COPD ELICITS REMODELING OF THE DIAPHRAGM AND VASTUS LATERALIS MUSCLES IN HUMANS

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ABSTRACT

A profound remodeling of the diaphragm and vastus lateralis (VL) occurs in patients with moderate-to-severe COPD. In this mini-review, we discuss the following costal diaphragm remodeling features noted in patients with moderate-to-severe COPD: (a) deletion of serial sarcomeres; (b) increased proportion of slow-twitch fibers; (c) fast to slow isoform shift in sarcoplasmic reticulum ATPase (SERCA); (d) increased capacity of oxidative metabolism; (e) oxidative stress; and (f) myofiber atrophy. We then present the sole feature of diaphragm remodeling noted in mild-to-moderate COPD under the heading “myosin heavy chain and contractile remodeling noted in mild-to-moderate COPD.” The importance of VL remodeling in COPD patients as a prognostic indicator as well as a major determinant of the ability to carry out activities of daily living is well-accepted. We present the remodeling of the VL noted in COPD patients under the following headings: (a) decrease in proportion of slow-twitch fibers; (b) decreased activity of oxidative pathways (c) oxidative and nitrosative stress; and (e) myofiber atrophy. For each of the remodeling features noted in both VL and costal diaphragm of COPD patients, we present mechanisms that are currently thought to mediate these changes as well as the pathophysiology of each remodeling feature. We hope that our mechanistic presentation stimulates research in this area that focuses on improving the ability of COPD patients to carry out increased activities of daily living.
Key words: diaphragm, vastus lateralis, COPD, fiber-type transformation, slow-twitch fiber, fast-twitch fiber
INTRODUCTION

Recently, we have learned a great deal about the remodeling of the diaphragm and limb muscles in patients with COPD. Indeed, for additional perspectives in this area, we refer the reader to several excellent recent review articles (10, 14, 24, 25, 39, 41, 47, 62, 65, 72, 74, 75). In this mini-review, we present selective aspects of this remodeling noted in the human diaphragm and vastus lateralis (VL)—the most commonly studied limb muscle—of COPD patients. For each feature of remodeling, we first provide a description of the particular characteristic(s) noted in the human diaphragm or VL; subsequently, we use experimental animal and in vitro studies in attempts to elucidate mechanisms underlying this remodeling, and then we provide our thoughts regarding the pathophysiological relevance of the remodeling feature (where appropriate). Although the features of COPD-induced remodeling of the diaphragm are numerous, many appear to represent transformation of protein isoforms noted in fast-twitch fibers to those noted in slow-twitch fibers; we use the “term fast-to-slow transformation” to describe these changes. In contrast, COPD-induced remodeling of the VL is characterized by transformation of many protein isoforms noted in slow fibers to those isoforms noted in fast-twitch fibers; accordingly, we use the term “slow-to fast transformation to describe these latter changes.”
Currently, it is common to organize features of COPD-induced diaphragm remodeling into those that occur in moderate to severe COPD and those that occur in mild to moderate COPD. In this mini-review, we follow this convention by first presenting those features of remodeling noted in moderate to severe COPD; we present these under the following headings: (a) deletion of serial sarcomeres; (b) increased proportion of slow-twitch fibers; (c) fast to slow isoform shift in sarcoendoplasmic reticulum ATPase (SERCA); (d) increased capacity of oxidative metabolism; (e) oxidative stress; and (f) myofiber atrophy. We then present features of diaphragm remodeling noted in mild-to moderate COPD under the single heading titled “myosin heavy chain and contractile remodeling noted in mild-to-moderate COPD.”

**Deletion of sarcomeres from the costal diaphragm.** Briefly, due to the length tension relationship, the length of costal diaphragm fibers is greatest at functional residual capacity (FRC) and then progressively decreases until total lung capacity (TLC) is reached. At FRC, the individual sarcomeres in the muscle fibers are at the optimum length for force generation (i.e. Lo), and as the diaphragm contracts to TLC, a progressive increase in distance from Lo occurs in each of the sarcomeres. Therefore, if there were no chronic muscle length adaptations to hyperinflation, the contribution of the diaphragm to inspiratory pressure as lung volume increases from FRC to TLC would be severely reduced. In seminal experiments in the early 1980s, two research
groups (11, 12, 44, 66) demonstrated that greater than three months after intra-tracheal administration of elastase to the hamster, they developed marked thoracic hyperinflation and the maximum tension generated by these hamster diaphragm fibers showed a prominent shift to the left of the length-tension relationships; i.e., maximum tension occurred at a shorter fiber length than in control hamsters. Moreover, the number of sarcomeres in series in the diaphragms of these emphysematous hamsters was markedly reduced in a manner such that the length-tension relationships of the emphysematous diaphragm sarcomeres did not differ from controls (who had been given intra-tracheal injections of saline). At the present time, there has been widespread confirmation of these findings in experimental animals, but the relevance of these studies to humans with COPD is still somewhat uncertain (2, 57). Additionally, we do not know the molecular mechanism effecting this phenomenon. Therefore, we suggest that the various signaling and proteolytic diaphragm pathways eliciting the sarcomere deletions in the elastase-treated group can be elucidated by serial observations during the time that this remodeling is occurring; e.g., first three months after intra-tracheal instillation of elastase or saline. We refer the reader to our prior review (10) for a more detailed discussion of this topic.

