Effects of emphysema and training on glutathione oxidation in the hamster diaphragm

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1Department of Pulmonary Diseases, University Hospital Nijmegen, 6500 HB Nijmegen; and 2Departments of Pharmacology and Toxicology, University of Maastricht, 6200 MD Maastricht, The Netherlands

Heunks, Leo M. A., Aalt Bast, Cees L. A. van Herwaarden, Guido R. M. Haenen, and P. N. Richard Dekhuijzen. Effects of emphysema and training on glutathione oxidation in the hamster diaphragm. J Appl Physiol 88: 2054–2061, 2000.—Loading of skeletal muscles is associated with increased generation of oxidants, which in turn may impair muscle contractility. We investigated whether the load on the hamster diaphragm imposed by pulmonary emphysema induces oxidative stress, as indicated by glutathione oxidation, and whether the degree of glutathione oxidation is correlated with contractility of the diaphragm. In addition, the effect of 12 wk of treadmill exercise training on contractility and glutathione content in the normal (NH) and emphysematous hamster (EH) diaphragm was investigated. Training started 6 mo after elastase instillation. After the training period, glutathione content and in vitro contractility of the diaphragm were determined. Twitch force and maximal tetanic force were significantly reduced (by ~30 and ~15%, respectively) in EH compared with NH. In sedentary hamsters, the GSSG-to-GSH ratio was significantly elevated in the EH compared with the NH diaphragm. A significant inverse correlation was found between GSSG-to-GSH ratio and twitch force in the diaphragm (P < 0.01). Training improved maximal tetanic force and reduced fatigability of the EH diaphragm but did not alter its glutathione content. In conclusion, 1) emphysema induces oxidative stress in the diaphragm, 2) training improves the contractile properties of the EH diaphragm, and 3) this improvement is not accompanied by changes in glutathione redox status.

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SKELETAL MUSCLES GENERATE reactive oxygen species (ROS) at rest, and generation is increased during contractile activity (17, 27). ROS are required for optimal contractile function (27). However, overproduction of ROS, so-called oxidative stress, is associated with impaired force generation (6, 28). Overproduction of ROS by skeletal muscle during fatiguing contractions may lead to a nonpathological form of oxidative stress (1). Indeed, in the rat diaphragm, an inverse correlation exists between superoxide anion release during fatiguing contractions and the final stress developed (28).

Antioxidant defenses protect skeletal muscles from deleterious effects of ROS. GSH is probably the most important antioxidant in skeletal muscle. Intracellular glutathione metabolism is regulated by complex pathways (21). GSH serves as an antioxidant by reacting directly with free radicals and by providing substrate for glutathione peroxidase (GPX). Both direct and enzymatic oxidation of GSH results in the formation of GSSG, which is reconverted to GSH by glutathione reductase. Thus tissue glutathione status depends on the direct interaction of GSH with oxidants, the activity of the GPX and glutathione reductase redox cycle, and the ability of tissues to synthesize GSH. Elevated glutathione oxidation is considered a marker for oxidative stress (5). For instance, it has been shown that acute loading of the respiratory muscles by inspiratory resistive breathing increases glutathione oxidation in the rat diaphragm (34).

In contrast to acute loading, little is known about the effects of chronic loading on ROS generation in the diaphragm. However, this may be of major relevance to patients with chronic obstructive pulmonary disease (COPD). Respiratory muscle dysfunction frequently occurs in patients with COPD (25). Pulmonary hyperinflation, which is one of the key features of COPD (10), puts the diaphragm on a disadvantageous position of the length-tension curve and thereby chronically increases the load on the diaphragm.

The first hypothesis of the present study was that impaired contractility of the diaphragm due to pulmonary emphysema is accompanied by elevated glutathione oxidation in the diaphragm. We tested this hypothesis in the emphysematous hamster (EH), which is a frequently used animal model to study the effects of pulmonary hyperinflation on diaphragm function and morphology (12, 20, 36).

