HIGHLIGHTED TOPIC | The Respiratory Muscles in Chronic Obstructive Pulmonary Disease

Respiratory muscle fiber remodeling in chronic hyperinflation: dysfunction or adaptation?

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Clanton TL, Levine S. Respiratory muscle fiber remodeling in chronic hyperinflation: dysfunction or adaptation? J Appl Physiol 107: 324–335, 2009. First published April 9, 2009; doi:10.1152/japplphysiol.00173.2009.—The diaphragm and other respiratory muscles undergo extensive remodeling in both animal models of emphysema and in human chronic obstructive pulmonary disease, but the nature of the remodeling is different in many respects. One common feature is a shift toward improved endurance characteristics and increased oxidative capacity. Furthermore, both animals and humans respond to chronic hyperinflation by diaphragm shortening. Although in rodent models this clearly arises by deletion of sarcomeres in series, the mechanism has not been proven conclusively in human chronic obstructive pulmonary disease. Unique characteristics of the adaptation in human diaphragms include shifts to more predominant slow, type I fibers, expressing slower myosin heavy chain isoforms, and type I and type II fiber atrophy. Although some laboratories report reductions in specific force, this may be accounted for by decreases in myosin heavy chain content as the muscles become more oxidative and more efficient. More recent findings have reported reductions in Ca2+ sensitivity and reduced myofibrillar elastic recoil. In contrast, in rodent models of disease, there is no consistent evidence for loss of specific force, no consistent shift in fiber populations, and atrophy is predominantly seen only in fast, type IIX fibers. This review challenges the hypothesis that the adaptations in human diaphragm represent a form of dysfunction, secondary to systemic disease, and suggest that most findings can as well be attributed to adaptive processes of a complex muscle responding to unique alterations in its working environment.

diaphragm; fiber type; sarcomere; skeletal muscle; emphysema

LENGTH PLASTICITY

The diaphragm, like all other muscles, exhibits a characteristic length-tension relationship analogous to the Frank-Starling curve for the heart. As length is increased in the relaxed muscle, a passive length-tension curve can be generated, representing the inherent static, viscoelastic properties of the tissue. Such a curve can be seen in Fig. 1A, adapted from Ref. 85. As the muscle is stretched from a slackened state and then stimulated, there is a rise in maximum active force, over and above passive force, reaching a peak value. The muscle length at which peak active force is obtained is referred to as the muscle’s optimum length (L0), most often equivalent to resting length in its working environment. It is generally considered equivalent to the average sarcomere length, resulting in maximum myosin-actin interaction, as shown in Fig. 2B, although clearly other molecular mechanisms are involved.

For the costal diaphragm, L0 has been shown to roughly correspond to the length at functional residual capacity (FRC), although this may vary somewhat with body position and...
From FRC, as lung volume is increased, either passively or actively, the diaphragm is shortened and moves down its length-tension relationship. This is shown in Fig. 1, C and D, adapted from Ref. 3. Therefore, if there were no chronic muscle length adaptations to chronic hyperinflation, the diaphragm would remain in a preshortened or slackened state during tidal breathing, and its capacity to contribute to inspiratory pressure would be reduced. An important question is whether the diaphragm, in a patient with a chronically hyperinflated chest wall, fully adapts to counteract this effect.

It has been well established from the work of Goldspink and colleagues in the 1970’s that, when limb muscles are chronically shortened or lengthened in an immobilized position, they respond by changing the number of sarcomeres in series, thus readjusting their $L_o$ to a new operating length, as reviewed in Ref. 27 and 45. Chronic lengthening responses, i.e., adding sarcomeres in series (sarcomerogenesis), are necessary for...
rapid limb growth in all organisms and are important in preventing length-induced injuries during growth phases. The early studies of responses to chronic lengthening and shortening could draw only limited conclusions, because the limb muscles being tested neither were allowed to change length with normal movement, nor were they activated periodically. However, subsequent work, using surgical reinsertion of limb muscles (6, 45), has shown that fiber length adaptation could be specifically attributed to chronic changes in overall length and not to changes in activation or periodic muscle excursion with movement.

The signaling mechanisms involved in chronic sarcomere deletion or addition are not well understood, but the two processes may represent fundamentally different molecular events. Removing sarcomeres in series requires increased protein degradation and possibly myonuclear apoptosis, whereas sarcomerogenesis is believed to occur through migration of satellite cells, fusion, development of mini-sarcomeres, and production of new nuclear domains (8). Although the latter is thought to occur near the myotendinous junctions (17), there is little evidence for a localized site for chronic shortening adaptations, nor are the signaling mechanisms clearly understood.