**Increased proportion of slow-twitch fibers.** Over the past decade, multiple investigators (27-30, 40) have demonstrated that the diaphragms of patients with severe COPD exhibit an increased proportion of slow-twitch fibers and decreases in the
proportion of fast-twitch fibers. Figures 1A and 1B show representative slides prepared from diaphragm biopsies followed by histochemical staining for myosin heavy chain (MyHC)-determined fiber-types (6). The section from COPD patients (i.e., Figure 1B) shows an increased proportion of slow fibers and a decrease in fiber size of both type I and II fibers when compared to the control section (i.e., Figure 1A). Since it appears that this COPD-related muscle fiber remodeling is due to fast fibers changing their phenotype to slow fibers, we will refer to this aspect of remodeling as fast-to-slow-fiber transformation. Additionally, biochemical analyses of diaphragm homogenates indicated that the severe COPD diaphragms had higher expression of the slow myosin heavy chains as well as the slow isoforms of myosin light chains, troponins, and tropomyosins, whereas diaphragm fibers from control subjects had higher percentages of the fast isoforms of these proteins (28). These data suggest that COPD diaphragms had a higher proportion of slow-twitch motoneurons and a decreased proportion of fast-twitch motoneurons.

Since slow-twitch motoneurons are more fatigue-resistant than fast-twitch motoneurons (31), this feature of remodeling suggests that the diaphragms of severe COPD patients should be more fatigue-resistant than control diaphragms. (We recognize that this statement pertains only to in vitro fatigue measurements using the nerve-muscle preparation as summarized by Leiber et al (31). However, in vivo, fatigue can be initiated at many points in the motor pathway from cortical neurons to various
intracellular processes in the myofiber. A discussion of this topic is contained in another article in this series entitled “neuromotor control of skeletal muscle in COPD.” However, considering only diaphragmatic motoneurons, the maximum isometric force generated by slow motoneurons is less than that of fast-twitch motoneurons (17); therefore, this postulated increase in fatigue-resistance comes at the expense of decreased strength.

**Mechanisms of fast-to-slow fiber transformation. Calcineurin.** Calcineurin (Cn) is a Ca\(^{2+}\)/calmodulin-regulated protein phosphatase that acts on transcription factors that were first noted in the nucleus of activated T-cells; indeed the abbreviation NFAT (nuclear factor of active T cells) is used to describe these factors. Cn is a heterodimer of catalytic (CnA) and regulatory (CnB) subunits. Both Cn subunits and NFATs are comprised of various isoforms. All of the four Cn-dependent NFATs (7) are expressed in skeletal muscle.

Several lines of evidence indicate that the transcription factors of the NFAT family act as nerve activity sensors in skeletal muscle and control activity-dependent fiber-type specialization (9, 32). First, when NFATc1-GFP fusion protein is expressed in isolated, unstimulated fibers from adult mouse flexor digitorum brevis, a predominantly fast-twitch muscle, it shows a cytoplasmic localization but translocates to the nucleus in fibers stimulated with a low-frequency pattern typical of slow motor units (9). Second, in vivo studies reveal that NFATc1-GFP has a predominantly
cytoplasmic localization in the fast tibialis anterior muscle but a predominantly nuclear localization in the slow soleus muscle (70). Last, NFATc1 nuclear import is rapidly induced in fast tibialis anterior muscle fibers by low frequency electrical stimulation, whereas nuclear export is rapidly induced in slow soleus muscle fibers by inactivity consequent to denervation or anaesthesia.

Figure 3A presents a schematic illustrating the concept that the increased neural activity to the COPD diaphragm changes the cytoplasmic calcium activity from high amplitude pulses of short duration to low amplitude pulses of long duration. The literature indicates that due to this change, the activated calcineurin then binds to a combination of transcription factors that code for various aspects of the “slow fiber developmental” program. It is important to note that these slow program transcription factors are essential to the fast-to-slow fiber type myosin heavy chain remodeling since calcineurin alone does not activate this transformation (9). This postulated mechanism is important in explaining the finding that fast-to-slow MyHC transformation in the COPD diaphragm is accompanied by similar fast-to-slow transformation in all of the following myofibrillar proteins: myosin light chains 1, 2, 3; alpha and beta units of tropomyosin, and troponins C, T, and I (28).

