The Contractility of the Diaphragm under Hypoxic Conditions in Aged Rats

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ABSTRACT

Background: Hypoxia is a common feature in several respiratory diseases. It is known to impair force generation and increases fatigability of respiratory and peripheral skeletal muscles. The precise mechanisms involved in hypoxia-induced impairment in contractile performance are incompletely understood, but oxidative and nitrosative stress could be at play. Little is known about the effects of hypoxia on the contractility of aged muscles. Aim: To investigate the effect of aging on the contractility of unfatigued diaphragm under hypoxic conditions. And to ask whether there is an age-specific difference in oxidative damage that could partially account for the differential response of the contractility of the diaphragm to hypoxia with age.

Materials and Methods: This experimental work was conducted on 2 groups of albino rats. Group I was the young adult rats (aged 8mo n=10). Group II was the old one (aged 24mo, n=10). Rat diaphragm muscle strips from each group were studied in vitro while aerated with 95% O₂-5% CO₂ (hyperoxia ,n=10) or 95% N₂-5% CO₂ (hypoxia,n=10). Results: The contractility was significantly decreased in old rats when compared with young rats specially under hypoxic conditions. On the other hand the markers of oxidative stress; MDA and nitrotyrosine were significantly higher in old rats than adults. Conclusion: There is an age-specific difference in oxidative damage that could partially account for the differential response of the contractility of the unfatigued diaphragm to hypoxia with age.

Keywords: Aging; Hypoxia; oxidative stress; diaphragm contractility; nitrotyrosin.

INTRODUCTION

HYPOXIA, A COMMON FEATURE in several respiratory diseases, such as chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), and severe pneumonia, impairs force generation and increases fatigability of respiratory and peripheral skeletal muscles[1,2,3,4,5]. Hypoxia is known to reduce the excitability of the sarcolemma[6] and to decrease Ca²⁺ release from the sarcoplasmic reticulum[7]. Hypoxia may impair intracellular Ca²⁺ homeostasis, which in turn could increase NO production by NOS[8]. The precise mechanisms involved in hypoxia-induced impairment in contractile performance are incompletely understood, but oxidative and nitrosative stress could be at play[1,9].

Skeletal muscles are composed of fibres of different types, each type being identified by the isoform of myosin heavy chain which is expressed as slow1, fast 2A, fast 2X, and fast 2B. Slow fibres are resistant
to fatigue due to their highly oxidative metabolism whereas 2X and 2B fibres are easily fatiguable and fast 2A fibres exhibit intermediate fatigue resistance\textsuperscript{10}. Slow fibres and fast fibres are present in equal proportions in the adult human diaphragm while intercostal muscles contain a higher proportion of fast fibres. A small fibre size, abundance of capillaries, and a high aerobic oxidative enzyme activity are typical features of diaphragm fibres and give them the resistance to fatigue required by their continuous activity. Because of their fibre composition, intercostal muscles are less resistant to fatigue. The structural and functional characteristics of respiratory muscle fibres are not fixed, however, and can be modified in response to several physiological and pathological conditions such as training (adaptation to changes in respiratory load), adaptation to hypoxia, age related changes, and changes associated with respiratory diseases\textsuperscript{10}. Dysfunction of these muscles negatively influences patient’s functional status and exercise tolerance, health care utilization and possibly survival\textsuperscript{11}.

Nitric oxide (NO), a highly reactive second messenger, plays an important role in skeletal muscle physiology, including contractility\textsuperscript{16}. This uncharged and diffusible molecule is produced in mammalian cells by the enzymatic cooxidation of L-arginine and NADPH by O\textsubscript{2} to yield NADP, L-citrulline, and NO. Three main isoforms of NO synthase (NOS) with distinct genomic localization are known: the two constitutive enzymes, neuronal NOS (nNOS; 160 kDa) and endothelial NOS (eNOS; 140 kDa), and the inducible NOS (iNOS; 135 kDa). The Ca\textsuperscript{2+} dependence of NO synthesis distinguishes the NOS isoforms, with nNOS and eNOS
having a much higher Ca\textsuperscript{2+} requirement than iNOS\textsuperscript{[17]}.

It is unknown whether peroxynitrite is generated in the diaphragm and plays a role in impaired force generation induced by hypoxia. This is an important question, since peroxynitrite is a potent nitrating and oxidizing agent\textsuperscript{[18]}. It can result in cellular injury and cell death by causing oxidation of sulfhydryls, lipid peroxidation, and nitration of tyrosine residues in protein to form nitrotyrosine\textsuperscript{[18]}.

