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% O_2-5% CO_2 (hyperoxia ,n=10) or 95% N_2-5% CO_2 (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 Ca^{2+} release from the sarcoplasmic reticulum[7]. Hypoxia may impair intracellular Ca^{2+} 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[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[10]. Dysfunction of these muscles negatively influences patient's functional status and exercise tolerance, health care utilization and possibly survival[11].

Aging is accompanied by a progressive loss of skeletal muscle mass and strength, leading to the loss of functional capacity and an increased risk of developing chronic metabolic disease[12,13]**.** Sarcopenia, the loss of muscle mass and strength with age, is becoming recognized as a major cause of disability and morbidity in the elderly population. Sarcopenia is part of normal aging and does not require a disease to occur, although muscle wasting is accelerated by chronic diseases. Sarcopenia is thought to have multiple causes, although the relative importance of each is not clear. Neurological, metabolic, hormonal, nutritional, and physical-activity–related changes with age are likely to contribute to the loss of muscle mass[14].

OXIDATIVE damage to cellular macromolecules has long been implicated in the aging process. The "Free Radical Hypothesis of Aging," first introduced by Denham Harman in 1956, has been modified to the "Oxidative Stress Hypothesis of Aging." Oxidative stress is defined as an imbalance of pro-oxidants and antioxidants that results in the accumulation of oxidative damage to a variety of macromolecules[15]. The Oxidative Stress Hypothesis of Aging postulates that the cumulative oxidative damage that occurs during aging results in a progressive loss in function of various cellular processes[15].

Nitric oxide (NO), a highly reactive second messenger, plays an important role in skeletal muscle physiology, including contractility[16]. This uncharged and diffusible molecule is produced in mammalian cells by the enzymatic cooxidation of L-arginine and NADPH by O_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^{2+} dependence of NO synthesis distinguishes the NOS isoforms, with nNOS and eNOS

having a much higher Ca^{2+} requirement than iNOS[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[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[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[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*:**

20male albino rats, 10 adult group (8mo) with a mean body weight of 140± 8 g and 10old group (24mo) 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± 1ºC 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_2 and 5% CO_2) Krebs solution at pH 7.40. This Krebs solution consisted of 137 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM KH_2PO_4, 24 mM $NaHCO_3$, 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_2–5% CO_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}, 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[20].

Nitrotyrosine Measurement:

Nitrotyrosine was detected by performing Western immunoblotting with a monoclonal anti-nitrotyrosine antibody of high specificity (StressGen Biotechnologies,Victoria Canda). 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 a 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/cm^2. 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/cm^2 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).

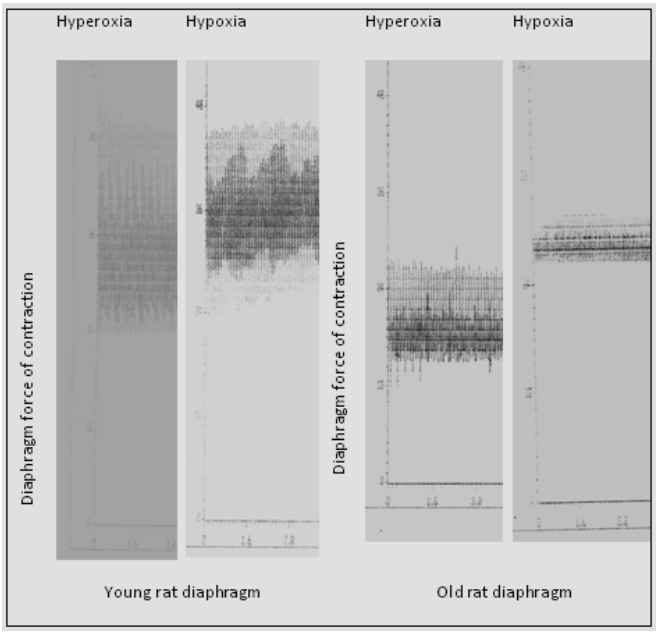

Fig.1: Effect of 60min hyperoxia and hypoxia on force of contraction of the rat diaphragm from young and old rats (30 Hz).

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

Peak force / Condition	1 Hz	15 Hz	30 Hz	100 Hz
Hyperoxia	7.3 ± 0. 3	11.9 ± 0.3	15.2 ± 0.3	16.2 ± 0.3
Hypoxia	6.8 ± 0.4□	**9.7 ± 0.4***	**11.8 ± 0.3***	12.5 ± 0.4*

* = 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/cm^2 for 1,15,30 and 100Hz of stimulation respectively after 60min 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).

Peak force / Condition	1 Hz	15 Hz	30 Hz	100 Hz
Hyperoxia	6.8 ± 0 .4	9.7 ± 0.4	11.8 ± 0.3	12.5 ± 0.4
Hypoxia	3.4 ± 0.1*	3.8 ± 0.1*	4.4 ± 0.1*	4.8 ± 0.2*

* = 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).

