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Diaphragm Muscle Adaptations in Health and Disease

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Abstract

Breathing is achieved without thought despite being controlled by a complex neural network. The diaphragm is the predominant muscle responsible for force/pressure generation during breathing, but it is also involved in other non-ventilatory expulsive behaviors. This review considers alterations in diaphragm muscle fiber types and the neural control of the diaphragm across our lifespan and in various disease conditions.

Keywords

Spinal cord; neuromotor control; phrenic motor neuron; diaphragm motor unit

Introduction:

The diaphragm muscle (DIAm) is unique to mammals, and separates the abdominal and thoracic cavities. The DIAm is the principal inspiratory pump muscle generating a negative intrathoracic pressure (P_{th}) that drives air into the lungs during breathing. However, the DIAm also contributes to the generation of a positive intra-abdominal pressure (P_{ab}), necessary for higher force expulsive behaviors (defecation, coughing and sneezing). Th pressure difference across the thoracic/abdominal surfaces of the DIAm is the transdiaphragmatic pressures (P_{di}) and can be measured as a surrogate for DIAm force generation (Figure 1). Repetitive activation of the DIAm during breathing involves a high duty cycle (time active versus inactive), but the P_{di} generated during even the most strenuous breathing efforts represent less than half of the maximum P_{di} ($P_{di_{max}}$) that can be generated by the DIAm. In contrast, higher force expulsive behaviors are shorter in duration and non-repetitive. Thus, the design features for DIAm fibers activated during ventilatory versus expulsive behaviors are quite different. To accomplish repetitive lower force breathing efforts, it is necessary to recruit only fatigue resistant DIAm fibers. In contrast, for shorter duration higher force expulsive efforts, more fatigable DIAm fibers are recruited. The nervous system controls the recruitment of DIAm fibers through phrenic motor neurons

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(PhMNs) located in the cervical spinal cord. Collectively, a PhMN and the group of muscle fibers it innervated is termed a motor unit (Figure 2). The mechanical and fatigue properties of DIAm motor units are determined by the properties of the muscle fibers comprising the motor unit. Thus, there are slow- and fast-twitch motor units, and fatigue resistant and fatigable motor units. In this review, the effect of different disease conditions on DIAm motor units will be considered in the context of the impact on ventilatory versus expulsive behaviors of the DIAm.

DIAm Fiber Type Classification:

Different isoforms of myosin heavy chain (MyHC) are expressed in the DIAm that determine both the contractile and fatigue properties of muscle fibers [1]. Some MyHC isoforms normally exist only during the embryonic ($MyHC_{Emb}$) and neonatal ($MyHC_{Neo}$) developmental periods of the DIAm [2, 3]. However, with muscle injury, the expression of $MyHC_{Emb}$ and $MyHC_{Neo}$ reappears, perhaps due to the fusion of satellite cells into injured fibers for repair [4–6]. In the normal adult DIAm, there are four MyHC isoforms that comprise four fiber types [1, 7–13]. A “slow” MyHC isoform ($MyHC_{slow}$) is expressed in type I fibers, and there are three “fast” MyHC isoforms ($MyHC_{2A}$, $MyHC_{2X}$, $MyHC_{2B}$) expressed in type IIa, and IIx and/or IIb fibers [8]. Type I fibers have a slower time to peak isometric force (i.e., slow twitch time), a slower maximum shortening velocity, generate lower specific force (force normalized per cross-sectional area), and are fatigue resistant during repetitive activation [1, 7, 14–16]. Type IIx and/or IIb DIAm fibers co-express $MyHC_{2X}$ and $MyHC_{2B}$ isoforms in varying proportions. These fast-twitch fibers have faster shortening velocities, generate greater specific force, and are more fatigable during repetitive stimulation. DIAm fiber type proportions can change under certain conditions, and this will influence the contractile and fatigue properties of DIAm [4–6]. However, caution should be taken in interpreting changes in fiber type proportions, since this may reflect dysregulation of MyHC isoform expression leading to co-expression of isoforms and ambiguous fiber type classification. Co-expression of MyHC isoforms in DIAm fibers has been noted in a variety of conditions [5, 17, 18].