**Increased capacity for oxidative metabolism.** Due to the increase in type I fibers and the accompanying decrease in type II fibers (see above), one would expect an increase in oxidative capacity and a decrease in glycolytic capacity in the COPD
diaphragm. Indeed, the studies of Wijnhoven (79) et al demonstrate that diaphragms from COPD patients exhibit increases in 3-hydroxyacylCoA-dehydrogenase (HADH—biomarker for β-oxidative capacity), and those of Sanchez and colleagues (59) show decreases in both lactate dehydrogenase and hexokinase (biomarkers for glycolytic capacity). Importantly, Levine et al (27) used quantitative histochemical determinations of succinic dehydrogenase (SDH) activity (77) to compare the fiber-type specific SDH activity in diaphragm biopsies of COPD and controls—see Figures 1C, 1D, and 1E. A comparison of Figures 1C and 1D indicates that COPD diaphragms exhibited appreciably greater SDH activity than control diaphragms. Moreover, Figure 1E shows that in each of the fiber types (i.e., I, Iia, and IIax), SDH activity in COPD diaphragms was increased approximately 100% over controls. These latter observations indicate that the increase in oxidative enzyme activity in COPD diaphragm homogenates cannot be solely due to the fast-to slow fiber type transformation; rather, they suggest that in severe COPD patients, all fiber types become more oxidative.

Other investigators have obtained more direct mitochondrial measurements on diaphragm biopsies from COPD patients and controls. Wijnhoven et al. (79) noted an increase in mitochondrial electron transport system complexes III and IV and a statistically significant increase in the capacity for pyruvate oxidation as the severity of COPD increased. Moreover, the electron microscopy study of Orozco-Levi et al (45) demonstrated that compared to controls, the COPD diaphragm exhibited an increase in
volume fraction of mitochondria; indeed, these workers showed a statistically significant negative correlation between volume fraction of mitochondria and FEV1.0 (expressed as % predicted). Last, Ribera and co-workers (56) carried out studies on in situ mitochondrial preparations prepared from COPD and control diaphragm biopsies, and they noted that COPD mitochondria exhibited increases in both maximum oxygen consumption and acceptor control of respiration. Therefore, their work suggests that COPD mitochondria exhibited increased oxidative capacity as well as increased coupling of oxidative phosphorylation.

Recent work by Rasbach et al. (55) has demonstrated that a necessary component of the PGC-1α induced switch to oxidative fiber types is the PGC-1α induction of hypoxia inducible factor, HIF-2α. This pathway is also dependent on the activity of sirtuin-1 (SIRT1), a redox sensitive (NAD+-dependent) deacetylase enzyme (69, 78). The sensitivity of these systems to both tissue oxygenation and the redox state (NAD+/NADH) of the cytosol raises interesting possibilities for future research in understanding why respiratory muscles and limb muscles undergo different fiber type programs in COPD.

**Fast-to-slow isoform shift in sarcoendoplasmic reticulum calcium ATPase (SERCA).** Previous workers have noted that after the MyHC, SERCA uses more ATP than any other ATPase enzyme during isometric contraction (67). Additionally, investigators in the area of muscle remodeling have indicated that a decrease in
diaphragm ATP utilization would help prevent low frequency fatigue of this organ. Therefore, the Philadelphia group (43) hypothesized that a fast-to slow isoform transformation of SERCA would be present in the diaphragms of patients with severe COPD because it would decrease ATP utilization.

Using isoform specific antibodies (43), they showed that compared with control diaphragms, the severe COPD diaphragms exhibited a large decrease in fibers expressing only SERCA 1 (the fast isoform), a large increase in hybrid fibers containing both isoforms of SERCA, and a small increase in fibers containing only SERCA2 (the slow isoform). These features of COPD remodeling are shown in Figures 2A-2D. Additionally, immunoblot experiments carried out on diaphragm homogenates demonstrated that severe COPD diaphragms expressed only one-third the SERCA1 content noted in control diaphragms, whereas the two groups did not differ with respect to SERCA 2 content (43). The combination of these histological and immunoblot results are consistent with the hypothesis that diaphragm remodeling elicited by severe COPD is characterized by a fast-to-slow SERCA isoform transformation.