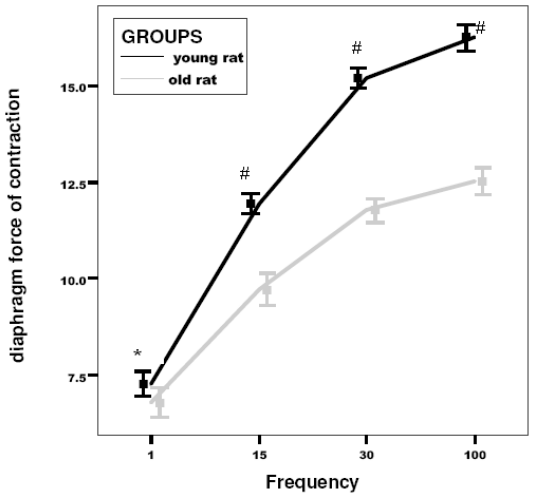

Fig. 2: Effect of hyperoxia on peak force of contraction in young and old rats under the frequency 1,15,30 & 100 Hz (n=10 in each group).

* = 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.

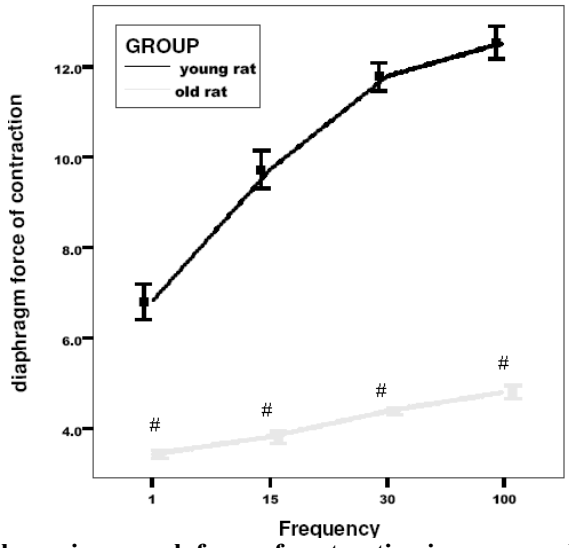

Fig 3: Effect of hypoxia on peak force of contraction in young and old rats under the frequency 1,15,30 & 100 Hz (n=10 in each group).
= Statistically significant difference between the force of contraction in young and old with the same frequency of stimulation ($P < 0.001$)

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 lincreased ($P < 0.005$)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).

The Variable / Condition	Nitrotyrosine (ug/mgptn)	MDA (nMol/mgptn)
Hyperoxia	108.3 ± 0.2	0.2 ± 0.05
Hypoxia	136.1 ± 5.7*	0.5 ± 0 .1*

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).

The Variable / Condition	Nitrotyrosine (ug/mgptn)	MDA (nMol/mgptn)
hyperoxia	120.3 ± 3.1	0.3 ± 0.06
hypoxia	168.0 ± 18.1*	0.9 ± 0 .18*

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.

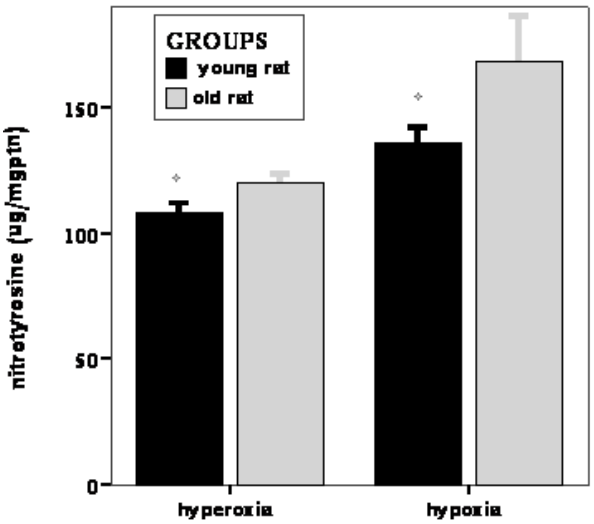

Fig. 4: The effect of hyperoxia and hypoxia on the development of Nitrotyrosine in the diaphragm of young and old rat (n=10 in each group).
* =Statistically significant difference between the amount of nitrotyrosine developed in young and old rat diaphragm under the same condition($P < 0.001$).

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[1].

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[1,5,26,27]. **Duranteau** et al.[26] showed that the generation of oxidants in cardiomyocytes is increased, depending on the degree of hypoxia: the lower the 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[1]. Little is known about the effects of acute hypoxia on NOS regulation or the rate of NO generation in skeletal muscle. **Javeshghani** and coworkers[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*[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[5]. 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[22] and thereby muscle contractility. **Zhu** and coworkers[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[28]. Supinski *et al*.[28] concluded that peroxynitrite impaired- force generation in skinned single skeletal muscle fibers, indicating a direct effect on contractile proteins. Interestingly **Zhu** *et al*.[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[22].