DIAm Fiber Force:

The sarcomere is the basic structural and functional unit of DIAm fibers. Actin filaments are anchored to Z-discs and project towards a midline (M-line). Each myosin filament is surrounded by six actin filaments, and Ca^{2+} binding to troponin C on the actin filament regulates the attachment of myosin heads (MyHC) to the actin filament, forming cross-bridges that provide the molecular basis for contraction and force generation [19]. The force (F) generated by a single muscle fiber is estimated by the equation:

$$F = n \bullet f \bullet \alpha_{fs}$$

Where n is the MyHC concentration per half sarcomere, f is the force contributed by each cross-bridge and α_{fs} is the proportion of MyHC heads forming tightly bound cross-bridges. As muscle fiber cross-sectional area increases (hypertrophy) or decreases (atrophy), n increases or decreases proportionately, such that the force per cross-sectional area (specific

force) of a fiber remains unchanged. However, there are many natural and pathological conditions where specific force of muscle fibers decreases (i.e., weakness). For example, during the perinatal period, the specific force of DIAm fibers is lower compared to adult fibers, due in part to a reduced n [8, 20]. Similarly, in old age, DIAm specific force is reduced compared to younger ages – sarcopenia [21–26]. Furthermore, with chronic obstructive pulmonary disease (COPD) [27–30], cachexia [31, 32], corticosteroid treatment [33–37], hypothyroidism [38–40], and malnutrition [41–50], specific force of the DIAm is reduced, indicating that n can be affected, most likely via proteolysis rather than reduced synthesis of MyHC [4].

The force generated by an individual cross-bridge (*f*) is mainly determined by MyHC isoform, being lower in fibers expressing $MyHC_{slow}$ compared to fast MyHC isoforms [8, 51]. The *f* for a specific MyHC isoform may be affected in some conditions; however, this can only be determined using a single fiber preparation where force and n are both measured. For example, following denervation, DIAm specific force decreases due to a decrease in both n and *f* [52, 53]. Similarly, with COPD in humans, DIAm fiber specific force decreases due to both a decrease n and *f* [30].

Ca^{2+} Sensitivity of DIAm Force:

At optimal sarcomere length (i.e., greatest extent of overlap of myosin heads with actin binding sites), the primary determinant of α_{fs} is Ca^{2+} regulation of myosin binding to actin (force-Ca^{2+} relationship). Type I DIAm fibers display greater Ca^{2+} sensitivity compared to all type II fibers (leftward shift in the force-Ca^{2+} relationship) [51]. This most likely reflects the expression of a “slow” isoform of troponin C with only two Ca^{2+} binding sites. In COPD patients, Ca^{2+} sensitivity of DIAm fiber force decreases, possibly reflecting the co-expression of MyHC and troponin C isoforms [30].

DIAm Force-Length Relationship:

At sub-optimal length, some myosin heads cannot bind to actin; thus, even during maximum Ca^{2+} activation, α_{fs} is reduced and less force is generated (force-length relationship). In COPD, the DIAm flattens due to retention of air in the lungs, and at least initially, this will affect the DIAm force-length relationship contributing to a reduction in force. However, with time, there is length adaptation in the COPD DIAm, with a reduction in the number of sarcomeres in series [27, 54]. Thus, an optimal length for force is re-established.

DIAm Fiber Energetic Balance:

Cross-bridge cycling requires ATP hydrolysis and depends on external load (Figure 3). Cross-bridge cycling and ATP hydrolysis rates vary across DIAm fiber types, being greater in type II fibers comprising $MyHC_{2A}$, $MyHC_{2X}$, $MyHC_{2B}$ isoforms compared to type I fibers comprising the $MyHC_{slow}$ isoform. The consumption of ATP for any MyHC isoform is described by the equation:

$$\text{ATP Consumption} = n \bullet b \bullet \alpha_{fs} \bullet g_{app}$$

Where b is the number of half sarcomeres in series and g_{app} is the apparent rate constant for cross bridge detachment.