The first hypothesis of the present study was that hypoxia-induced impairment of in vitro force generation in the rat diaphragm is affected with increased age. To test this hypothesis we measured in vitro force generation of the rat diaphragm under hypoxic and hyperoxic conditions in two age groups. The second hypothesis was, there is an age-specific difference in oxidative damage that could partially account for the differential response of the contractility of the diaphragm to hypoxia with age. To test it, total nitrotyrosine formation was measured in these muscle bundles as a marker for peroxynitrite formation\textsuperscript{[19]} after completion of contractile experiments. In addition, concomitant measurement of malondialdehyde (MDA) was made to determine whether the lipid membranes or the protein components are the plausible target of peroxynitrite.

**MATERIALS & METHODS**

The experiment was conducted in the Animal House of Faculty of Medicine, Cairo University.

**Experimental animals:**
20 male albino rats, 10 adult group (8 mo) with a mean body weight of 140 ± 8 g and 10 old group (24 mo) with a mean body weight of 322 ± 10 g were used in this study. There was no significant difference in the body mass index among groups. They were kept in the animal house of Kasr Al-Aini Faculty of Medicine, Cairo University. The rats had free access to standard rat chow and water. They were kept at 22 ± 1°C temperature at 12 h dark-light cycles.

**Animal Preparation**
The rats were anesthetized with pentobarbital sodium (70 mg/kg body wt ip). The diaphragm and adherent lower ribs were quickly excised after a combined thoracotomy and laparotomy and were immediately submersed in cooled oxygenated (95% O\textsubscript{2} and 5% CO\textsubscript{2}) Krebs solution at pH 7.40. This Krebs solution consisted of 137 mM NaCl, 4 mM KCl, 2 mM CaCl\textsubscript{2}, 1 mM MgCl\textsubscript{2}, 1 mM KH\textsubscript{2}PO\textsubscript{4}, 24 mM NaHCO\textsubscript{3}, 7 mM glucose. From the central costal region of the hemidiaphragm a muscle strip was dissected along the parallel axis of the muscle fibers. Silk sutures were tied firmly to both ends of the muscle strip.

**Contractile Measurements:**

Effect of hyperoxia:
The effect of hyperoxia on contractility, nitrotyrosine formation, and tissue MDA level were determined. The strips (no. of muscle strips = 10 from each group) were mounted vertically in tissue baths containing Krebs solution bubbled with 95% O\textsubscript{2}–5% CO\textsubscript{2} with a pH of 7.4. Temperature of the solution was maintained at 37°C. The muscle was stimulated directly by using platinum
plate electrodes placed in close apposition of the bundle. Stimuli were applied with a pulse duration of 0.2 ms and train duration of 400 ms. Muscle preload force was adjusted until optimal fiber length \( L_o \) for maximal twitch force \( P_t \) was achieved. After 10 min of thermoequilibration, baseline measurements were determined. After 60 min of hyperoxia, force was measured at 1, 15, 30, and 100 Hz \( (P_1, P_{15}, P_{30}, \text{and} P_{100}) \) respectively) at 2-min intervals. Isometric force was measured with a high-sensitivity force transducer, and force records were collected and stored on the hard drive of a computer for later data analysis. Data analysis was performed off-line with use of manually controlled cursors for measurement of peak force of contraction. Subsequently bundles were blotted dry, quickly frozen in liquid nitrogen, and stored at –80°C.

Effects of hypoxia.

The effect of hypoxia on contractility, nitrotyrosine formation, and tissue MDA level were determined. After an initial measurement of force-frequency characteristics, the Krebs solution was replaced by fresh standard Krebs solution, and the gas mixture was maintained 95% \( N_2 \) and 5% \( CO_2 \) (hypoxia, \( n = 10 \) for each group). After 60 min, the force-frequency relationship was remeasured, and subsequently bundles were blotted dry, quickly frozen in liquid nitrogen, and stored at –80°C.

Biochemical measurements:

Diaphragm bundles were divided into two parts. The first part was stored in lysis buffer for detection of nitrotyrosine residues. The second part was put in phosphate buffer saline for MDA assay and both were stored at -80°C till the assay. Concomitant measurement of malondialdehyde (MDA) was made to determine whether the lipid membranes or the protein components are the plausible target of peroxynitrite.