Additionally, using serial section histochemistry, Nguyen et al (43) determined the co-expression pattern of SERCA and the myosin heavy chain (MyHC) and the results of these studies are shown in Figure 2E; it shows coordinate remodeling of SERCA and MyHC isoforms and these data are once again consistent with the hypothesis that diaphragm remodeling elicited by severe COPD should decrease ATP
utilization by diaphragm myofibers relative to control fibers at a given time tension index.

Last, these changes in SERCA expression should decrease the rate of pumping calcium from the myoplasm into the sarcoplasmic reticulum (at the termination of the contractile phase) and this should shift the force-frequency curve to the left; i.e., this will permit a given level of force generation with a lower frequency of stimulation which in turn will reduce the ATP cost of calcium pumping. These phenomena are known to occur in laboratory experiments, and they may be pertinent to the in vivo diaphragm as well. Nonetheless, more studies are needed to elucidate this area.

**Oxidative stress.** Oxidative stress is commonly defined as an imbalance of pro-oxidants and antioxidants with this inequality documented by the accumulation of oxidized molecules in tissue (23, 53). To evaluate the possibility that diaphragms of severe COPD patients exhibited oxidative stress, Barreiro and colleagues (3) compared diaphragm biopsies from groups of subjects afflicted with severe COPD, moderate COPD, or no COPD (i.e., controls) and they noted that severe COPD patients exhibited higher levels of protein carbonyls compared to controls. Indeed, in the two COPD groups, they noted a statistically significant negative correlation between the carbonylation level and airway obstruction (assessed by % predicted FEV1.0). In contrast to these finding, Barreiro and co-workers (3) noted no differences in 3-nitrotyrosine levels between the diaphragms of severe COPD subjects and those of
controls and they also noted no up-regulation of any of the three nitric oxide synthases in COPD diaphragms. These latter data indicate that nitrosative stress does not occur in the COPD diaphragm. More importantly, this paper demonstrates that oxidative stress does occur and the severity of this latter stress is related to the degree of airway obstruction.

Subsequently, from the same laboratory, Marin-Corral et al (36) noted that four proteins (i.e., MyHC, creatine kinase, α1-sarcomeric actin, carbonic anhydrase) were carbonylated in all diaphragms from each group; however they noted no difference in the levels of carbonylation between controls and moderate COPD. In contrast, severe COPD diaphragms exhibited a five-fold increase in MyHC carbonylation above controls and this was accompanied by a decrease in non-carbonylated MyHCs to one third of that noted in controls. These data suggest that in vivo, the MyHC was being rapidly carbonylated and this carbonylated MHC was undergoing rapid degradation. The magnitude of the observed decreases in non-carbonylated MyHC appeared more than sufficient to account for the decreases in Pdi max and Pi max noted in these severe COPD patients. Importantly, this decrease in functional MyHC is consistent with our overall hypothesis that the severe COPD diaphragm exhibits decreased strength when compared to control diaphragms.

**Myofiber atrophy.** Muscle fiber cross-sectional area (CSA) is commonly used to quantify the magnitude of fiber atrophy. Multiple studies (27-29) by the Philadelphia
group indicate that the CSAs of all fiber-types are decreased approximately 30-to-40% and the recent paper by Testelmans et al. (68) show similar myofiber atrophy in the severe COPD diaphragm. Skeletal muscle fiber size depends upon a dynamic balance between anabolic (hypertrophic) and catabolic (atrophic) processes. Figure 3B (taken from reference (60)) indicates that a decrease in the phosphorylation level of cytoplasmic protein kinase B (Akt) elicits myofiber atrophy by eliciting increases in proteolysis and decreases in protein synthesis. First, the decrease in Akt phosphorylation level effects increased binding of forkhead box O (FOXO1) to nuclear DNA (including the consensus sequence coding for Atrogin-1 and MuRF-1). This results in increased transcription of Atrogin-1 and MuRF-1 that increase the proteolytic activity of the ubiquitin-proteasome pathway (UPP) thereby increasing protein degradation via the UPP. Additionally, Figure 3B shows that a decrease in the phosphorylation level of Akt is associated with a decrease in protein synthesis due to dephosphorylation of GSK, mTOR, and S6K.

It is generally accepted that the atrophy mechanism (presented above and in Figure 3B) is operative in COPD diaphragm fibers exhibiting atrophy. However, the literature does not contain any papers documenting increased activity of this pathway in human COPD diaphragms. However, we (26) recently demonstrated that this pathway plays an important role in effecting the marked fiber atrophy noted by human
diaphragm myofibers in ventilator-induced diaphragm atrophy. We believe that similar types of measurements are needed on COPD diaphragm biopsies.