Velocity of shortening and g_{app} are dependent on external loading; thus, ATP consumption changes with external load and the velocity of shortening, reaching a maximum at the peak power output of a muscle fiber (Figure 3) [9, 55, 56]. In order to accommodate the sizeable range of ATP consumption demands across different DIAm fiber types, there is also considerable range in the reserve capacity for ATP production via mitochondrial oxidative phosphorylation. Due to the higher mitochondrial volume densities of type I and IIa fibers DIAm fibers and thus their higher oxidative capacities, the reserve capacity for ATP production in these fibers is quite high compared to type IIx and/or IIb fibers [57, 58]. Type IIx and/or IIb fibers depend more on glycolytic pathways for ATP production, with a much lower functional reserve capacity. Accordingly, it is likely that the higher rates of ATP consumption, lower mitochondrial volume density and oxidative capacity, and thus, lower reserve capacity for ATP production in type IIx and/or IIb fibers underlies their greater fatigue susceptibility [7, 13]. Mitochondrial production of ATP via oxidative phosphorylation in type I and IIa DIAm fibers is much higher, which when combined with their slower cross-bridge cycling rates and ATP consumption rates [57, 58] contributes to their increased endurance (fatigue resistance). In this respect, it is noteworthy that with aging, the residual force maintained by the DIAm after repetitive activation is unaffected [23], indicating the energetic resilience of type I and IIa fibers. Unfortunately, very few if any studies have systematically explored the impact of diseases (e.g., COPD, diabetes) or conditions (e.g., aging, malnutrition) on energetic balance of DIAm fibers.

Training Effects:

Throughout life, skeletal muscle is constantly remodeled, adjusting to vagaries in activity, load, or innervation. Thus, within limits, muscle fibers can adapt to environmental conditions, changing their structure and function to suit new natural or imposed scenarios. This is commonly observed in sports, where athletes train to achieve desired muscle adaptations that optimize performance needs. For example, mitochondrial biogenesis can improve muscle endurance via an increase in mitochondrial volume density and oxidative capacity. An increase in the external load on muscle fibers leads to an increase in fiber cross-sectional area thereby adding sarcomeres in parallel, increasing MyHC concentration per half sarcomere (n in the equation above) and increasing muscle strength. There is no reason to doubt that the DIAm responds in a similar fashion to improve endurance and/or strength. However, it should be considered that the DIAm is a highly active muscle with a breathing duty cycle (time active versus inactive) of ~40%. Thus, those DIAm fibers that are active with breathing are essentially endurance trained and may not respond to a further increase in activity compared to limb muscle fibers with a much lower duty cycle. In contrast, it is very likely that DIAm fibers will respond to resistance/strength training, but this does not target breathing efforts.

The importance of skeletal muscle remodeling extends far beyond exercise physiology to many clinical conditions and diseases. The strength and endurance of various muscles changes throughout life, even without exercise (e.g., early postnatal development and old

age). Chronic diseases, including diabetes, heart disease, cancer cachexia, etc. result in muscle wasting, weakening and diminished endurance. Additionally, neuromuscular or muscular diseases such as muscular dystrophy and other congenital muscular disorders, amyotrophic lateral sclerosis (ALS), and spinal cord injury may impair DIAm performance. A variety of therapeutic conditions including corticosteroid treatment and mechanical ventilation may also impact DIAm function. Of considerable interest is the fact that these effects are predominantly reflected by an atrophy (reduced cross sectional area) of type IIx and/or IIb fibers, resulting in weakness (reduced maximum force) with little initial impact on endurance during breathing [14, 23, 26, 57, 59–63]. Consistent with these results, endurance training has not been shown to be effective. Just as the mode of exercise varies for training marathon runners versus body builders, there is not a single therapeutic approach to mitigate the impact of disease on the DIAm.

