contrast, treadmill running was associated with increased activation (tonic or phasic expiratory) burst of the SH in five of the six animals studied. In addition, the recruitment of the SH noted during exercise was markedly re-

TABLE 2. LIPID HYDROPEROXIDE CONTENT OF UPPER AIRWAY MUSCULATURE IN CONTROL (n = 10) AND EXERCISE (n = 8) SPRAGUE-DAWLEY RATS

Muscle	Experimental Group*		p Value
	Control Rats	Trained Rats	
Sternohyoid	6.5 ± 1.0	4.6 ± 0.1	<0.05*
Omohyoid	5.3 ± 0.7	4.8 ± 0.5	NS
Digastric	10.4 ± 3.1	7.2 ± 0.5	<0.05*
Genioglossus	14.7 ± 1.7	10.2 ± 2.9	NS
Diaphragm	8.5 ± 1.2	6.9 ± 1.0	<0.05*

Definition of abbreviations: NS = not significant.

* Units are expressed as cumene hydroperoxide equivalents (mmol/μg protein).

duced with stoppage of the treadmill, suggesting that part of the increased recruitment noted in the SH during exercise was due to locomotion.

The DG muscle exhibited small tonic activity with occasional bursts that were unrelated to phasic breathing events before exercise. Treadmill running revealed no phasic activation of the DG, but tonic activation was detected in four of six animals studied during exercise. Stoppage of treadmill running was associated with a consistent decrease in DG activation. In comparison, the GG was phasically active, albeit at very low levels, during inspiration during the pre-exercise period in five of the six rats studied. At rest, however, larger EMG bursts were often noted that were related to postural, grooming, or exploratory behavior. Treadmill running produced little change in the phasic recruitment of the GG, whereas the effect on the burst activation was inconsistent across animals studied and appeared little affected by stoppage of the treadmill.

All four upper respiratory muscles studied appeared to be activated in response to postural, grooming, feeding, or exploratory behavior to a greater extent than that noted during exercise. Our results are summarized for all four muscles studied in Table 3. Note, for example, that drinking elicited recruitment of the DG and GG muscles that exceeded any recruitment noted during exercise (Figure 2).

DISCUSSION

Overview of Principal Findings

Our data indicate that chronic endurance exercise training is associated with metabolic adaptations in two major upper airway muscles (i.e., DG and SH). Major exercise-induced changes in the DG and SH muscles include (1) an increased oxidative capacity; (2) elevated antioxidant enzyme activity; (3) reduced lipid peroxidation; and (4) a relative increase in Type I MHC content and a relative decrease in Type IIb content. Similar training-induced changes were also observed in the costal diaphragm. In contrast, endurance exercise training did not significantly affect these parameters in the GG or the OH.

Exercise and Oxidative Capacity of Airway Muscles

In these experiments, we measured the activity of CS, a Krebs cycle enzyme, as a marker of oxidative capacity. In control animals, the CS activity of the GG muscle was significantly greater than those of the SH, OH, and DG muscles. This implies that the activation pattern of the GG muscle during daily activities is greater than that of the SH, OH, and DG. Note that in untrained (control) animals, CS activity of the GG muscle was similar to that of the costal diaphragm. This high oxidative capacity in the GG may be related to the fact that the GG muscle is not only recruited to maintain airway patency but is also recruited during nonventilatory behavior such as eating, drinking, grooming, and thermoregulation. Therefore, based on these collective functions and the high basal oxidative capacity, it is likely that the GG muscle plays an important physiologic role in the rat.

Our hypothesis that the oxidative capacity of these four upper airway muscles would adapt similarly to that of the DIA in response to endurance training was only partially supported by our data. Indeed, compared with control animals, endurance exercise did not alter CS activity in the OH and GG muscles. In contrast, CS activity was elevated in the SH and DG muscles of exercise-trained animals. This observation indicates that regular treadmill exercise is associated with increased recruitment of these muscles either during exercise or perhaps during recovery from exercise. The EMG data col-