Additionally, the recent work by Testelmans et al (68) on human COPD diaphragms indicates that several other signaling pathways (i.e., myostatin, NF-Kappa B) are involved in the myofiber atrophy of moderate to severe COPD. A proposed mechanism for myostatin-induced atrophy modified from reference (33) is presented in Figure 4. It shows that myostatin up-regulates components of the ubiquitin proteolysis system, including Atrogin-1, through a FoxO1- and Smad3-dependent signaling mechanism. Enhanced activation of the ubiquitination system leads to degradation of the majority of sarcomeric proteins, which are required for normal muscle growth and development. Myostatin also inhibits protein synthesis by decreasing the phosphorylation of Akt and thereby reduces protein synthesis, and this leads to enhanced progression of skeletal muscle atrophy. Last, despite the myostatin-induced proteolysis, the IGF-1-PI3K pathway can still stimulate AKT; indeed, laboratory experiments (18, 71) indicate that sufficiently intense stimulation of AKT by this growth pathway can overcome the myostatin effects.

Additionally, in their landmark study, Testelmans and co-workers (68) noted changes in the NF Kappa B signaling pathway(s) that can effect myofiber atrophy in COPD diaphragms (22). Specifically, Testelmans and co-workers reported decreased cytoplasmic levels of the inhibitor proteins IκBα and IκBβ, decreased NF-κB P-50 DNA
binding capacity, and increased Atrogin-1 transcripts. These data indicate that the NF-
κB (i.e., the systemic pathway) was up-regulated and played a role in the atrophy of the
diaphragm myofibers.

Further, Testelmans et al. reported decreases in mRNA coding for myoD as well
as decreases in myoD protein levels that were accompanied by no changes in myogenin
at either the transcript or protein level. They also noted a negative correlation between
the proportion of slow myofibers and the myoD protein levels. We agree with these
authors that the remodeling changes in myoD are probably related to the fast-to-slow
fiber type transformation.

**Myosin heavy chain and contractile remodeling noted in mild-to-moderate COPD.** As previously noted, the earliest studies on remodeling of the COPD diaphragm focused on moderately severe to severe COPD (28, 40). However, relatively early in the studies on remodeling of the human diaphragm, Ottenheijm et al. (50) used the permeabilized single fiber preparation to test the hypothesis that contractile abnormalities exist in mild-to-moderate COPD. Specifically, they compared fiber types I and IIa from diaphragm biopsies of these COPD patients with those of normal controls. Among other observations, Ottenheijm and colleagues noted that in comparison to controls, COPD fibers exhibited reduced maximum force generation per unit CSA (i.e., specific force); and reduced MyHC content and concentration. These findings suggested that decreases in MyHC concentration (per half sarcomere)
accounted for the decreases in specific force. They also performed biochemical measurements on diaphragm homogenates and noted that diaphragms from COPD patients contained high levels of ubiquitin-protein conjugates. Ottenheijm et al concluded that the contractile abnormalities in mild to moderate COPD were largely due to increased degradation of the myosin heavy chain by an up-regulation of the ubiquitin-proteasome pathway, and they provided strong support for this conclusion in a subsequent study(49). However, these authors do not present any history of smoking or smoke exposure data on their patients. This is important because recent work by Barreiro et al (4) raises the possibility that smoking-induced oxidative stress per se—and not COPD—may have elicited the findings of Ottenheijm et al.

VASTUS LATERALIS

In this segment, we will discuss the muscle fiber remodeling that occurs in the VL muscle of COPD patients, under the following headings: (a) decrease in proportion of slow-twitch fibers; (b) decreased activity of oxidative pathways (c) oxidative and nitrosative stress; and (e) myofiber atrophy.

**Decreased proportion of slow-twitch fibers.** Gosker and colleagues (19) carried out a systematic review and meta-analysis of fiber-types in patients with various severities of COPD; they noted a slow-to-fast fiber-type shift in COPD patients (i.e., a decrease in the proportion of type I fibers and an increase in the proportion of type II
fibers). Indeed, after analyzing 11 previous studies, their data indicate that in 60-70 year old males, a proportion of type I fibers <27% and/or a proportion of type IIx > 29% should be considered pathological. Importantly, these workers concluded (in this subset of COPD patients) that the proportion of type I fibers was highly negatively correlated with the severity of airway obstruction assessed by either % predicted FEV1.0 or the ratio of FEV1.0 to FVC expressed as a percent. These latter observations have been confirmed by several other investigators (42, 73).