During general exercise training sessions, the diaphragm faces an elevated load because of increased ventilation. Previous research in healthy rodents revealed that training-induced elevation in oxidative capacity of the diaphragm is accompanied by increased antioxidant enzyme activity (23, 26). This indicates that the antioxidant screen of the diaphragm adapts to the elevated oxygen consumption rate. However, it is unknown whether training affects the glutathione sta-
tus of the diaphragm. Only one study has been published on the effects of training on contractility of the EH diaphragm (11). In that study, general exercise training did not affect in vitro contractility of the diaphragm. In contrast to our EH model, in the study by Farkas and Roussos (11), pulmonary emphysema did not impair in vitro contractility of the diaphragm. This difference in baseline contractility of the diaphragm may affect the course of training responses. In a separate study, the same authors reported that training did not increase oxidant capacity of the EH diaphragm as expressed by citrate synthase (CS) activity (12). To our knowledge, the effects of training on antioxidant status in the EH diaphragm have not yet been studied.

The second hypothesis of the present study was that training improves contractility of the EH diaphragm, which is accompanied by a reduction in glutathione oxidation at rest. To answer this question, we trained EH on a motor-driven treadmill for 12 wk. Subsequently, the degree of glutathione oxidation and in vitro contractility of the diaphragm were assessed.

METHODS

Animals, Induction of Emphysema, and Study Design

All experiments were conducted on male Syrian hamsters. Animals were housed in a specific pathogen-free area, with controlled day and night cycle and fed ad libitum. Hamsters were studied for the following reasons. First, antioxidant enzyme activities in humans are more similar to those in hamsters compared with rats (9). Second, the EH is a frequently used animal model to study the effects of pulmonary emphysema on diaphragm contractility and morphology. The hamster model has several advantages. The pathological lesions in the lung induced by elastase instillation have been studied in detail (31) and are quite similar to those in patients with emphysema. Furthermore, lung function changes in the EH resemble changes in patients with emphysema: increased pulmonary compliance, functional residual capacity, residual volume, and total lung capacity. In the present study, hamsters at 40 wk of age were intratracheally instilled with either porcine pancreas elastase [24 U/100 g body wt (EPC, Owensville, MI) in 0.50 ml 0.9% NaCl/100 g body wt] or an equal volume of 0.9% saline, as was described in detail previously (36). Six months after instillation, 15 EH and 15 normal hamsters (NH) were trained (EH-Tr and NH-Tr, respectively) on a motor-driven treadmill. Sedentary NH and EH were used as controls (n = 17 each group, NH-Sed and EH-Sed, respectively). These studies were approved by the Animal Ethics Committee, University of Nijmegen.

Training

The day-and-night cycle was reversed to train the hamsters during their naturally most active period of the day. Training started 6 mo after instillation. In the week preceding the start of training, NH-Tr and EH-Tr were acclimatized to the treadmill by walking at a low speed for 15 min/day (5° inclination). An electrical grid in the rear of each running compartment was used to encourage running. The animals were exercised 5 days/wk for a 12-wk period. In the first week of the training program, running speed was 11.4 m/min for 40 min/day. In the following 5 wk, running speed and duration were gradually increased up to 20 m/min for 60 min/day. An attempt to increase running speed further was abandoned because EH were not able to maintain this speed. Thus, from the sixth until the twelfth week, both NH and EH ran 20 m/min, 60 min/day at 5° inclination for 5 days/wk. Subsequent experiments were performed 24–36 h after the last training session.