Although NO release has been measured from intact skeletal muscle tissue in vitro[29], no data are available on NO concentrations within the subcellular compartments. This is relevant because within muscle fibers NOS expression is not uniform[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[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[31,32]. An alternative scenario is that, a few proteins, such as cytochrome *c*, which acquires a strong peroxidase activity after nitration[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[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[34]. Peroxynitrite can also affect calcium homeostasis by inactivating Ca^{2+}-ATPase[35]. Studies with cultured cardiac myocytes revealed that plasma membrane proteins and myofibrillar creatine kinase are potential targets for peroxynitrite as well[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[35].

Indeed, it has been shown that the effects of NO on muscle contractility depend on muscle PO_2[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[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[38] and in vivo[39]. In turn, TNF- has been shown to increase oxidant levels in diaphragm muscle fibers[40] and also to increase the generation of NO in peripheral skeletal muscle[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[15]. Several studies reported a substantial reduction in transcript levels of MHC type II with age compared with MHC type I[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[15] 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[24,25]. Although some antioxidants are increased in aging muscle, the extent of increase is muscle-specific and not global to all enzymes[44]. 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[45-47], 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[48-51] which suggested that protein nitration may contribute to underlying mechanisms in age-related functional decrement .

Thompson *et al.*[15] *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[15] 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.[5] who revealed that hypoxia-induced rat diaphragm dysfunction was associated with elevated diaphragm muscle nitrotyrosine levels. Additionally, Fugere and coworkers[52] observed a significant age-associated increase in nitrotyrosine-modified proteins. The modified proteins include the sarcoplasmic reticulum Ca^{+2}-ATPase, aconitase, ß-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[52].

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 الانقباضية لعضلة الحجاب الحاجز تحت تأثير نقص الأوكسجين في الفئران المسنة

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كان الغرض من هذا البحث دراسة تأثير التقدم في العمر على انقباضية عضلة الحجاب الحاجز في حالات نقص غاز الأوكسجين ، وتحديد ما إذا كان هناك تأثير مختلف لعوامل الأكسدة على العضلة في الفئران المسنة التي قد تلعب دورا في تباين استجابة العضلة تحت تأثير نقص الأكسيجين .

أستخدم في هذه الدراسة ١٠ فئران بالغة عمرها (٨ أشهر) و ١٠ فئران متقدمة في العمر عمرها (عامين). وقد تم تعريض نصف عضلة الحجاب الحاجز لكل حالة لتأثير كهربي ترددات ١، ١٥، ٣٠ و ١٠٠ هرتز في وسط تهويته ٩٥% أوكسجين و ٥% ثاني أوكسيد الكربون (عالي الأوكسجين) . بينما تعرض النصف الآخر للعضلة لنفس الترددات ولكن في وسط تهويته ٩٥% نيتروجين و ٥% ثاني أوكسيد الكربون (قليل الأوكسجين) . وبعد قياس قوة انقباض العضلة في كل حالة تم قياس المؤشران الدالين على التوتر الأوكسيدي ، ملونديالديهايد (MDA) ونيتروتيروزين.

وقد تبين أن قوة انقباض عضلة الحجاب الحاجز في الفئران المسنة أضعف منه في الفئران البالغة ضعفا ذو دلالة إحصائية، خاصة للترددات العالية وذلك في وسط عالي الأكسيجين. وقد ظهر أنَّ قوة انقباض عضلة الحجاب الحاجز في وسط نقص الأكسيجين في الفئران المسنة أضعف بمعدل كبير بدلالة إحصائية عالية لكل الترددات عند مقارنتها بتلك في الفئران البالغة.

ويكشف المؤشران المستخدمان في هذه الدراسة للدلالة على التوتر الأوكسيدي في العضلة على زيادة ذات دلالة إحصائية في تكوينهما في عضلات الفئران المسنة عند مقارنتها بالفئران البالغة. وقد لوحظ زيادة في تكوينهما بمعدل أكثر في الوسط قليل الأوكسجين وذلك مصاحب للضعف الأكثر في قوة انقباض عضلة الحجاب الحاجز عند الفئران المسنة.

وهذه النتائج تدل على أن التوتر الأوكسيدي يلعب دورا في نقص قوة انقباض عضلة الحجاب الحاجز عند الفئران المسنة تحت تأثير نقص الأوكسجين .وربما علاجه بمضادات الأكسدة يحسن الأضرار المصاحبة للأمراض التنفسية الحادة عند كبار السن.ومازال ذلك يحتاج إلى العديد من الدراسات السريرية لبحث دور التوتر الأوكسيدي ومضادات الأكسدة المختلفة في التأثير على قوة أداء عضلة الحجاب الحاجز عند المسنين