After reviewing the literature, Caron et al (8) suggested that these slow-to-fast vastus lateralis fiber-type changes cannot be due to age. In 2001, Gea and colleagues (15) concluded that much greater COPD-induced muscle remodeling occurs in the lower limbs than in the upper limbs. In contrast to the marked remodeling noted in the lower limbs, they concluded that upper limb muscle structure and function were relatively well preserved due to the maintenance of some daily activities involving the arms or even the use of some of these muscles in ventilatory efforts. While this postulate of Gea et al is intuitively attractive, over a decade later, we still do not have adequate biopsy-derived information on the upper limb muscles—in COPD patients—to test their hypothesis.

**Mechanisms mediating slow-to-fast fiber transformation.** Shi et al (64) carried out a seminal series of experiments to test the hypothesis that activation of the ERK 1/2 subfamily of the MAPK signaling pathway mediated a slow to fast fiber transformation,
whereas blockade of this pathway elicited a fast-to-slow fiber shift. First, in tissue culture experiments, these investigators demonstrated that pharmacological blocking of the ERK1/2 pathway increased slow-twitch fiber-type specific reporter activity and repressed that associated with the fast-twitch fiber phenotype; in contrast, over-expression of a constitutively active ERK2 had an opposite effect. Second, inhibition of ERK signaling in cultured myotubes increased slow-twitch fiber specific protein accumulation while repressing those characteristic of fast-twitch fibers. Third, over-expression of MAP kinase phosphatase-1 (MKP1) in mouse and rat muscle fibers containing almost exclusively type IIb or IIx fast MyHC isoforms induced de novo synthesis of the slower, more oxidative types IIa and I MyHCs in a time-dependent manner. Similar in vivo experiments were also performed and this work reveals the conversion of fast fibers to the slower phenotype as indicated by the up-regulation of slow reporter gene activity and down-regulation of fast reporter activity. Fourth, they noted that activation of ERK2 signaling induced up-regulation of the fast-twitch fiber program in the slow-twitch soleus muscle. These experiments of Shi and colleagues (64) demonstrate that the ERK1/2 subfamily of the MAPK family can elicit a slow-to fast fiber-type transformation in experimental studies. Therefore, a similar mechanism may be responsible for the slow-to-fast fiber-type transformation noted in the VL of COPD patients, whereas blockade of this pathway may eliminate or attenuate the slow-to-fast fiber transformation in the VL of patients with COPD.
**Decreased activity of oxidative pathways.** Several studies have demonstrated that the activity of oxidative enzymes such as 3-hydroxyacyl-CoA-dehydrogenase (HADH) (21, 34, 35) and citrate synthase (34, 35), is reduced in the vastus lateralis of patients with moderate to severe COPD. Cytochrome c oxidase, a component of the electron transport system, is also decreased in these individuals (21), although the current literature is not in full agreement in this regard (54, 61). Glycolytic enzymes are not unambiguously affected in the presence of COPD (21). However, when oxidative-to-glycolytic enzymatic ratios are considered, predominance of a glycolytic metabolism appears to be a common feature in the quadriceps of patients with COPD (21). This metabolic pattern of the lower limbs differs from what is seen in the upper extremity muscles in which an increased citrate synthase and lactate dehydrogenase activity is observed in severe COPD patients (16). These observations corroborate the putative importance of local factors in the development of muscle dysfunction in the VL of COPD patients.

**Oxidative and nitrosative stress.** The relationships among oxidative stress, nitrosative stress, and exercise is important since regular exercise is usually a component of rehabilitation programs for COPD. We believe that these relationships can be best understood by comparing two studies carried out three years apart in patients with severe COPD by the Barcelona group. In the first study, Barreiro and colleagues (5) analyzed biopsies of severe COPD and control VL before and after a three
week cycle ergometer exercise training program. Prior to the exercise program, protein carbonylation levels, hydroxynonenal (HNE)-protein adducts, superoxide dismutase activity (SOD), and inducible nitric oxide synthase (NOS) were higher in patients than in controls. Importantly, 3-nitrotyrosine immunoreactivity levels were also statistically significantly increased in the quadriceps of patients compared with controls. In patients, the 3-week daily training period induced a significant rise in inducible NOS levels and a four-fold increase in protein nitration. Specifically, the proteins that underwent nitration were some involved in glycolysis (enolase, aldolase A, triosephosphate isomerase); ATP distribution (creatine kinase); muscle oxygen transfer (myoglobin); carbon dioxide hydration (carbonic anhydrase III); and DNA repair (uracil DNA glycosylase). Additionally, the contractile protein α-1 actin was nitrated only in patients exhibiting muscle loss, whereas superoxide dismutase (SOD) only increased in controls. These findings argue against including daily cycle ergometer training as part of a rehabilitation program for patients with severe COPD.