General Procedures and Tissue Treatment

The hamsters were anesthetized with pentobarbital sodium (Nembutal, 70 mg/kg ip). A tracheotomy was performed, and a polyethylene cannula was inserted. The animals were mechanically ventilated with 100% O2. The diaphragm and adherent ribs were quickly excised after combined laparotomy and thoracotomy, and they were immediately submerged in cooled oxygenated Krebs solution at pH 7.40. This Krebs solution consisted of 137 mM NaCl, 4 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 1 mM KH2PO4, 24 mM NaHCO3, 7 mM glucose, and 25 μM d-tubocurarine (Sigma Chemical, Bornem, Belgium). The right middle costal hemidiaphragm was used for in vitro contractile measurements. The remainder of the right costal hemidiaphragm was used for CS activity measurement. From the left hemidiaphragm, ribs and connective tissue were removed, and the remainder was quickly frozen in liquid N2 and stored at −80°C for later glutathione measurements. Meanwhile, the posterior-inferior area of the liver and the intact soleus muscles were removed from the animal, quickly frozen in liquid N2, and stored at −80°C for glutathione measurements. There is no good peripheral counterpart in terms of fiber-type composition of the diaphragm. The soleus muscle (80% type I fibers (37)) was chosen arbitrarily as a control muscle for the diaphragm to investigate whether any changes in glutathione concentration of the diaphragm were the result of a systemic response.

Measurement of Contractile Properties

From the middle lateral costal region of the right hemidiaphragm, two rectangular strips were dissected parallel to the long axis of the muscle fibers. Silk sutures were tied firmly to both ends of the strip. The strips were suspended in two tissue baths containing Krebs solution, maintained at 37°C, pH 7.35–7.45, and perfused with a mixture of 95% O2 and 5% CO2. The central tendon end of each strip was connected to an isometric force transducer (model 31/1437–10; Sensotec, Columbus, OH) mounted on a micrometer. Two large platinum stimulation electrodes were placed parallel to the muscle strips. Stimuli were applied with a pulse duration of 0.2 ms and a train duration of 250 ms and were delivered by a stimulator (ID-electronics; University of Nijmegen, Nijmegen, The Netherlands) activated by a personal computer. To ensure supramaximal stimulation, the strips were stimulated at ~20% above the voltage at which maximal forces were obtained (~6 V applied through the stimulating electrodes). Data acquisition and storage of the amplified signal were done with a Dash-16 interface (Twist-trigger software, ID-electronics, University of Nijmegen) on a personal computer. Both strips were placed at their optimal length, defined as the length at which peak twitch tension (Pt) was obtained. The following protocols were performed in either both or one of the strips.

Twitch characteristics. In each strip, two twitches were recorded to determine maximal Pt, contraction time (CT), and half relaxation time.

Maximal tetanic force. Both bundles were stimulated twice at 160 Hz to obtain a plateau in force generation. The maximal force was defined as the maximal tetanic force (Pt).
Force-frequency characteristics. One of the bundles was randomly selected for studying force-frequency characteristics. The bundle was stimulated with 2-min intervals at the following frequencies: 15, 25, 50, 80, and 120 Hz. Both the absolute force and the percentage of Po were calculated.

Fatigability. To avoid impairment in force generation at the start of the fatigue protocol because of the measurement of force-frequency characteristics, in vitro fatigability was studied in the strip not used in force-frequency experiments. The strip was stimulated once every 2 s at 25 Hz, 330-ms train duration, for 120 s. After the completion of these measurements, the length of each strip was measured twice by using a micrometer (model 560–128, Mitutoyo, Veenendaal, The Netherlands), and the strips were weighed. The cross-sectional area (CSA) was calculated by dividing diaphragm strip weight (g) by strip length (cm) times specific density (1.056). Forces were expressed per CSA (N/cm²), and the Pt to Po ratio was calculated for each muscle strip.

Verification of Emphysema

After the diaphragm was removed, the trachea was exposed, and an endotracheal cannula was inserted and held in place with silk sutures. Subsequently, the lungs were inflated with 0.9% saline to a pressure of 25 cmH₂O. Fluid displacement with silk sutures. Subsequently, the lungs were inflated and an endotracheal cannula was inserted and held in place with silk sutures. The bundle was stimulated with 2-min intervals at the randomly selected for studying force-frequency characteristics. The bundle was stimulated with 2-min intervals at the