In the early 1980s two research groups, one at Meakins-Christie Laboratories in Montreal (20, 21) and the other at Case Western University in Cleveland (35, 85), reported almost simultaneously that the diaphragms of hamsters exposed to elastase-induced emphysema for 6–18 mo demonstrated a net diaphragm muscle shortening due to loss of sarcomeres in series. These changes shifted the \( L_o \) of the diaphragm to a shorter length, thus preserving contractile force at hyperinflated lung volumes, as shown in Fig. 1A. The two studies differed in that Farkas and Roussos (21) also described small reductions in average sarcomere length from \( 2.6 \) to \( 2.5 \mu m \), whereas Supinski and Kelsen (85) saw no such changes (Fig. 1B). Subsequent work by Poole and colleagues (72), in a similar animal model, addressed this discrepancy and found no differences in diaphragm sarcomere length in emphysematous hamsters, but confirmed the changes in overall fiber length and sarcomere deletion. More recent work has described diaphragm fiber shortening and sarcomere deletion in a rat model of elastase-induced emphysema (81, 82).

The fiber length changes observed in animal models of emphysema have been considered prototypes of adaptation to chronic human hyperinflation, although adaptation occurs over several decades in humans compared with several months in animals. The idea that nearly complete length adaptations of the diaphragm occur in humans was supported for many years by an observation that diaphragm twitch force in hyperinflated chronic obstructive pulmonary disease (COPD) patients is similar to that of the normal population when normalized to lung volume (83). However, whether diaphragm fiber lengths physically shorten by deletion of sarcomeres in series in chronically hyperinflated humans has never been clearly established, in part because of the difficulty in accurately measuring sarcomere length in vivo, at FRC. It is a critically important problem to understand because of the obvious implications for lung volume reduction surgery, as discussed in the accompanying review in this series (19). Some studies have measured human diaphragm length using computed tomography, coupled with three-dimensional modeling, e.g., Ref. 9, combinations of chest wall circumference and ultrasound of the area of apposition, e.g., Ref. 28, or by measuring AP and lateral chest radiographs, e.g., Ref. 3, as shown in Fig. 1D. Although all have found a shorter net diaphragm length at FRC or residual volume (RV) in emphysema, when costal diaphragm length is adjusted to a predicted lung volume, it is not distinguishable from that in normal subjects (9, 28). However, the problem with comparing the diaphragm lengths between normal subjects and COPD patients at the same lung volumes (RV and FRC) is that these are elevated in hyperinflated subjects, by definition (see Fig. 1C). Conversely, the problem with comparing the lengths following adjustment to predicted lung volumes is that there is no longer any reference point corresponding to an optimum sarcomere length. To illustrate, normalization of a COPD patient’s diaphragm length to a predicted FRC rather than the actual FRC could result in a lung volume well below the patient’s actual RV (Fig. 1D), where the diaphragm no doubt must be stretched far beyond anything close to its resting length. The idea that stretch of the diaphragm could account for the apparent normalized isovolume length in human emphysema is supported by three other observations: 1) the diaphragm at isovolume appears thinner in hyperinflated subjects (28); 2) the relationship between maximum transdiaphragmatic pressure (\( P_{di max} \)) and diaphragm length are shifted to stronger \( P_{di max} \) values at lower lengths in emphysema patients, as shown in Fig. 1D (3); and 3) autopsy measurements of diaphragm surface area consistently show reductions in length that cannot be attributed to changes in thickness (7). Because of these considerations and because of the striking improvements in diaphragm configuration and apparent length-force relationships after lung volume reduction surgery, e.g., Refs. 3, 29, the literature consensus at this time supports the idea that muscle fiber length must be reduced in severe emphysema patients by some degree of sarcomere loss, much like the responses seen in animal models. Although these adaptations serve to optimize force-generating capacity and are, in part, successful, with increasing dynamic hyperinflation, “acute on chronic” challenges ensue that continue to stress the optimal force/length relationships in many conditions for the patient.

Others have challenged the extent to which the diaphragm length adapts, by evaluating absolute sarcomere length in frozen biopsy specimens from COPD patients (59). They observed that average sarcomere length was shorter in emphysema than in comparable controls and that the greater degree of hyperinflation or disease severity corresponded with shorter sarcomere lengths. This suggests that human diaphragm does not fully adapt to length changes like the hamster or rat diaphragm (72, 85). However, muscle sarcomere length measurements from open-lung biopsy specimens are unlikely to be an accurate measure of in vivo sarcomere length, because the preload or lung volume at which the fiber is obtained is not controlled. Unless lung volume is rigidly controlled during thoracotomy or video-assisted thoracoscopy, it is likely that diaphragm length would approach that seen in supine total lung capacity, or at least at chest wall relaxation volume, unopposed by lung elastic recoil. Therefore, diaphragms would be artificially shortened from resting length, and it is difficult to predict how the chest wall mechanics in COPD might influence this. Nevertheless, this interesting observation, when coupled with our greater basic science understanding that not all types of limb skeletal muscles fully respond to chronic shortening by sarcomere deletion, as reviewed in Ref. 45, suggests the need.
for further investigation. Evaluation of biopsies taken at controlled and accurately measured lung volumes and diaphragm lengths, over a wide range of disease severities, will be necessary to resolve the issue completely.