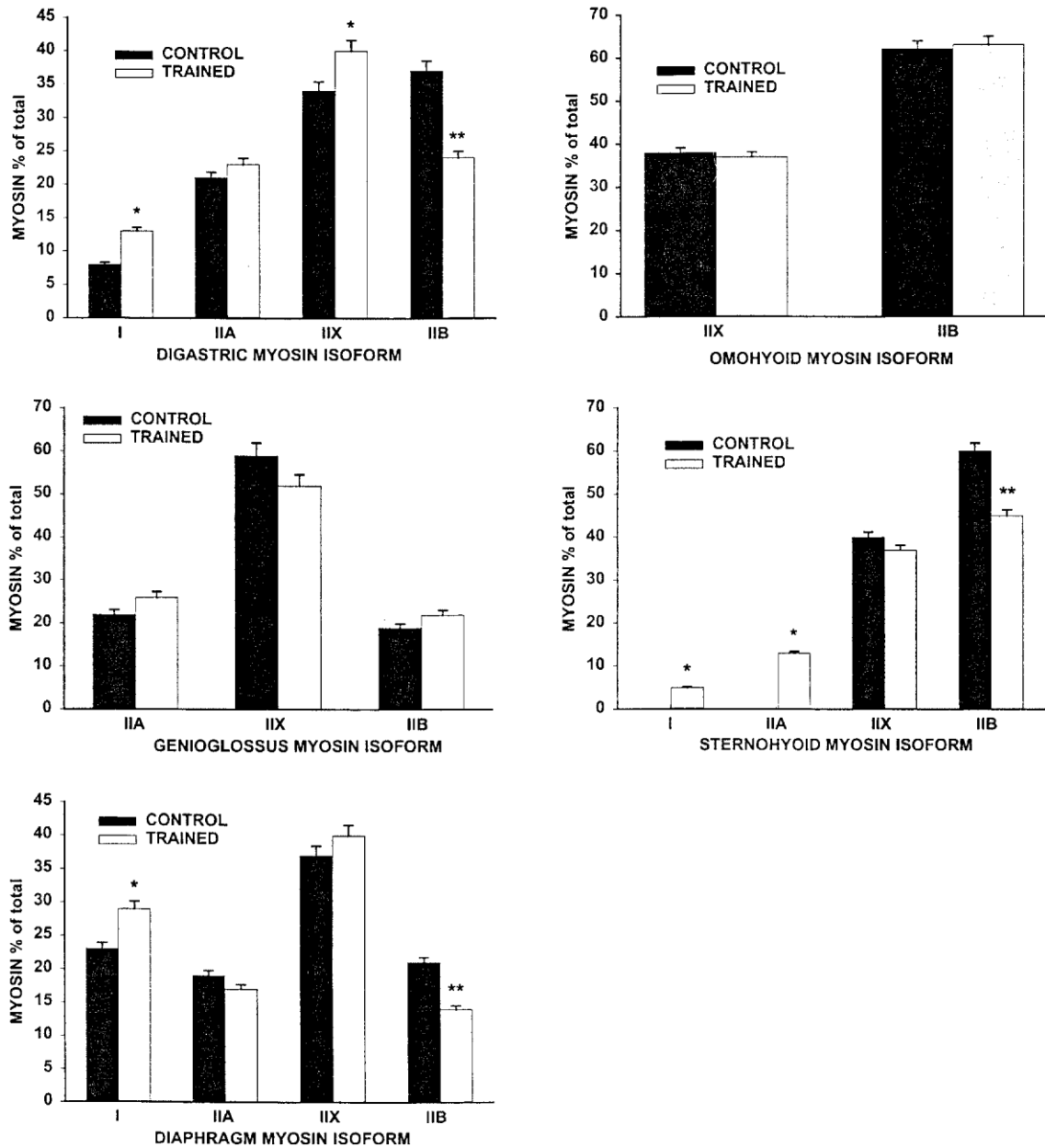


Figure 1. MHC profiles of the DG, OH, GG, SH, and costal diaphragm muscles of sedentary and endurance-trained Sprague-Dawley rats. Values are means \pm SE. *Significantly different between groups at $p < 0.05$.

lected in these experiments provide qualitative insight as to the nature of the recruitment of these muscles during exercise but does not provide information about the activation pattern of these muscles during nonexercise periods. Our EMG recordings did not detect phasic recruitment of the four upper airway muscles during any phase of the respiratory cycle (Table 3; Figure 2). However, a consistent increase in tonic activity was detected during exercise in both the SH and DG muscles. This type of tonic activity was continuous throughout the exercise bout and suggests that these muscles were used to stabilize the upper airway or neck position during exercise. Hence, it is tempting to speculate that the training stimulus for these upper airway muscles originated from functions that were not entrained with ventilation, but may play a significant respiratory role. However, we cannot rule out the possibility that these upper airway muscles were recruited to perform

nonrespiratory tasks during recovery from exercise. For example, possible nonventilatory activities that could be associated with increased contractile activity in upper airway muscles include postural changes, increased eating/drinking behavior, thermoregulation, and even contact with the electrical grid (17) (Table 3). Indeed, the largest activation of these muscles was consistently observed during grooming/thermoregulatory behavior before, or soon after, exercise. Which of these nonventilatory behaviors, if any, contributed to the training adaptation in these airway muscles is difficult to predict from the current data alone.