Relative force and the percentage of Po were calculated. After the diaphragm was removed, the trachea was exposed, and an endotracheal cannula was inserted and held in place with silk sutures. Subsequently, the lungs were inflated with 0.9% saline to a pressure of 25 cmH₂O. Fluid displacement with silk sutures. Subsequently, the lungs were inflated and an endotracheal cannula was inserted and held in place with silk sutures. The bundle was stimulated with 2-min intervals at the randomly selected for studying force-frequency characteristics. The bundle was stimulated with 2-min intervals at the

| Table 1. Effects of elastase instillation and training on body weight and lung volume |
|-----------------------------------|---------------------------------|-----------------|-----------------|
|                                   | Normal Hamster                 | Emphysematous Hamster |                 |
|                                   | Control (n = 17) | Training (n = 15) | Control (n = 17) | Training (n = 14) |
| Body weight, g                   | 147 ± 4 | 166 ± 3 | 131 ± 4* | 138 ± 3*         |
| Body weight, g                   | 146 ± 4 | 153 ± 3 | 129 ± 3* | 131 ± 5*         |
| Lung volume, ml                  | 9.8 ± 0.3 | 10.9 ± 0.4 | 13.2 ± 0.5* | 15.0 ± 0.9†         |

Values are means ± SE; n, no. of hamsters. *Significantly different due to elastase treatment (P < 0.05). †Significantly different due to training (P < 0.05).

RESULTS

Animal Characteristics

One of the EH did not complete the training program because of serious limb injury. The remaining 29 hamsters successfully completed all training sessions. After several days of treadmill exercise, electrical stimulation was needed only sporadically to encourage running. At the end of each training session, EH were severely fatigued as indicated by tachypnea and lethargy. Some EH were evidently cyanotic, and wheezing was audible after the training session. Instillation with elastase had profound effects on body weight and lung volume of hamsters (Table 1). Elastase treatment resulted in severe pulmonary emphysema, as indicated by an ~35% increase in lung volume. Exercise training further increased lung volume in EH.

In Vitro Contractile Properties of the Diaphragm

Pulmonary emphysema significantly reduced Pt, Po, and optimal length of the diaphragm strips compared with NH-Sed (Table 2). Pt and Po were ~30 and ~15% lower in EH-Sed compared with NH-Sed, respectively. In the EH-Sed, normalized force-frequency responses were significantly impaired at frequencies up to 60 Hz compared with that in NH-Sed (Fig. 1). This indicates

Table 2. Effects of elastase instillation and training on diaphragm length and contractile properties

<table>
<thead>
<tr>
<th></th>
<th>Normal Hamster</th>
<th>Emphysematous Hamster</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 17)</td>
<td>Training (n = 15)</td>
</tr>
<tr>
<td>L₀, mm</td>
<td>15.5 ± 0.4</td>
<td>15.5 ± 0.3</td>
</tr>
<tr>
<td>CT, ms</td>
<td>27.8 ± 0.2</td>
<td>28.2 ± 0.3</td>
</tr>
<tr>
<td>RT₁, ms</td>
<td>26.4 ± 0.5</td>
<td>26.1 ± 0.5</td>
</tr>
<tr>
<td>Ft, N/cm²</td>
<td>7.14 ± 0.26</td>
<td>7.30 ± 0.17</td>
</tr>
<tr>
<td>P₀, N/cm²</td>
<td>25.92 ± 1.10</td>
<td>26.33 ± 0.79</td>
</tr>
<tr>
<td>P₁/P₀</td>
<td>0.28 ± 0.01</td>
<td>0.28 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of hamsters. *Significant difference due to elastase instillation (P < 0.05). †Significant difference due to training (P < 0.05).
that, because of elastase treatment, besides a down-
ward shift, a rightward shift of the force-frequency
curve also occurred. Furthermore, fatigability of the
EH-Sed was significantly higher compared with that of
the NH-Sed diaphragm (Fig. 2).

General exercise training did not significantly change
\( P_t \) in NH or EH but significantly increased \( C_T \) in EH.
Maximal force-generating capacity of the EH dia-
phragm increased by \(-15\%\) due to training (\( P < 0.05 \), Table 2). In fact, training abolished the detrimental
effects of emphysema on \( P_o \). In addition, training did
not affect the shape of the normalized force-frequency
curve in NH or EH. Training had a small but significant
beneficial effect on fatigability of the EH diaphragm.