**CHANGES IN SPECIFIC FORCE**

Maximum specific force is an indicator of the fundamental contractile state of the muscle. It is defined as the maximum tetanic force per cross-sectional muscle area. It is relatively independent of muscle length, but is dependent on myosin content, a measure of cross-bridge density (26). It is well known that patients with COPD and chronic hyperinflation exhibit reductions in maximum inspiratory pressures and reduced Pdimax, e.g., Ref. 4. This results in a lowered inspiratory effort, a measure of cross-bridge density (26). It is well established that specific force is a component of the systemic muscle mechanics, or whether there is some intrinsic loss of force, or specific force that is a component of the systemic disease. Despite years of work, the data are still not completely clear regarding this question, and the clinical implications are great. If respiratory muscles are inherently weak or dysfunctional, they could be responsive to therapy.

As mentioned, for many years, it was thought that force-generating capacity of the diaphragm was relatively well preserved in COPD, when normalized to lung volume and chest wall configuration (83). Bellemare et al. (3) have more recently supported this observation by measuring Pdimax in emphysema patients at measured diaphragm lengths and lung volumes, shown in Fig. 1. C and D. At most overlapping absolute lung volumes, Pdimax was greater in emphysema patients. However, when analyzed as a function of diaphragm length, the authors concluded that there must be a decrement in strength (Fig. 1D) that cannot be accounted for entirely by length adaptation (3). This conclusion is in line with other investigators measuring reduced magnetic stimulation twitch forces and sniff Pdi of the diaphragm at FRC (68). Bellemare et al. (3) argue that this could represent a limit of the ability of fiber length to fully adjust to the shortened length in humans, resulting in sarcomeres contracting at inherently shortened state. This would be in agreement with the study by Orozco-Levi et al. (59) that has shown shorter sarcomeres in the diaphragm of COPD patients. Thus the area remains unresolved.

In most studies of rat or hamster elastase-induced emphysema, there is little or no biologically significant reduction in maximum specific force (<10% change) of muscle bundles or individual fibers (20, 21, 31, 36, 49, 85), as summarized in Table 1. The largest exceptions to this are the work of Lewis et al. (43), who showed reductions in in vitro specific force of >20%, and Huensks et al. (32), who demonstrated reductions of ~14%. In human studies of isolated fibers from biopsy specimens, two groups, Levine et al. (40) and Ottenheijm et al. (64), have found significant reductions in specific force in individual costal diaphragm fibers (Table 2), an observation recently confirmed in another group of COPD patients by these authors (86). Ottenheim et al. (64) demonstrated that this could be accounted for by lower myosin heavy chain (MHC)
Table 2. Changes in fiber characteristics of respiratory muscles from chronic obstructive pulmonary disease patients

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**Costal diaphragm**

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**Accessory muscles**

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twitch force to be reduced out of proportion to maximum tetanic force (twitch-to-tetanic ratio in Table 1). If true in humans, this could have the impact of requiring a greater neuronal stimulation to sustain a given Pdi during tidal breathing, possibly contributing to elevations of diaphragm muscle activation seen during resting breathing in COPD (11).

The changes in twitch force observed in some studies are also consistent with the observation from Ottenheijm et al. (62) that fibers from patients with mild to moderate COPD have lower Ca\(^{2+}\) sensitivities in both type I and IIA fibers. This has recently been confirmed by the same group in another set of mild to moderate COPD patients (86). Interestingly, changes in Ca\(^{2+}\) sensitivity cannot be solely explained by the more oxidative characteristics of diaphragm fibers in COPD, as discussed below. This is because it is opposite in direction of what would be expected to occur with shifts to slower, more oxidative fibers, which generally result in leftward shifts in the force-pCa\(^{2+}\) relationship (24). Furthermore, Levine and colleagues (38) have demonstrated that expression of tropomyosin and troponin isoforms (in part, responsible for characteristic single-fiber Ca\(^{2+}\) sensitivity) are shifted proportionately to slower isoforms, in synchrony with the shifts to slower MHC isoform expression. Therefore, the changes in Ca\(^{2+}\) sensitivity appear to reflect some other unknown process. It is important that this single observation is verified in other laboratories, is evaluated as a function of disease severity, and is explored for potential mechanisms underlying it. It is a characteristic that would be responsible to pharmacological treatment. In a recent study, the cardiac drug, levosimendan, the only pharmacological Ca\(^{2+}\) sensitizer approved for human use at this time, has been shown to improve the Ca\(^{2+}\) sensitivity of isolated biopsies of diaphragm from COPD patients (86).

There are only modest changes in the force-velocity relationship of single diaphragm fibers from COPD patients (84). However, the rate of isometric force development is reduced in type I fibers (84), and the rate of force redevelopment following shortening (a measure of the rate of cross-bridge kinetics) is reduced in both type I and IIA fibers (62). The mechanisms for these changes and the impact they might have on normal function are not entirely clear, but they probably contribute to accompanying improvements in contractile efficiency that have also been observed (84). Shifts in the sarcomeric Ca\(^{2+}\) ATPase isoforms (62) to slower, more oxidative isoforms parallel the shifts to slow MHCs, as observed by Nguyen et al. (56). This response could also contribute to improvements in efficiency of contraction.