Measurement of MDA:

MDA was measured in tissue homogenate after precipitation of protein by addition of trichloroacetic acid (TCA)- then thiobarbituric acid (TBA) reacted with malondialdehyde (MDA) to form thiobarbituric acid reactive product, which was measured at 532 nm according to Draper and Hadley.

Nitrotyrosine Measurement:

Nitrotyrosine was detected by performing Western immunoblotting with a monoclonal anti-nitrotyrosine antibody of high specificity (StressGen Biotechnologies, Victoria, Canada). Crude diaphragm homogenate proteins (10 µg) were heated for 5 min at 95°C, then briefly about 30 ul of homogenate supernatant was separated on 10% sodium dodecylsulfate- SDS-polyacrylamide gel electrophoresis at (200 V for 1 h). High- and low-molecular-weight standards of nitrotyrosine (Upstate Biotechnology) were run in parallel as positive controls and Proteins were transferred to nitrocellulose membrane using a semi-dry transfer apparatus membranes were subsequently incubated with primary monoclonal antibodies raised against nitrotyrosine in 0.1% bovine serum albumin (BSA) in phosphate-buffered saline with 1% Tween 20 (PBST). After three 10-min washes with PBST on a rotating
shaker, the membranes were incubated with a secondary antibody (polyclonal anti-mouse IgG horseradish peroxidase conjugated) for 1 h at room temperature. The membranes were finally washed twice for 10 min with PBST. Afterward, protein bands were visualized using an enhanced chemiluminescence detection kit (Amersham Biosciences Europe). The blots were scanned with an imaging densitometer, and optical densities (OD) of positive nitrotyrosine protein bands were quantified using documentation system supplied by (Biometra, Germany). Total nitrotyrosine OD was calculated for each samples by adding the OD of individual positive protein bands[21].

Data Treatment and Statistics
After completion of contractile experiments, the length and dry weight of the diaphragm muscle bundle were measured. Cross-sectional area (CSA) was calculated by dividing diaphragm strip weight (g) by strip length (cm) times specific density (1.056). Force is expressed per CSA in g/cm². Data are presented as means ± SD. Differences were analyzed with Non Parametrical Tests and either Mann-Whitney test for independent group comparisons or Wilcoxon Signed Rank for comparison of paired data. Statistical analysis was performed with the SPSS package version 15. Comparisons were considered significant at $P \leq 0.05$.

RESULTS

Contractile Studies
Figure 1 shows the effect of 60min hyperoxia and hypoxia on force of contraction of the rat diaphragm from young and old rats under 30 Hz frequency of stimulation. It was apparent that the hypoxia reduces the force of contraction in both young and aged rats. In addition the force of contraction in old rat diaphragm was less than that in the young.

Hypoxia in young adult rat diaphragm
The peak force of contractions under hyperoxic condition averaged 7.3 ± 0.3, 11.9 ± 0.3, 15.1 ± 0.6, and 16.2 ± 0.3 g/cm² at 1, 15, 30 and 100Hz of stimulation frequency, respectively. The force-frequency relationship was significantly different between hypoxia and hyperoxia ($P = 0.005$, except for the initial $P = 0.013$). Hypoxia resulted in a significant downward shift of the force-frequency compared with hyperoxia (table 1).
Fig. 1: Effect of 60 min hyperoxia and hypoxia on force of contraction of the rat diaphragm from young and old rats (30 Hz).

Table 1: The peak force of contraction (mean ±SD) of the diaphragm in the young group with different frequency of stimulation after 60 min in either hyperoxia or hypoxia conditions. (n=10 in each condition).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Peak force</th>
<th>1 Hz</th>
<th>15 Hz</th>
<th>30 Hz</th>
<th>100 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperoxia</td>
<td></td>
<td>7.3 ± 0.3</td>
<td>11.9 ± 0.3</td>
<td>15.2 ± 0.3</td>
<td>16.2 ± 0.3</td>
</tr>
<tr>
<td>Hypoxia</td>
<td></td>
<td>6.8 ± 0.4</td>
<td>9.7 ± 0.4*</td>
<td>11.8 ± 0.3*</td>
<td>12.5 ± 0.4*</td>
</tr>
</tbody>
</table>

* Statistically significant difference between the effect of hypoxia and hyperoxia with the same frequency of stimulation (P = 0.005)
□ = Statistically significant difference between the effect of hypoxia and hyperoxia with the same frequency of stimulation (P = 0.013)

Hypoxia in old rat diaphragm:

The force-frequency relationship was significantly reduced under hypoxic conditions (P = 0.005) and averaged 6.8 ± 0.4, 9.7 ± 0.4, 11.8 ± 0.3, and 12.5 ± 0.4 g/cm² for 1, 15, 30, and 100 Hz of stimulation respectively after 60 min of hyperoxia. (Table 2)
Table 2: The peak force of contraction (mean ±SD) of the diaphragm in the old group with different frequency of stimulation after 60min in either hyperoxia or hypoxia conditions. (n=10 in each condition).