Regardless of the stimulus responsible for the increase in CS activity in the SH and DG muscles, the current data unequivocally demonstrate that exercise training is associated with a training effect in some of the respiratory muscles of the upper airway. Upregulation of CS activity is indicative of a

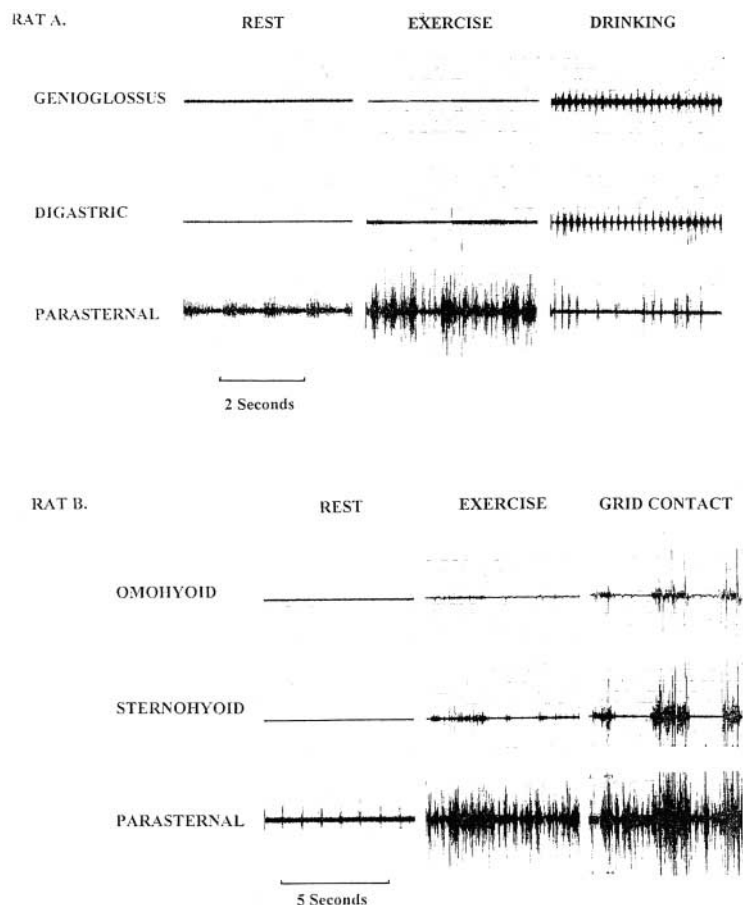


Figure 2. EMG tracings from two animals during both rest and exercise. EMG activity was recorded for the GG, DG, and PS muscles in Rat A, whereas activity from the OH, SH, and PS muscles were recorded in Rat B. Note that exercise did not activate the GG or DG muscles. In contrast, exercise resulted in a tonic increase in activity in both the OH and SH muscles. Please note the different time scales in the *upper* and *lower* portions of this figure.

greater oxidative capacity and is correlated to increased fatigue resistance (18). Given that the DG and the SH muscles move the hyoid bone caudally to widen the aperture of the larynx (19), improving the endurance of these muscles should be physiologically advantageous in maintaining airway patency.

Note that our failure to observe a consistent and marked increase in EMG activity within the GG muscle of exercising rats differs from recent data on humans. Although some controversy exists, recent work by Williams and colleagues (20) indicate that neural drive to the GG muscle is increased during exercise in humans. The explanation for the apparent spe-

cies difference in the recruitment of this upper airway is unclear and warrants further study.

Antioxidant Enzyme Activity and Lipid Peroxidation

Recent evidence suggests that endurance exercise can improve the enzymatic antioxidant defense system within locomotor skeletal muscles (10). Our laboratory and others have shown that chronic endurance exercise elevates GPX and Mn-SOD activities in the DIA (21, 22). Because Mn-SOD is located in the mitochondria, it is likely that the stimulus for this upregulation is due to the leakage of electrons from the mito-

TABLE 3. QUALITATIVE ELECTROMYOGRAPHIC RESPONSE IN UPPER AIRWAY MUSCLES AND THE PARASTERNAL INTERCOSTAL MUSCLES DURING TREADMILL EXERCISE COMPARED WITH REST

Muscle	Training-induced Changes in Muscle Oxidative Capacity	EMG Activity (Exercise versus Rest)	Activities That Elicited Muscle EMG Activation
Parasternal intercostals	No observations	Phasic increase*	-
Sternohyoid	Oxidative capacity increased	Tonic increase	Postural, grooming, exploratory behavior, electric shock
Digastric	Oxidative capacity increased	Tonic increase	Drinking, grooming, exploratory behavior
Omohyoid	No change	Limited change	Postural, grooming, exploratory behavior, electric shock
Genioglossus	No change	Limited change	Drinking, grooming, exploratory behavior

Definition of abbreviation: EMG = electromyographic.