**CHANGES IN FIBER PHENOTYPE AND SIZE**

In animal models of elastase-induced emphysema, after periods of 6 mo or more, most studies have found that changes in fiber distributions between type I, IIA, IIB, and IIX are modest and quite variable (22, 31, 32, 36, 43, 44, 49, 52, 85), as summarized in Table 1. None have found a significant elevation in the proportion of type I fibers, and there has been only one report of significant increases in the proportion of type IIA fibers (49), with an accompanying reduction in IIX fibers. Changes in type IIB fibers are sometimes present, but these represent 5–10% of the total fibers in the rodent diaphragm and probably are nonexistent in the human diaphragm. The lack of consistent shifts in fiber populations is also consistent with one study by Kim et al. (36) that showed only minor changes in the proportions of MHC isoforms in the rat emphysema model.

The changes in the cross-sectional areas (CSA) of the individual fibers also reveal extreme variability in animal models. Whereas Marchand et al. (49) found a significant reduction of type I fiber CSA (~17%), this has not been a typical finding (Table 1). One study found an elevation in the fiber areas of type IA fibers (52), but a more typical response has been a general reduction in the size of large glycolytic fibers (22) or, specifically, type IIX (36, 49).

The results in animals are in sharp contrast to adaptation in human costal diaphragm biopsy specimens (Table 2). Multiple studies from several laboratories have shown statistically significant increases in the relative proportions of type I fibers in the costal diaphragm (18, 37, 38, 40, 84), with two of the laboratories demonstrating a strong correlative relationship of the proportion of type I oxidative fibers with deterioration of lung function (40, 84). Figure 2, A and B, illustrates the contrasting differences in fiber populations in control vs. severe emphysema patients. There is also reported a reduction in the percentage of IIA (18, 38) and IIX (38) fibers. These results are complimented by analysis of MHC expression (Table 2). A general consensus finds a large increase in the MHC I isoform, corresponding to the myosin predominant in type I fibers (38, 40, 54, 77) and relative decreases in MHC IIA (38) and IIX(38, 77) isoforms. In addition, Nguyen et al. (57) have shown that the proportion of fibers in the diaphragm that contain MHC I isoforms is significantly increased, whereas the proportion expressing IIA or IIB (probably IIX) isoforms is reduced. Therefore, data from multiple sources point to a general redistribution of fiber populations and myosin isoforms in diaphragms of human COPD to more oxidative, slow fibers and away from faster, more glycolytic phenotypes. Furthermore, the increase in type I fiber populations is clearly related to the severity of disease and the extent of hyperinflation, as shown in Fig. 2, C and D, from Levine et al. (40).

There is less clarity on whether there is a significant change in diaphragm fiber CSA in patients with COPD. Sanchez et al. (78) reported a general loss in diameter of both type I and type II fibers. This was also addressed by Levine et al. (37) and in the recent work of Stubbings et al. (84). Both report significant reductions (41% in Ref. 37 and 16% in Ref. 84) in fiber size of type I fibers with relatively little effect on the size of all categories of type II fibers. Scott et al. (79) showed overall reductions in median fiber area of all muscle cells in the diaphragm (~14%), but did not evaluate individual categories of fiber. Doucet et al. (18) found only small changes in fiber size in COPD patients, and Ottenheijm et al. (64) found a small but insignificant increases in type I fiber CSA in mild COPD patients. Therefore, human type I fibers may undergo selective atrophy in patients with COPD, but it is not a universal finding and is probably more likely to be present in patients with severe disease (40).

**ALTERATIONS IN THE PASSIVE MECHANICAL PROPERTIES OF RESPIRATORY MUSCLES**

One of the more interesting recent observations in the human diaphragm from emphysema patients relates to the alterations seen in passive mechanical characteristics (55, 63). In animal models of elastase emphysema, there is currently no evidence...
for substantial changes in the passive elastic properties of whole, intact muscle, when normalized to sarcomere length (85). However, single-cell measurements are necessary to reveal the delicate elastic properties of the myofibers in the absence of influence from other parallel elements in the extracellular compartment, and these have not been measured in rodent models. In permeabilized human diaphragm fibers from biopsy specimens, there is a decrease in the overall elastic “restoring force” (55), as shown in Fig. 3A, or the passive tension of single-fiber segments as they respond to lengthening (63). This reduction in the parallel elastic behavior of single fibers is largely attributed to the properties of the protein, titin, a large, unfolding macromolecule that spans the distance between Z disk and the M region within the sarcomere. It is considered the largest known protein in vertebrates (30) (Fig. 3B). The alterations in passive mechanical properties are associated with splice variants of the elastic components of this molecule, thus changing the viscoelastic properties of the sarcomere. Although gross measures of titin isoform expression could theoretically be attributed to the concurrent shift in fiber populations to slower, more oxidative fibers, where titin variants are known to be more flexible (33), both Moore et al. (55) and Ottenheijm et al. (63) have demonstrated that the changes in stiffness can be observed in both type I and type II cells, suggesting that this may be a more general response. The phenomenon also suggests that diaphragm fibers might be more susceptible to stretch-induced injury, a fact that could contribute to the observed evidence for diaphragm injury in human COPD patients, at autopsy (79). The changes in splice-variant expression responsible for the elevation in elastic recoil of the fibers could also reflect a compensatory mechanism for increases in the stiffness of the extracellular matrix, another important source of parallel elastic behavior of the intact muscle (30), as illustrated in Fig. 3B. Elevations in collagen content have been described in human diaphragm from COPD patients (79).