<table>
<thead>
<tr>
<th>Condition</th>
<th>1 Hz</th>
<th>15 Hz</th>
<th>30 Hz</th>
<th>100 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperoxia</td>
<td>6.8 ± 0.4</td>
<td>9.7 ± 0.4</td>
<td>11.8 ± 0.3</td>
<td>12.5 ± 0.4</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>3.4 ± 0.1*</td>
<td>3.8 ± 0.1*</td>
<td>4.4 ± 0.1*</td>
<td>4.8 ± 0.2*</td>
</tr>
</tbody>
</table>

* = Statistically significant difference between the effect of hypoxia and hyperoxia with the same frequency of stimulation (P =0.005)

The figures 2&3 would be studied to investigate the differential response of the contractility of the diaphragm to hyperoxia and hypoxia between young and old rats. They showed the differences between the peak force of contraction in young and old rats under hyperoxic and hypoxic conditions respectively.

There were significant differences between the contractile response of the diaphragm of the young and old rats (p =0.005, < 0.001, < 0.001, < 0.001) for the frequencies 1,15,30 &100 respectively, under hyperoxic conditions. The peak force of contraction was significantly reduced in the old rat diaphragm under all frequencies used compared with that of the young, specially more prominent with the higher frequencies (Fig. 2).

![Fig. 2](image)

**Fig. 2**: Effect of hyperoxia on peak force of contraction in young and old rats under the frequency 1,15,30 & 100 Hz (n=10 in each group).

* = Statistically significant difference between the force of contraction in young and old with the same frequency of stimulation (P =0.005)

# = Statistically significant difference between the force of contraction in young and old with the same frequency of stimulation (P < 0.001)
In addition, there were significant differences between the contractile response of the diaphragm of the young and old rats \( (p < 0.001) \) for all frequencies used under hypoxic conditions. It was apparent that the peak force of contraction was significantly reduced in the old rat diaphragm under all frequencies used as compared with that of the young, specially more prominent with the higher frequencies(Fig. 3). Furthermore, the force frequency curve for the old rat diaphragm under hypoxic conditions appeared slowly rising curve.

![Graph showing the effect of hypoxia on peak force of contraction in young and old rats under the frequency 1, 15, 30 & 100 Hz (n=10 in each group).](image)

# = Statistically significant difference between the force of contraction in young and old with the same frequency of stimulation \( (P < 0.001) \)

**Nitrotyrosine Formation and Lipid peroxidation in the Rat Diaphragm**

Hypoxia significantly increased diaphragm nitrotyrosine level \( (P < 0.005) \) in the young as well as in the old rats diaphragm.

Malondialdehyde (MDA) was used as a marker for lipid peroxidation. It was significantly increased \( (P < 0.005) \) in the rat diaphragm of both groups after 60 min of hypoxia. (table 3 and 4). These results were signifying oxidative stress as a result of hypoxia.
Table 3: Effect of hyperoxia & hypoxia on the development of Nitrotyrosine and MDA in diaphragm of the adult young group after contractile measurement. (n=10 in each condition).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Nitrotyrosine (ug/mgptn)</th>
<th>MDA (nMol/mgptn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperoxia</td>
<td>108.3 ± 0.2</td>
<td>0.2 ± 0.05</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>136.1 ± 5.7*</td>
<td>0.5 ± 0.1*</td>
</tr>
</tbody>
</table>

Results are mean ±SD.

* = Statistically significant difference between the effect of hypoxia and hyperoxia in the same variable (P =0.005)

Total nitrotyrosine & MDA were significantly higher under hypoxic conditions.