However, in a 2012 study by Rodriguez et al (58), the Barcelona groups tested the hypothesis that high-intensity exercise training of long duration (i.e., 8 weeks) on the cycle ergometer does not cause a deterioration in muscle redox status of severe COPD patients. At baseline, in comparison to controls, COPD subjects exhibited greater levels of both muscle protein carbonylation and muscle protein nitration. Nonetheless, following the eight week training period, the levels of both protein carbonylation and
nitration did not change in either COPD or controls. Moreover, these workers noted that both COPD and controls exhibited increases in peak work-rate, peak oxygen consumption, distance walked in 6 minutes, and decreases in arterial lactate concentration. Rodriguez and colleagues concluded that high intensity cycle ergometer exercise training of long duration improves exercise capacity in patients with severe COPD in the absence of any increases in muscle protein oxidation or muscle protein nitration. Therefore, the results of this latter study indicate that daily high intensity exercise training for greater than 8 weeks with a cycle ergometer should be included in rehabilitation programs for patients with severe COPD.

We postulate that the combination of these two studies with disparate results have physiological relevance. That is, the dose response curve for their exercise intervention could be time-dependent; early response seems deleterious, but a longer training period engages additional mechanisms that are adaptive and/or mechanisms are activated to prevent the deleterious aspects of muscle remodeling. We believe that uncovering the physiology underlying these mechanisms is important and warrants further investigation.

Roles of inactivity and pulmonary pathology in producing oxidative stress and decreases in oxidative capacity in VL. Severe COPD patients are more sedentary than healthy age and sex matched subjects and therefore the possibility exists that inactivity per se might account for some of the remodeling noted in the VL of these COPD
patients. Since muscle inactivity can elicit remodeling (51, 52), Mattson et al (37, 38) developed a protocol for producing elastase-induced emphysema in hamsters that elicited 100% increases in lung volumes but the carefully measured activity level of these hamsters did not differ from those of controls. Surprisingly, these emphysematous hamsters exhibited decreases statistically significant decreases in VL citrate synthase (a biomarker for oxidative metabolism), increases in malondialdehyde (a biomarker for lipid peroxidation), and statistically significant decreases in glutathione peroxidase (an antioxidant buffer). Although the authors do not demonstrate a mechanistic explanation for these results, their publications raise the possibility that—at least in this animal model of emphysema—lung pathology can elicit decreases in oxidative capacity and oxidative stress in leg muscles.

Myofiber atrophy. A landmark study by Fermosele and colleagues (13) has provided much human information about the relationships among severity of COPD, muscle wasting, protein carbonylation, redox status, ubiquitin proteasome pathway (UPP), superoxide anion production; and FOXO and NF-Kappa B transcription factors. They carried out comparisons of VL biopsy features among the following three groups: (a) a group of severe COPD patients without muscle wasting, (b) a group of severe COPD patients with muscle wasting, and (c) an age-matched healthy control group. Compared to controls, in the VL of muscle-wasted COPD patients, levels of protein carbonylation, oxidation of MyHC and myonuclei, superoxide anion
production, superoxide dismutase, total ubiquitin-protein conjugates, E214k, atrogin-1, FoxO1, and p65 were higher, while the contents of MyHC, creatine kinase, carbonic anhydrase-3, myogenin, and fast-twitch fiber size were decreased. Importantly, in non-wasted COPD patients, whereas MyHC was more oxidized than in controls, its content was preserved. Muscle inflammation and glutathione levels did not differ between patients and controls. In all patients, muscle structure abnormalities were increased, while muscle force and exercise capacity were reduced.

Fermosele et al (13) concluded that in severe COPD, while muscle oxidative stress occurs regardless of the presence or absence of muscle wasting, protein ubiquitination and loss of MyHC were enhanced only in those patients exhibiting muscle atrophy. These investigators interpreted their data as evidence that oxidative stress does not directly modulate muscle loss in severe COPD patients. We believe that equally important conclusions from this study are that the UPP appears to mediate the protein degradation and the transcription factors eliciting this up-regulation of the UPP are FOXO1 and the P65 protein of the NF-Kappa B signaling pathway.

LIMITATIONS OF THIS MINI-REVIEW

Due to space constraints, we limited our discussion to selected topics in the area of COPD-induced remodeling of the diaphragm and VL. We believe that virtually all other topics may be covered in other articles in this Highlighted Topic series. However, we briefly mention two topics that may not be discussed in other articles in this series.
The first is recent developments in the area of skeletal muscle angiogenesis, vascular endothelial growth factor (VEGF) (1, 20, 76, 80) and relationship between myofibers and capillaries (8), whereas the second is the vulnerability of the diaphragm to injury (46, 48, 63) and the mechanisms involved in repair of this pathology. We provide some guidance to the interested reader by the references cited in this paragraph.