**Tissue Glutathione Measurements**

Emphysema did not affect glutathione levels in the
diaphragm (Table 3). Although GSSG concentration in
the EH-Sed was \(-34\%\) higher compared with that in
NH-Sed, this did not reach statistical significance (\( P =
0.18, \text{ANOVA} \)). However, the GSSG-to-GSH ratio (GSSG/
GSH) was significantly higher in the EH diaphragm
compared with that in the NH diaphragm (\( 9.1 \pm 0.8 \) vs.
\( 5.9 \pm 0.6\%\), respectively, Fig. 3A).

Endurance training did not significantly affect GSH
or GSSG levels in the diaphragm of NH or EH. Also, the
GSSG/GSH in the diaphragm was not affected by
training (\( 7.8 \pm 0.7 \) vs. \( 10.5 \pm 1.1\%\) in NH-Tr and EH-Tr,
respectively).

Neither emphysema nor training had significant
effects on GSH or GSSG levels or GSSG/GSH in soleus
muscle or liver (Table 3, Fig. 3, B and C). T-Glu, GSH,
and GSSG levels were significantly higher in the
EH diaphragm compared with that in soleus muscle for all
groups. Furthermore, liver glutathione content was
significantly higher compared with that in diaphragm
and soleus muscle in all groups.

**Correlation Between Force Generation
and Glutathione Oxidation**

To test the first hypothesis of the present study, we
made further correlations between glutathione oxida-
tion and in vitro force generation of the diaphragm. If
oxidative stress impairs contractility of the diaphragm,
an inverse correlation should be present between mark-
ers for these variables. Indeed, we found a significant
inverse correlation between GSSG/GSH and \( P_t \) (Pear-
son correlation coefficient \( r = -0.48, P < 0.01 \), Fig. 4A).
No correlation exists between GSSG/GSH and \( P_o \) (Pear-
son correlation coefficient \( r = -0.26, P > 0.05 \), Fig. 4B).

**Oxidative Capacity of the Diaphragm**

CS activity was used as a marker of oxidative capac-
ity of the diaphragm. No significant differences in CS
activity were observed between NH-Sed and EH-Sed
(\( 1.24 \pm 0.12 \) vs. \( 0.98 \pm 0.11 \), respectively, \( P > 0.05 \)).
General exercise training did not affect CS activity in
the NH diaphragm (\( 1.24 \pm 0.12 \) vs. \( 1.02 \pm 0.60 \) in
NH-Sed and NH-Tr, respectively, \( P > 0.05 \)). However,
in the EH, training increased oxidative capacity of the
diaphragm (\( 0.98 \pm 0.11 \) vs. \( 1.35 \pm 0.14 \) in EH-Sed and
EH-Tr, respectively, \( P < 0.05 \), Student t-test).

**DISCUSSION**

The results of our experiments show that, in ham-
sters, the load on the respiratory muscles imposed by
pulmonary emphysema impairs in vitro contractility of
the diaphragm, which is accompanied by increased
generation of ROS, as indicated by elevated glutathione
oxidation in the diaphragm. In fact, a significant in-
verse correlation exists between GSSG/GSH and \( P_t \) in the diaphragm. Second, general exercise training improves maximal force-generating capacity of the diaphragm in EH. However, contrary to our expectation, exercise training did not affect glutathione redox status of the diaphragm in EH.

### Effects of Emphysema on Diaphragm Force Generation and Glutathione Content

Reduction in diaphragm muscle strip length and impairment in in vitro contractility after treatment with elastase have been reported previously by others and by studies from our laboratory (19, 20, 36), although some other groups did not find changes in in vitro contractility (11, 33). Possible explanations for the reduction in contractility due to emphysema have been discussed previously (20), such as alterations in the contractile protein composition, changes in noncontractile proteins (sarcoplasmic, stromal, and mitochondrial), and changes in the intrinsic characteristics of muscle fibers or disturbances in calcium uptake and release. Changes in fiber-type frequency or CSA may also contribute. Data on fiber-type composition in the EH diaphragm are conflicting. It has been shown that the relative contribution of type I fibers is increased in the EH diaphragm (12), whereas other studies reported hypertrophy of type II fibers in the EH diaphragm (20). We did not find any effect of emphysema on diaphragm muscle fiber frequency or CSA (36). Thus, in our animal model of emphysema, it is unlikely that changes in diaphragm morphology fully explain impaired in vitro force generation. It is likely that other factors contributed to the negative effects of emphysema on contractility, such as oxidative stress.