Table 4: Effect of hyperoxia & hypoxia on the development of Nitrotyrosine and MDA in diaphragm of the old group after contractile measurement (n=10 in each condition).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Nitrotyrosine (ug/mgptn)</th>
<th>MDA (nMol/mgptn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperoxia</td>
<td>120.3 ± 3.1</td>
<td>0.3 ± 0.06</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>168.0 ± 18.1*</td>
<td>0.9 ± 0.18*</td>
</tr>
</tbody>
</table>

Results are mean ±SD.

* = Statistically significant difference between the effect of hypoxia and hyperoxia in the same variable (P =0.005).

Total nitrotyrosine & MDA were significantly higher under hypoxic conditions.

Figure 4 demonstrated that the rise in nitrotyrosine in the groupII was significantly higher when compared with group I under hyperoxic and hypoxic condition (P < 0.001). This was also the case for MDA, where it was significantly higher in the old rat diaphragm compared with that of the young(P < 0.001) under both hyperoxic and hypoxic conditions. In fact the aged rat diaphragm apparently more susceptible to the oxidative damage than the young as the percentage of rise in both nitrotyrosine and MDA in the group II under hypoxic condition is more than group I.
DISCUSSION

The severity of hypoxia used in the present work is considered as severe hypoxia, according to previous studies using the same model [1,22].

The present study showed that hypoxia tends to reduce in vitro force generation of the young rat diaphragm. This is in agreement with several studies which showed that hypoxia impairs diaphragm muscle function [1,22]. Also, it was found that hypoxia depresses isotonic contractile properties and power output, and reduces endurance time during repeated isotonic contractions [23]. The precise mechanisms of hypoxia-induced impairment in muscle contractility are unknown. Several recent studies indicate that free radicals may be at play [11].

It has been shown that, skeletal muscles produce reactive oxygen and nitrogen species including superoxide and nitric oxide at low levels under resting conditions and at higher levels during contractile activity [24,25]. The reaction of superoxide and nitric oxide form peroxynitrite, a highly reactive molecule that can covalently modify cysteine and tyrosine residues [19]. In this study, we focused on nitrotyrosine, a stable product of peroxynitrite-induced tyrosine nitration that was monitored using an antibody that recognized this posttranslation modification.

Our data showed that acute hypoxia increases diaphragm muscle
nitrotyrosine level. These finding indicated that there is elevated generation of NO and/or superoxide which enhance peroxynitrite formation. The previous data are supported also by the presence of significantly higher MDA in hypoxic adult rat diaphragm as compared with that under hyperoxia. That results were in agreement with many previous studies on skeletal and cardiac myocytes\cite{1,5,6,27}. Duranteau et al.\cite{26} showed that the generation of oxidants in cardiomyocytes is increased, depending on the degree of hypoxia: the lower the \( \text{PO}_2 \), the higher the oxidant generation. In addition, antioxidants reduce fatigability of the rat diaphragm under hypoxic conditions, indicating that reactive oxygen species affect force generation under hypoxic conditions\cite{10}. Little is known about the effects of acute hypoxia on NOS regulation or the rate of NO generation in skeletal muscle. Javeshghani and coworkers\cite{27} found that sixty days of hypobaric hypoxia increases eNOS and nNOS expression and activity in rat diaphragm muscle, but prolonged (9 months) exposure decreased NOS activity and expression.

In addition, Ottenheijm et al.\cite{5} found that nitrotyrosine levels were increased in hypoxic rat diaphragm muscles. They concluded that acute hypoxia induces dysfunction of skinned muscle fibers, reflecting contractile protein dysfunction. Also, these data indicate that reactive nitrogen species play a role in hypoxia-induced contractile protein dysfunction. They also found that reoxygenation of the muscle bundle partially restores bundle contractility but completely reverses contractile protein dysfunction\cite{8}. Nitrotyrosine formation has been suggested as just "footprints" of peroxynitrite formation. However, evidence is accumulating that nitrotyrosine formation is a posttranslational mechanism for altering protein function\cite{22} and thereby muscle contractility. Zhu and coworkers\cite{22} observed a similar pattern of reduced force generation after peroxynitrite exposure under hyperoxic conditions. The peroxynitrite-induced decrease in force generation is in line with other studies\cite{28}. Supinski et al.\cite{28} concluded that peroxynitrite impaired force generation in skinned single skeletal muscle fibers, indicating a direct effect on contractile proteins. Interestingly Zhu et al.\cite{22} didn't found a causal relationship between total nitrotyrosine level in diaphragm muscle and contractility. However, this is not surprising, since the effect of protein nitration on contractility depends on the final effect of all proteins nitrated\cite{22}.