CONCLUSIONS.

We have presented the remodeling that occurs in the costal diaphragm and vastus lateralis of COPD patients. Surprisingly, some aspects of remodeling in these two muscles occur in opposite directions. For example, the COPD diaphragm is characterized by a fast-to slow fiber transformation, whereas the VL of these types of patients undergoes a slow-to-fast fiber transformation. Hopefully, we can exploit these discrepant aspects of muscle remodeling in the same patient to more fully arrive at cellular and molecular mechanisms in human experiments.
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Due to space constraints, we could only reference some of the laudatory papers in the topics that we covered; therefore, we apologize to our many colleagues whose work was not presented. During the preparation of this manuscript, SL was supported by a grant from the NHLBI (HL078834).


Figure 1: Staining of serial diaphragmatic sections for fiber typing (A and B) and succinate dehydrogenase (SDH) activity (C and D). Labeled fibers in SDH sections are the same fibers labeled in the histochemical myosin ATPase (hATPase) sections (6). Calibration bars represent 50 µm. A and B: Fiber-type-stains. Dark stained fibers are type I fibers, light stained fibers are type IIa fibers, and intermediate-staining fibers are type IIax fibers. C and D: Quantitative SDH stain. The intensity of the stain is directly related to SDH activity; i.e., the darker the fiber, the greater the SDH activity (77). E. Fiber-type specific activities of SDH. In each of the fiber types (i.e., I, IIa, and IIax), SDH activity in COPD diaphragms was increased approximately 100% over controls (****, P<0.0001). Reproduced, with permission, from reference (27).

Figure 2: Serial sections from control (A & C) and COPD diaphragms (B & D). A and B are stained with monoclonal antibody to the slow isoform (SERCA2) of sarcoendoplasmic reticulum Ca\(^{2+}\)ATPase (SERCA), whereas C and D are stained with monoclonal antibody to the fast SERCA isoform (SERCA1). In all panels, the light fibers are the ones that reacted with the antibody. The figure shows that both diaphragms contain three types of fibers; representative fibers of these types are indicated by the following
symbols: *, fiber expressing only SERCA1; o, fiber expressing only SERCA2; □, fiber expressing both SERCA isoforms (i.e., a hybrid fiber).

Comparison of Figures 2A and 2B indicates that the COPD diaphragm contains a larger proportion of SERCA2 fibers than the control diaphragm, whereas comparison of Figures 2C and 2D indicate that the control diaphragm contains a larger proportion of SERCA1 fibers than the COPD diaphragm. Calibration bars represent 50 µm. 2E: Comparison of COPD and control diaphragms with respect to SERCA and myosin heavy chain (MyHC) co-expression patterns: These data indicate coordinate changes in expression of MyHC and SERCA. A multivariate analysis of variance showed highly statistically significant differences between COPD and control data sets and the differences between groups for each of the comparisons is indicated by the asterisk code: *P < 0.05; **P <0.01; ***P <0.001. Reproduced, with permission, from reference (43).

Figure 3: A. Model for a calcineurin-dependent pathway linking specific patterns of motor nerve activity to distinct programs of gene expression that establish phenotypic differences between slow and fast myofibers. MEF2 is shown to represent the requirement for collaboration between activated NFAT proteins and muscle-restricted transcription factors in slow-fiber-specific gene transcription, but other proteins (not shown) also are likely
to participate. Reproduced, with permission, from reference (9).

Summary of the roles of the IGF-1/AKT Pathway and FOXO in muscle atrophy (right image) and hypertrophy (left image). The schematics indicate that a decrease in Akt phosphorylation elicits increased binding of forkhead box O (FOXO1) to nuclear DNA that results in increased transcription of Atrogin-1 and MuRF-1 (i.e., ubiquitin ligases) and thereby increases protein degradation. Additionally, a decrease in the phosphorylation level of Akt is associated with a decrease in protein synthesis due to dephosphorylation of GSK, mTOR, and S6K. Factors and pathways in bold are activated. Reproduced, with permission, from reference (60).

**Figure 4:** Overview of the myostatin pathway. The schematic shows that myostatin (Mstn) up-regulates genes coding for components of the ubiquitin-proteasome pathway via FOXO and Smad-3 signaling pathways. Additionally, the increased level of FOXO transcription factors decreases protein synthesis (see Figure 3B). Reproduced, with permission, from reference (33)—See text for description.
Mstn → ActRIIB

Smad3 → Akt

pSmad3 → Smad4

pSmad3/4 Complex → Nucleus

pSmad3/4 → FoxO

Ubiquitination of sarcomeric proteins and reduced protein synthesis rate → Skeletal Muscle Wasting