GSSG/GSH is frequently used as a marker for oxidative stress. The latter is defined as a disturbance between the prooxidant and antioxidant balance, in favor of the former. GSSG/GSH is a good indicator of oxidative stress (5) and is considered an important regulator of the function of enzymes, receptors, transporters, and transcription factors (14). Accurate measurement of GSH/GSSG is difficult because of GSH autooxidation. Because GSSG is only present in minimal amounts compared with GSH, a small GSH autooxidation during sample processing can give erroneously high GSSG levels. To prevent GSSH autooxidation, GSH trapping agents, such as N-ethylmaleimide and 2-vinyl pyridine, have been used. Although HPLC is assumed to be the state-of-the-art technique for measurement of GSH and GSSG, it has been shown that, at least in solid tissue, an enzymatic assay as used in the present study is a valid alternative to the much more time-consuming HPLC technique (13).

In the present study, GSSG/GSH in the diaphragm was significantly higher in EH-Sed compared with NH-Sed. This indicates that, even at rest, the EH diaphragm is in a more oxidized state compared with the NH diaphragm. In vitro studies using rat diaphragm showed that oxidants accumulate intracellularly and contribute directly to the loss of contractile function, which occurs in muscular fatigue (27). Other studies have confirmed the association between increased generation of ROS and muscular fatigue (17), but the present study is the first to investigate the oxidant status of the EH diaphragm. In other models for loading of the respiratory muscles, such as inspiratory resistive breathing, in vitro force generation is also impaired, and glutathione oxidation increased (4, 34). In these latter studies, GSSG/GSH in the rat diaphragm increased up to \( \sim 28\% \), whereas in the present study this ratio was \( \sim 9\% \) in the EH. These differences in the degree of glutathione oxidation are probably the result of differences in the duration and severity of loading. In inspiratory resistive breathing, a strenuous load is acutely applied, resulting in ventilatory failure and apnea within \( \sim 30\) min, whereas in the EH a less severe load is applied continuously for a prolonged period of time. Indeed, when inspiratory resistive breathing was discontinued before the development of apnea (7) or when the loading was applied by tracheal banding for 4–12 days (32), changes in diaphragm glutathione content were less severe (7). The associa-