Although NO release has been measured from intact skeletal muscle tissue in vitro\cite{29}, no data are available on NO concentrations within the subcellular compartments. This is relevant because within muscle fibers NOS expression is not uniform\cite{30}, suggesting that localized areas of higher NO concentration may exist. Also, localized areas of high superoxide generation may exist within muscle fibers. Therefore, measuring the average concentration of NO, superoxide, or peroxynitrite does not provide accurate information
regarding maximal concentration within muscle fibers\cite{30}.

It is to be noted that tyrosine nitration can inhibit protein function, for example, by altering a protein's conformation, imposing steric restrictions to the catalytic site, and preventing tyrosine phosphorylation\cite{31,32}. An alternative scenario is that, a few proteins, such as cytochrome c, which acquires a strong peroxidase activity after nitration\cite{31}.

The functional consequences for tyrosine nitration of the SR Ca-ATPase have been demonstrated using peroxynitrite following in vitro chemical modification of the protein. Viner and colleagues\cite{33} showed that peroxynitrite induced an increase in nitrotyrosine content that correlated with a significant loss in Ca-ATPase activity.

The potential targets for peroxynitrite include myosin, actin, and troponin I, which have tyrosine groups that can be nitrated\cite{34}. Peroxynitrite can also affect calcium homeostasis by inactivating \(Ca^{2+}\)-ATPase\cite{35}. Studies with cultured cardiac myocytes revealed that plasma membrane proteins and myofibrillar creatine kinase are potential targets for peroxynitrite as well\cite{34}. In addition, mitochondria potentially provide an abundant source of superoxide, and high concentrations of NOS are localized to mitochondria in skeletal muscle. Consequently, peroxynitrite may reach high concentrations in mitochondria, affecting nitration of mitochondrial proteins, and thereby impair mitochondrial function\cite{35}.

Indeed, it has been shown that the effects of NO on muscle contractility depend on muscle PO\(_2\)\cite{36}. The PO\(_2\) dependence for peroxynitrite in skeletal muscle is not known. Indeed, it has been suggested that not all tyrosine groups in biological environment are equally susceptible to nitration. Protein characteristics, such as the location of tyrosine on a loop structure and its association with a neighboring negative charge, determine susceptibility to nitration by peroxynitrite\cite{37}.

The mechanisms of increased peroxynitrite formation under hypoxic conditions have not been investigated. It can be speculated that elevated generation of peroxynitrite is the result of hypoxia-induced inflammatory response. Evidence is emerging that hypoxia induces expression of cytokines such as tumor necrosis factor (TNF) - both in vitro\cite{38} and in vivo\cite{39}. In turn, TNF- has been shown to increase oxidant levels in diaphragm muscle fibers\cite{40} and also to increase the generation of NO in peripheral skeletal muscle\cite{41}. However, whether the hypoxia-induced elevation in nitrotyrosine formation in skeletal muscle is related to the activation of proinflammatory cytokines remains to be investigated.

The decline in peak force of contraction observed in our study in the diaphragm from aged rats is partially explained by the age-related loss in muscle mass, other yet undefined mechanisms contribute\cite{15}. Several studies reported a substantial reduction in transcript levels of MHC type II with age compared with MHC type I\cite{15,42,43}. Thus, over time, this selective reduction of myosin relative to actin in the type II fibers muscle would result in a change in optimal stoichiometry of the myofibrillar
proteins relative to each other. Thompson and coworkers concluded that the loss of optimal stoichiometry between these two key proteins critical for force production likely results in decreased force generation in the affected muscle.

It has been shown in the present work the oxidant production is increased in aged diaphragm as indicated by the significantly raised MDA under hyperoxia as compared with that of the young. This is in agreement with many studies. Although some antioxidants are increased in aging muscle, the extent of increase is muscle-specific and not global to all enzymes. Thus, the burden of defending against the increased load of free radicals may be greater than the compensatory change in antioxidants. Thus, the fundamental changes in cell redox status, and the ability to remove free radical–damaged proteins likely contribute to the age-related increase in oxidized proteins observed in this study. This is in accordance with many studies which suggested that protein nitration may contribute to underlying mechanisms in age-related functional decrement.