Ottenheijm et al. (63) have also observed reductions in the expression levels of the protein nebulin in patients with COPD. Nebulin is another large cytoskeleton protein, the function of which is still speculative (53) but may be involved, not only in cytoskeleton structure, but also in Ca^{2+} regulation (61).

**METABOLIC ADAPTATIONS AND FATIGUE RESISTANCE**

There is a general consensus that the diaphragms in patients with COPD and in animal models of COPD show a shift toward higher metabolic capacity and an increased fatigue resistance. Isolated diaphragms from rodents with elastase-induced emphysema exhibit a more or less universal 10–20% improvement in fatigue index, i.e., the ratio of force generated after 2–5 min of repeated contractions compared with the force generated at baseline, before fatigue (20, 36, 44, 49). These in vitro data suggest that some component of the increases in endurance can be attributed to adaptations of the muscle metabolic machinery and are independent of changes in blood perfusion or oxygen delivery.

In human COPD patients, it is very difficult to compare the susceptibility of the diaphragm to fatigue with normal subjects. This is, in part, due to the relative uncertainties in maintaining comparable preloads and afterloads on the muscle, as well as factors such as the effect of dyspnea, hyperinflation, and air trapping on task performance. However, a number of studies have evaluated the susceptibility of the diaphragm to fatigue in hyperpnea (69) or in exhaustive exercise (4, 70), and the general consensus is that the diaphragm in COPD does not show signs of increased susceptibility to fatigue, and possibly an inherent fatigue resistance. This is in contrast to the concepts of Bellamare and Grassino (4) and colleagues who suggested that the diaphragm in COPD normally exists close to a threshold of pressure-time product that would make it highly susceptible. One of the weaknesses of this argument has always been that this theoretical threshold has never been identified accurately in COPD patients and is assumed to be the same as in normal humans. However, with the increased endurance and efficiency characteristics measured at the cellular and biochemical level, it is relatively clear that the human diaphragm in COPD certainly has the capacity for greater fatigue resistance.

Mechanisms for increased fatigue resistance can most likely be attributed to increased activity of oxidative enzymes and to elevations in mitochondrial function in COPD. Two studies in animal models of emphysema demonstrated elevations in the concentration of mitochondrial enzymes, citrate synthase (22) and succinate dehydrogenase (SDH) (44), the latter being increased in both type I and type II fibers. Farkas and Roussos (22) also observed reductions in the glycolytic enzyme, phosphofructokinase.

In severe human emphysema, the elevations in oxidative SDH have been shown by Levine and colleagues (37) to be greatly elevated to >100% of control, when all fiber types of the costal diaphragm are evaluated together. The increases are

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**Fig. 3.** A: restoring force (a measure of passive elastic properties) of single fibers from biopsy specimens of human diaphragm in normal subjects (●) and patients with moderate hyperinflation (○). B: schematic of the relationship of the macromolecule titin and the extracellular elastic matrix, both of which contribute to the parallel elastic elements of the intact muscle. Alterations in extracellular matrix elasticity could be compensated for by alterations in titin splice variant expression. [A, reproduced from Moore et al. (55).]
manifested across all phenotypes, i.e., even fast fibers become more aerobic. The SDH data were further evaluated as a fraction of myosin ATPase (mATPase), the energy-consuming pathway in contracting muscle. In that analysis, despite consistent reductions in fiber mATPase activities, the ratio of SDH to mATPase was elevated. The significance of this finding is highlighted by the idea that SDH-to-mATPase ratio is considered a marker of fatigue resistance (26). A parallel study by Wijnhoven et al. (88) in a group of patients with largely moderate disease found increases in β-oxidation capacity and in mitochondrial respiratory enzyme activity that increased as a function of worsening disease severity. Parallel to the increases in oxidative enzyme capacity, diaphragm mitochondrial density, measured with electron microscopy, has been shown to increase in patients with COPD by an average of ~50% (59), and the mitochondrial oxidative capacity of permeabilized fibers increases as well (77). Both of these variables were also shown to increase as a function of disease severity (59, 77).