### Table 3. Effects of elastase instillation and training on tissue glutathione content

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control (( n = 16 ))</th>
<th>Training (( n = 14 ))</th>
<th>Control (( n = 17 ))</th>
<th>Training (( n = 14 ))</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Diaphragm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-Glu, ( \mu \text{mol/g} )</td>
<td>1.13 ± 0.06</td>
<td>1.21 ± 0.08</td>
<td>1.11 ± 0.07</td>
<td>1.01 ± 0.08</td>
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<tr>
<td>GSH, ( \mu \text{mol/g} )</td>
<td>1.00 ± 0.05</td>
<td>1.05 ± 0.07</td>
<td>0.95 ± 0.07</td>
<td>0.84 ± 0.06</td>
</tr>
<tr>
<td>GSSG, ( \mu \text{mol/g} )</td>
<td>0.062 ± 0.009</td>
<td>0.080 ± 0.008</td>
<td>0.083 ± 0.006</td>
<td>0.091 ± 0.013</td>
</tr>
<tr>
<td>Soleus muscle</td>
<td></td>
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<tr>
<td>T-Glu, ( \mu \text{mol/g} )</td>
<td>0.55 ± 0.06</td>
<td>0.60 ± 0.06</td>
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<tr>
<td>GSH, ( \mu \text{mol/g} )</td>
<td>0.48 ± 0.05</td>
<td>0.51 ± 0.04</td>
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<tr>
<td>GSSG, ( \mu \text{mol/g} )</td>
<td>0.036 ± 0.005</td>
<td>0.043 ± 0.008</td>
<td>0.031 ± 0.005</td>
<td>0.054 ± 0.006</td>
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<tr>
<td>Liver</td>
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</tr>
<tr>
<td>T-Glu, ( \mu \text{mol/g} )</td>
<td>7.44 ± 0.61</td>
<td>7.23 ± 0.69</td>
<td>6.38 ± 0.34</td>
<td>5.88 ± 0.51</td>
</tr>
<tr>
<td>GSH, ( \mu \text{mol/g} )</td>
<td>6.93 ± 0.59</td>
<td>6.67 ± 0.62</td>
<td>5.85 ± 0.32</td>
<td>5.35 ± 0.50</td>
</tr>
<tr>
<td>GSSG, ( \mu \text{mol/g} )</td>
<td>0.26 ± 0.02</td>
<td>0.28 ± 0.04</td>
<td>0.27 ± 0.02</td>
<td>0.26 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), no. of hamsters. T-Glu, total glutathione. ANOVA did not show differences in absolute glutathione content due to elastase instillation or training.
tion between oxidant status and force generation is also demonstrated by the significant correlation between GSSG/GSH and $P_t$ in the diaphragm in the present study (Fig. 4A), although no such correlation existed for GSSG/GSH and $P_o$ (Fig. 4B). It has been recognized previously that submaximal force is more sensitive to oxidative stress than maximal force. For instance, prolonged exposure of mouse skeletal muscle fibers to $H_2O_2$ reduced maximal force generation by 23%, whereas submaximal force was reduced by 79% (3). Reduction in (sub)maximal force generation by oxidants could arise from modification of different steps in the excitation-contraction coupling process, including oxidation of hyperreactive thiols on the myosin head (24), modification of intracellular sulphhydryl groups on Ca$^{2+}$ release channels (8), and reduced Ca$^{2+}$ sensitivity (3). The present study was not designed to identify the target for elevated generation of oxidants. However, because in addition to a downward shift a rightward shift of the force-frequency curve also occurred (Fig. 1), it is likely that oxidants have effects on noncontractile proteins as well.

The liver plays a prominent role in GSH homeostasis, and hepatic export of GSH is the major source for plasma glutathione (22). Thus, theoretically, it is possible that emphysema altered GSH status of the liver. However, this did not occur in the present study, indicating that the liver could fulfill the glutathione demands of the other tissues.

Soleus muscle glutathione content and GSSG/GSH were not affected by emphysema. This indicates that the alterations in GSSG/GSH in the diaphragm were not the result of a systemic inflammatory response following elastase instillation. Although EH had significantly lower body weight compared with NH, it is unlikely that a catabolic response attributed to changes in glutathione status in the diaphragm, because no such changes were observed in the limb skeletal muscles. Thus changes in diaphragm GSSG/GSH in EH are most likely the direct result of the increased loading.

CS activity in the diaphragm tended to be lower in the EH-Sed compared with NH-Sed. This is in apparent contrast to other studies (12, 20) in which CS activity...
was increased. However, our findings are in line with the increased fatigue rate of the EH compared with NH diaphragm found in the present study. The degree of hyperinflation due to elastase treatment was less severe in the present study compared with previously published studies (i.e., Ref. 20). This may have contributed to the lack of response of CS activity in the diaphragm due to hyperinflation.