*Phasic increase in EMG activity associated with inspiration.

chondrial membrane-bound electron transport chain (9). Specifically, one electron reduction of oxygen generates the superoxide radical (O_2^-); O_2^- is then dismutated to H_2O_2 by SOD. This production of H_2O_2 serves as a stimulus to increase the expression of GPX. Airway muscles that incurred a training-induced increase in oxidative capacity (i.e., SH and DG) also experienced increased Mn-SOD activity following training. However, training-induced increases in GPX activity occurred only in the DG. The explanation as to why training did not elevate GPX activity in the SH muscle is unclear.

To our knowledge, this is the first study to investigate whether training-induced increases in antioxidant enzyme activities of the upper airway musculature alter the basal levels of lipid peroxidation in resting animals. To evaluate the effects of exercise training on airway muscle lipid peroxidation, we compared the lipid hydroperoxide content of upper airway muscles in both control and trained animals. Our results revealed that the exercise-induced increase in antioxidant enzyme activity (i.e., SOD) was associated with a reduction in lipid peroxidation in the DG and the SH. Although GPX activity was not significantly increased in the DIA and the SH muscles of the trained group, the protection afforded by SOD appears adequate to protect against exercise-induced lipid peroxidation. Alternatively, it is possible that lipid peroxidation was blunted by changes in antioxidant elements that were not measured in the current protocol.

Myosin Phenotype

This is the first investigation to explore the effects of chronic endurance exercise on the myosin phenotype of upper airway muscles. We hypothesized that endurance exercise would result in an increase in contractile activity in upper airway muscles and thus promote a shift in fast-toward-slow MHC isoforms similar to that observed in exercise-induced adaptations in the diaphragm. This postulate was only partially supported as a fast-to-slow shift MHC isoforms only occurred in the DG and SH muscles. Specifically, the MHC profiles of the DG and the SH muscles from exercise-trained animals were characterized by significant reductions in the relative content of Type IIb MHC and increases in the slower Type II MHC isoforms, Type II_{d/x} and II_a. This finding is significant in that muscles containing a high proportion of Type IIb myosin, such as the SH, are vulnerable to fatigue (2). The increased content of slower MHC in the SH and DG muscles in response to training suggests that fatigue resistance and muscular efficiency increased in these upper airway muscles. Improved resistance to fatigue is a desirable adaptation for maintenance of airway patency under various ventilatory overload conditions.

Our finding that training results in a fast-to-slow shift in the MHC profile of the costal diaphragm agrees with previous training studies indicating that regular endurance exercise can promote fast-to-slow shifts in diaphragmatic myosin phenotype. Sugiura and coworkers (23, 24) reported that chronic training reduced the expression of Type IIb fast myosin and increased the expression of Types II_a and II_{d/x}. Recent work in our laboratory has also shown an exercise-induced shift toward increased expression of Type I myosin in the costal diaphragm (4).

Conclusion

These experiments tested two hypotheses. First, we postulated that chronic endurance training would improve the oxidative capacity of these muscles and promote a shift in fast-toward-slow MHC isoforms in upper airway muscles similar to that observed in exercise-induced adaptations in the diaphragm. Secondly, we hypothesized that endurance training would ele-

vate the antioxidant capacity of upper airway muscles and promote a reduction in basal levels of lipid peroxidation. These hypotheses were partially supported as endurance exercise training was associated with metabolic adaptations in two upper airway muscles (i.e., SH and DG); these adaptations were similar to those observed in the DIA. Specifically, endurance training resulted in a significant increase in the oxidative and antioxidant capacities of the SH and DG muscles and resulted in lower levels of lipid peroxidation. Further, exercise training also resulted in a fast-to-slow shift in the MHC isoform profile in these muscles. Collectively, these changes should result in improved protection from oxidative injury and fatigue resistance in these muscles.

Although it is clear that endurance exercise training resulted in metabolic adaptations of the SH and DG muscles, the stimulus responsible for this adaptation remains uncertain. EMG recordings from these muscles during exercise indicated an increase in tonic activity but revealed no evidence of phasic recruitment during the respiratory cycle. We also observed that the upper airway muscles are activated during nonventilatory behavior such as postural movements, exploratory behavior, grooming, thermoregulatory behavior, and eating/drinking. Therefore, it is possible that the metabolic adaptations in these respiratory upper airway muscles can be attributed to an exercise-induced increase in both respiratory and nonrespiratory tasks.

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