Adaptations of the microcirculation can also contribute to the overall endurance properties in the intact system. One study has addressed this in human diaphragm, but found no significant increase in the number of capillary contacts per fiber, although COPD patients trended toward having more contacts compared with controls (18). In the hamster model of elastase-induced emphysema, the number of capillaries per muscle fiber is increased, with no change in capillary density per fiber, due to a simultaneous fiber hypertrophy in this model (44). However, Poole and Mathieu-Costello (73) have carefully examined capillary tortuosity and concluded that the average capillary length per fiber volume increases by a value of ~183%, and, therefore, capillary surface area per fiber volume increased ~12%. Interestingly, Poole et al. (71) have also demonstrated that, despite the increase in capillary density, the microcirculatory Po2 is reduced in diaphragms of emphysematous hamsters during resting conditions, with greater severity seen in hypoxia. This could be due to elevated O2 requirements of the diaphragm during normal breathing or possibly altered tissue mechanics and tissue pressures during contraction because of chest wall distortion. Should this observation translate into the human disease, there are important implications that the unique cellular adaptations of the COPD diaphragm could be influenced by regional hypoxic signaling or some other pathway associated with disordered gas exchange.

EVIDENCE FOR RESPIRATORY MUSCLE INJURY, PROTEIN DEGRADATION, AND OXIDATIVE STRESS

A number of publications have pointed to the possibility that the diaphragm and other respiratory muscles may have significantly elevated degrees of muscle injury, myonuclear apoptosis, and/or oxidative stress. Diaphragms from hamsters with elastase-induced emphysema show ultrastructural alterations consistent with injury (48). Interestingly, emphysematous hamsters that have undergone lung volume reduction surgery show acute sarcocellular damage in over one-third of their fibers within the first few days after treatment (42). This was associated with elevations in a splice variant of insulin-like growth factor I associated with muscle stretch. This observation points to the importance of long-term length adaptation to protect muscles from stretch injury. In postmortem samples from patients with “acute on chronic” pulmonary disease, ~30% of the muscle fibers in the midcostal region showed some evidence of injury, with the largest proportion exhibiting abnormalities within the cytoplasm (79). The injury may have been accentuated by added respiratory loads related to the cause of death in these patients, but previous studies by Orozco-Levi et al. (60) have shown substantial evidence of sarcomere disruptions in presumably stable COPD patients. Interestingly, these effects were accentuated when the patients were given inspiratory loads to breathe before the biopsy specimens being taken, and the extent of the lesions was directly related to the degree of airway obstruction. The data suggest that ongoing injury in diaphragm fibers may be a component of remodeling and that it is related to the increased inspiratory loads and/or distortions of the chest wall. Of note, some evidence of injury is also seen in normal diaphragms, and, therefore, the extent to which these observations represent abnormality or simply ongoing adaptation or fiber disruption with training has not been resolved.

Another interesting finding is that hamsters with elastase-induced emphysema exhibit some evidence of myonuclear apoptosis (DNA fragmentation) in the diaphragm, as well as in the soleus muscle, but not in other less oxidative muscles of the limb (15). This occurred 1–5 mo after elastase administration. There was no elevation in caspase 3 activity in this model (another marker of apoptosis), but there were elevations in BAX/BCL-2 and apoptosis-inducible factor, markers of mitochondrial, noncaspase-dependent apoptotic pathways. Signs of apoptosis in multinucleated skeletal muscle are not likely reflections of myofiber cell death, but represent “myonuclear apoptosis” and remodeling of nuclear domains, or possibly apoptosis of satellite cells, which have a higher turnover rate. Unlike animal experiments, studies on diaphragm fibers from human COPD patients exhibit elevations in caspase-3 activity (67). They also reveal increased 20s proteosome activity and elevated levels of atrogin-1, an E-3 ligase, believed to be involved with the regulation of protein ubiquitination in atrophic muscles (67). Ubiquitination is one of the first steps in degrading damaged proteins via the proteosome or in tagging proteins targeted for degradation in the process of atrophy or fiber shortening. It is interesting that these findings occurred in patients with mild to moderate disease, which led the authors to speculate that the process of protein degradation and accompanying loss of MHC content might be characteristic of early disease and adaptation (62).

Several lines of evidence have suggested that the diaphragm in COPD exhibits elevated levels of oxidative stress. In the hamster model of emphysema, Heunks et al. (32) demonstrated an elevation in glutathione oxidation state in the diaphragms of emphysematous hamsters compared with controls that was significantly correlated to the loss of maximum specific force. Barreiro et al. (2) have reported elevations in markers of oxidative stress in diaphragms of patients with severe COPD, including increased protein carbonyl formation and hydroxynonenal-protein adducts, which are related to severity of disease and/or loss of respiratory muscle strength. These findings were recently corroborated in the diaphragms of patients with severe emphysema, which identified oxidation of specific proteins, including creatine kinase and contractile and cytoskeletal proteins within the sarcomere (51).
The important question regarding observations of diaphragm injury, oxidative stress, apoptosis, and increased activity of proteolysis pathways is to what extent these observations indicate an abnormal response to disease, a kind of manifestation of systemic effects of COPD, or whether they are more likely indicative of ongoing remodeling. Paradoxically, essentially all of these signaling and oxidative pathways have been implicated as important links in muscle atrophy responses to models of disuse of the diaphragm (74), but, in COPD, the diaphragm undergoes greater activation and is contracting against greater mechanical loads. Furthermore, although these atrophy and/or injury-associated signals are present to some extent in animal models of emphysema, there is little consistent evidence of muscle atrophy in animal models (Table 1). So, what is actually going on? Since most observations can be categorized at this time as correlations with disease and not necessarily causative of the actual changes in the muscle, it becomes a classic chicken and egg problem. Is adaptation driving apparent pathology, or is apparent pathology driving adaptation?