Effects of Training on Diaphragm Contractility and Glutathione Status

The beneficial effects of exercise training on contractility of the EH diaphragm are in apparent contrast to previous studies by Farkas and Roussos (11). These differences in outcome are probably the result of differences in experimental setup. As mentioned earlier, in the study by Farkas and Roussos, elastase instillation did not affect contractility of the diaphragm at optimal fiber length, indicating the absence of respiratory muscle dysfunction in their hamster model. Possibly the load on the respiratory muscles of the EH was higher in our study. It has been postulated that the threshold for obtaining a training effect is much higher in the diaphragm compared with limb skeletal muscle (16). Because of the absence of respiratory muscle dysfunction, it is possible that this threshold was not exceeded in the study by Farkas and Roussos (11), and thus no effect of training on contractility was achieved. Second, in the present study, training started 6 mo after instillation with elastase, whereas Farkas and Roussos started training 3 wk after instillation. It has been demonstrated that changes in lung volume occur up to 26 wk after instillation with elastase (31). Thus part of the differences in outcome between their and our data may be explained by differences in the experimental setup.

Training reduced fatigability of the EH diaphragm, which is in accordance with training-induced elevation in CS activity of the EH diaphragm. In contrast, contractility of the NH diaphragm was not affected by endurance exercise training. This is probably due to the fact that the training stimulus was not strenuous enough for the NH diaphragm. This is in line with our expectations, as the training program was designed for EH. Indeed, increasing running speed beyond 20 m/min was abandoned because the EH could not sustain this speed. In contrast to the NH, the EH appeared severely fatigued at the end of each training session.

Training-induced elevation in CS activity in the diaphragm of EH increases oxygen flux through the mitochondria, which may favor generation of ROS. Despite the effects of training on contractility and oxidative capacity of the EH diaphragm, training did not affect glutathione status of the diaphragm at rest. No other studies have been published regarding the effects of exercise training on glutathione status in the (EH) diaphragm. In addition, scarce literature is available concerning the effects of exercise training on glutathione status of limb skeletal muscles. In line with our observations, training did not affect the T-Glu level of highly oxidative rat limb skeletal muscles such as the red gastrocnemius (29) and the soleus muscle (18). However, the absence of a response on muscle glutathione status after training does not necessarily imply that antioxidant capacity was unaffected. Sen et al. (29) found that GPX activity increased in response to training, whereas glutathione content was unaltered. Upregulation of antioxidant enzyme activity was reported in other studies as well. Superoxide dismutase activity of the diaphragm increased after 10 wk of endurance training in rats (26) and mice (23). Also, in rats GPX activity of the diaphragm increased after training (23, 26). Moreover, a significant correlation existed between GPX and CS activity (26). Thus it is conceivable that endurance training increases enzymatic antioxidant capacity of skeletal muscles, including the diaphragm, without affecting glutathione status at rest. This suggests that, after training, the diaphragm is better equipped to sustain increased generation of oxidants, for instance, as induced by an acute bout of exercise.

Clinical Relevance

Pulmonary hyperinflation, which is a key feature of COPD, has detrimental effects on diaphragm function (10). This is of importance because a significant correlation exists between respiratory muscle strength and exercise tolerance in COPD (15). Thus strategies to improve respiratory muscle function in COPD are of major clinical importance. The present study shows that pulmonary hyperinflation is associated with oxidative stress in the diaphragm and that a significant correlation exists between in vitro contractility and oxidant status of the diaphragm. Based on these findings, we speculate that, in patients with COPD, the diaphragm is continuously exposed to oxidant stress. This implies that treatment with antioxidants might be able to improve respiratory muscle function. Indeed, it has been demonstrated that administration of the antioxidant N-acetylcysteine, a precursor of glutathione, to healthy subjects before inspiratory resistive breathing attenuates the fall in transdiaphragmatic pressure and increases task endurance (35). However, no such studies have been performed in patients with COPD. Furthermore, we showed that endurance training reversed the detrimental effects of hyperinflation on contractility of the diaphragm, indicating that, in patients with COPD, exercise training may improve diaphragm function and thus exercise tolerance.

In conclusion, elastase-induced pulmonary emphysema impairs in vitro contractility of the hamster diaphragm, which is accompanied by alterations in the redox status of the diaphragm. The significant inverse correlation between submaximal force generation and GSSG/GSH suggests an important role of oxidative stress in impaired force generation in the diaphragm of EH. Endurance training did not affect the glutathione status of the diaphragm but reversed the detrimental effects of emphysema on maximal force-generating capacity of the diaphragm.

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REFERENCES