Thompson et al. observed that, although myosin and actin were modified with 3-nitrotyrosine and 4-hydroxy-2-nonenal, the extent of chemical modification did not increase with age. So, Thompson and coworkers suggested that the decline in force production with age was not due to the accumulation of these two specific markers of protein oxidation on the myofibrillar proteins. This was in contrast to the present study, as nitrotyrosine was found to be markedly elevated in the diaphragm muscles from old rats. These differences might be explained by the different muscles used in both studies. On the other hand, our finding go with that of Ottenheijm et al. who revealed that hypoxia-induced rat diaphragm dysfunction was associated with elevated diaphragm muscle nitrotyrosine levels. Additionally, Fugere and coworkers observed a significant age-associated increase in nitrotyrosine-modified proteins. The modified proteins include the sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase, aconitase, β-enolase, triosephosphate isomerase, and carbonic anhydrase III. These proteins, involved in metabolism and calcium homeostasis, exhibited an age-dependent increase in 3-NT content in both (type II) and (type I) muscles.

Clinical Relevance

The adverse effects of hypoxia on respiratory muscle function are commonly recognized, but relatively little is known about the underlying pathophysiology. Recent studies have shown that oxidants play a prominent role in muscle physiology at different steps in excitation-contraction coupling. Recent studies indicate that oxygen free radical scavengers improve muscle endurance under hypoxic conditions. Whereas hypoxia may directly increase oxidant generation by the respiratory muscles, the hypoxia-induced elevated work of breathing will further enhance oxidant generation by the respiratory muscles. Further understanding of the role of oxidants in respiratory muscle function is needed to develop successful strategies for preventing...
hypoxia-induced respiratory muscle failure in aged population.

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الخواص الاقطاطية لعضلة الحجاب الحاجز تحت تأثير نقص الأوكسجين في الفئران المسنة

علا تورك

قسم الفسيولوجي بكلية الطب - جامعة القاهرة

كان الغرض من هذا البحث دراسة تأثير التقدم في العمر على اقتطاعية عضلة الحجاب الحاجز في حالات نقص غاز الأوكسجين، وتحديد ما إذا كان هذا تأثير مختلف لعوامل الأكسجين على العضلة في الفئران المسنة التي قد تلعب دوراً في تكيف استجابة العضلة تحت تأثير نقص الأكسجين.

استخدمت هذه الدراسة 10 فئران بالبلعة عمرها (8 أشهر) و10 فئران مقدمة في العمر. وتم تعريض نصف الحجاب الحاجز لكل حالة لتلفاز كهربائي تردداته 3000 و4000 (عليم). وقد تم تأثیر نصف عضلة الحجاب الحاجز نصف تلفاز كهربائي تردداته 4000 و5000 (عليم) في الفئران المسنة. بينما تعرض النصف الآخر للعامة للفص الترددات ولكن في وسط ترددات 5000 و6000 (عليم) الأكسجين. وبعد قياس قوة انقباض العضلة في كل حالة تم قياس الفئران الدالين على الأوكسجين، ملئه بمليونية (MDA) (أوكسجين تأثیر وتروتوترزين).

وقد تبين أن قوة انقباض عضلة الحجاب الحاجز في الفئران المسنة أضعف من نصف الفئران البالغة. وتحت ملاحظة إحصائية، خاصة للترددات العالية، وذلك في وسط عالي الأكسجين. وظهر أن قوة انقباض عضلة الحجاب الحاجز في وسط نقص الأكسجين في الفئران المسنة أضعف بعدد كبير بدالة إحصائية عالية لكل الترددات عند مقارنتها بالتي في الفئران البالغة.

وينكشف المؤشر المستخدم في هذه الدراسة لدراسة الدالة على التردد الأكسجيني في العضلة على زيادة ذات دالة إحصائية في تكوينهما في عضلات الفئران المسنة عند مقارنتها بالفئران البالغة. وقد وجدت زيادة في تكوينهما بعدد أكثر في الوسط قبل الأكسجين ونسبة مصداقية للضعف الأكبر في قوة انقباض عضلة الحجاب الحاجز عند الفئران المسنة.

وهذه النتائج تدل على أن التردد الأكسجيني يلعب دوراً في قوة قوة انقباض عضلة الحجاب الحاجز عند الفئران المسنة تحت تأثير نقص الأكسجين. وربما علاجها بمضادات الأكسدة يمكن أن يكون دالة للمرض، إضافة إلى العديد من الدراسات السريرية لبحث دور التوتر الأكسجيني. ومضادات الأكسدة المختلفة في التأثير على قوة أداء عضلة الحجاب الحاجز عند المسنين.