ADAPTATIONS IN OTHER RESPIRATORY MUSCLES

Much less work has been done on the effects of hyperinflation on accessory muscles of respiration, and only a brief review of what is known is included here for completeness. The parasternal intercostals are extremely important for normal inspiration in humans and are coactivated with the diaphragm (12). Although we know less about their importance in animal models of emphysema, in hamsters with elastase-induced emphysema, there is a no change in fiber length of this muscle group and no change in maximum force generation (35) (Table 1). In human parasternal intercostals, Levine and coworkers (39) have demonstrated a shift in fiber characteristics, much like what is seen in the diaphragm (Table 2), with a fast-to-slow transformation of the fibers and their myosin isoforms.

The external and internal intercostal muscles have been studied in the hamster model. Farkas and Roussos (22) showed a modest shift away from faster fiber to slower fiber types in the external intercostals, with no consistent changes in the internal intercostals. There was also a general increase in citrate synthase activities in the intercostal muscles taken as a whole (22). In human COPD, the external intercostals have been shown to exhibit only modest changes in MHC expression compared with control subjects (77).

Arnold et al. (1) analyzed the responses of the expiratory muscles, the transverse abdomenus and the external oblique muscles, in emphysematous hamsters and found that there were no changes in the specific force or twitch contractions, but saw significant improvements in endurance capacity.

The scalenus muscles, important inspiratory accessory muscles both in humans and animals, have been shown by Fournier and Lewis (25) to be recruited during resting breathing in emphysematous hamsters. After 1 yr of elastase-induced emphysema, there were no effects on muscle length at \( L_o \), specific force, twitch characteristics, or fatigue resistance, but, in the medial scalene, there was a greater proportion of fibers containing type IIA MHC, with a proportional reduction in the proportion of IIX fibers. Some evidence of atrophy of the type II fibers was also reported.

In general, we can conclude that the accessory muscles of inspiration and the expiratory muscles are also undergoing adaptations in COPD and hyperinflation that parallel, to some extent, the changes in the diaphragm and probably contribute to elevations in endurance of the respiratory pump. In general, however, the changes are not as profound as are seen in the diaphragm.

POTENTIAL SOURCES OF DIFFERENCES IN DIAPHRAGM REMODELING IN ANIMAL MODELS AND HUMAN DISEASE

From the above discussions, it is relatively clear that, although there are some commonalities between the effects of human COPD and elastase-induced emphysema in animals, there are many differences. Commonalities include apparent changes in fiber length and greater fatigue resistance. However, there is no consistent evidence of predominant shifts to type I fibers or to slow myosin isoforms in the animal models, whereas these are relatively consistent findings in humans. Also, whether specific force is reduced in humans is perhaps debatable, but it is relatively clear that there is little or no loss of specific force in most animal models of the disease. Furthermore, there is little evidence of muscle atrophy of type I fibers in animal models, but it seems to be predominant in at least severe COPD. What possible characteristics of rodent models or human disease could account for differences in response?

1) Human and rodent diaphragms have inherently different fiber populations in control conditions, no doubt reflecting the relatively rapid respiratory rate and contractile requirements of rodent breathing. Costal diaphragms from hamsters have about 23–27% type I, 33–47% IIA, and 28–44% IIX/IIB fibers (31, 49, 52). Humans have a greater predominance of slow fibers, 45–50% type I, 35–40% type IIA, and 15–20% type IIX (18, 38). These differences and differences in contractile patterns could be responsible for different adaptive strategies.

2) The length of time the animals are exposed to lung disease and hyperinflation is different than in elastase-emphysema vs. human COPD. In most of animal models, the respiratory muscles are studied around 6 mo after elastase infusion (Table 1). Since the life expectancy of hamsters is \( \sim 3 \) yr, this could be considered something like 12 human years, whereas the human disease might take 2 or 3 decades to develop. Unfortunately, there is no clear understanding of how life span influences the rates of adaptation.

3) Large mechanical and immunological profile differences may exist between the human disease and the elastase model in animals. Whereas the animal model is a relatively pure lung parenchymal disease, the human disease is generally characterized by both parenchymal and airway components. While airway resistance is elevated in elastase-induced emphysema, it is entirely accounted for by the elastic properties of the lungs and may not be large enough to load the diaphragm at levels comparable to human disease (5). To consider the implications of this, it is instructive to look at the responses of the rodent diaphragm to a pure chronic airway resistive loading, i.e., tracheal banding (34, 75). After \( \sim 6 \) mo of extratracheal banding, there are trends toward a reduction in maximum specific force and significant reductions in twitch force (75). In addition, there are marked shifts in fiber type, with an increase in type I fiber populations from 36 to 51%, and type I fiber area...
increases 41% (75), while type II fiber area diminishes. There is an increase in fatigue resistance and a fatigue-resistant metabolic profile. These results, in sum, resemble the influences of human emphysema on the diaphragm as much or even to a greater extent compared with elastase emphysema models, and most of the effects show up after as little as 1 mo of banding (34). Therefore, a reasonable hypothesis is that human adaptations to chronic obstructive disease might reflect a larger than expected component of adaptation to increases in airway resistance elements that simply are not present in the elastase model.

4) Systemic inflammation accompanies COPD in humans, including elevations of TNF and other cytokines (10, 14, 87). TNF-α is a well known mediator of muscle atrophy and cachexia (76). The origin of the systemic inflammatory signaling in COPD is not well known, but it is generally believed to reflect a spillover of airway inflammation into the circulation, or the systemic influence of smoke inhalation and disease exacerbation with accompanying proliferation and activation of circulating monocytes and inflammatory neutrophils, as reviewed in Ref. 10. Whether a substantial level of chronic inflammation exists in elastase-induced emphysema in animals is not well understood, although it has been proposed that a significant degree of lung injury in these models is influenced by ongoing local TNF-α and IL-1β signaling in the lung (46). Furthermore, TNF-α is elevated within skeletal muscle cells in elastase-induced emphysema (16). Nevertheless, it is difficult to equate the complexity of cytokine responses in human disease with what is seen in animal models.

5) Human COPD is often accompanied by hypoxemia and chronic CO2 retention, and both of these could have direct effects on muscle remodeling. Interestingly, chronic hypercapnia results in shifts toward more oxidative fiber populations and reduced muscle-specific force (80), which resembles the characteristics in human diaphragm of COPD patients, as discussed above. However, elastase-induced emphysema can also induce rather severe hypercapnia and hypoxia (71), and, therefore, both human and animals share these influences. Nevertheless, no one has ever evaluated the extent to which gas exchange abnormalities might relate to the kinds of unique adaptations and their magnitude in human disease or in animal models.

SUMMARY AND CONCLUSIONS

The diaphragm and other respiratory muscles appear to undergo profound adaptations to chronic hyperinflation and disease. Although some aspects of adaptation can be reproduced in animal models, it is clear that many of the responses in human disease are unique. Many important findings that are said to distinguish human adaptations, e.g., type I fiber atrophy, loss of specific force, changes in parallel elastic elements, and loss of Ca2+ sensitivity, are actually not that well defined or verified, particularly across levels of disease severity and across clinically distinguishable populations. Particularly surprising are the observations that some changes in single-fiber characteristics occur in relatively mild disease. Significant chest wall distortions are unlikely in mild disease, but modest elevations in work of breathing and increased levels of systemic inflammation might be expected. Much work remains to continue to understand the prevalence and origins of these findings as a function of disease severity.

It has been proposed that the adaptations of the diaphragm in COPD demonstrate a kind of muscle dysfunction or impairment (62–64, 84). This may be true, and findings such as loss of Ca2+ sensitivity and atrophy certainly lead us in this direction. However, an alternative hypothesis is just as valid, i.e., that the majority of the observations represent complex adaptations that are optimizing the performance of the diaphragm in a setting of greater metabolic and mechanical requirements. For example, lower specific forces might be accounted for largely by changes in myosin content (62), which, in turn, are likely to be a reflection of the higher mitochondrial or organelle density necessary for greater metabolic activity. The diaphragm is probably more fatigue resistant, shifting to slower, more efficient myofibrillar proteins, which can reduce the rate of energy utilization and improve oxidative capacity. It is likely that the diaphragm has undergone loss of series myofilaments and changes in architecture to adjust its length to appropriately match its new hyperinflated environment. Changes in its intrasarcromeric elastic properties may be adapting to other external elastic forces to keep average sarcomere length constant. The atrophy that is sometimes seen may be part of an attempt to improve efficiency of gas exchange, a strategy that limb muscles use in chronic hypoxic environments to match oxygen uptake with delivery (47). For example, at altitude, limb muscle cells become smaller but maintain capillary density, thus ensuring adequate O2 availability while reducing demand. Even injury, oxidative stress, and evidence of accelerated protein turnover can all be attributed to evidence for adaptation and not necessarily dysfunction. Nevertheless, it is possible that various combinations of what we might call adaptation could lead to an overall dysfunction for a particular requirement of the diaphragm. For this reason, it is easy to overemphasize one hypothesis vs. the other. On the other hand, if dysfunction or impairment is real in some patients, the diaphragm might be amenable to treatment or therapy, an important goal. If it is not real, imposed treatment may or may not benefit the patient, depending on whether the outcome can compensate for some aspect of the overall impact of the disease. Therefore, distinguishing between a primary “dysfunction hypothesis” and a primary “adaptation hypothesis” and whether each of these might be applicable to different types of patients at different stages of disease remains the most challenging frontier of this area of research and a target for personalized medicine.

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REFERENCES

Review

COPD RESPIRATORY MUSCLE CELL ADAPTATION