Motor Units in the DIAm:

The final executor of neural control of the DIAm is the PhMN, which evokes contraction of the muscle fibers it innervates (i.e., DIAm motor unit activation; Figure 2). PhMNs are located in the cervical spinal cord (C_3-C_6 depending on species). In the mouse and rat, there are ~200–240 PhMNs on each side [64–66], providing a total of ~400–480 DIAm motor units that affect motor control across a range of motor behaviors. In the adult rat, PhMNs vary ~8-fold in size with somal surface areas ranging from ~1,000 to 8,000 μm^2 [64–66] and this size variability plays an important role in motor control. During embryonic and early postnatal development, the size of PhMNs is more homogenous, with a subsequent growth of larger PhMNs into adulthood [65, 67, 68]. This increase in PhMN size corresponds to the emergence of type IIx and/or IIb fibers in the DIAm [65]. In older rats, there is an ~25% age-related loss of larger PhMNs that may underlie sarcopenia of old age [26, 64]. Recently, we reported that PhMN size varies with DIAm paralysis induced by spinal cord hemisection (shift to smaller size) compared to tetrodotoxin (TTX) blockade of phrenic nerve action potential propagation (shift toward larger PhMNs) [69]. Unfortunately, no studies have explored whether changes in PhMN morphology are induced by chronic disease.

The size of PhMNs is matched to the mechanical and fatigue properties of the DIAm fibers they innervate. Importantly, all DIAm fibers comprising an individual DIAm motor unit express the same contractile proteins and share similar biochemical properties (i.e., they are the same muscle fiber type; Figure 2) [1, 7, 11, 70–74]. Slow-twitch, type S DIAm motor units comprise type I fibers that generate less force but are fatigue resistant [13, 73]. It is likely that type S motor units comprise smaller PhMNs, although this has not been directly established. Fast-twitch, fatigue resistant (type FR) DIAm motor units comprise type IIa fibers that also generate lower specific force compared to other fast motor units but greater than type I fibers [8, 20, 52, 75]. More fatiguable fast-twitch motor units comprise type IIx and/or IIb DIAm fibers that co-express $MyHC_{2X}$ and/or $MyHC_{2B}$ isoforms in varying proportions accounting for intermediate (type FInt) to greater fatigability (type FF). The type IIx/IIb DIAm fibers comprising FInt and FF DIAm fibers have larger cross-sectional areas [47, 50, 65, 76] and generate greater specific forces compared to type I and IIa fibers [8, 51]. Thus, their contribution to total DIAm force is proportionately greater.

DIAm motor unit innervation ratio (i.e., number of muscle fibers innervated by a PhMN) is an important contributor to unit force generation. In rats, there are ~400 to 480 PhMNs [64], that innervate ~48,000 DIAm fibers, with an average innervation ratio ~100 to 120 fibers [77]. Of note, in the cat DIAm, we determined the innervation ratio of different motor unit types using a glycogen depletion method and found an innervation ratio of ~120 fibers per motor unit with no difference across motor unit types [12, 78, 79].

With advanced age, the total number of PhMNs decreases primarily impacting larger PhMNs that innervate type FInt and FF DIAm motor units [64]. This age-related loss of PhMNs results in initial denervation of type IIx and/or IIb DIAm fibers. Importantly, we previously found that DIAm denervation is associated with atrophy of type IIx and/or IIb fibers and a decrease in specific force [52, 76], similar to sarcopenia. Interestingly, neither denervation or age affects the cross-sectional areas of type I and IIa DIAm fibers. With time, the age-related loss of PhMNs is accompanied by axonal sprouting of surviving PhMNs, reinnervation of type IIx and/or IIb fibers and expansion of motor unit innervation ratio.

During embryonic and early postnatal development, DIAm fibers can be innervated by more than one PhMN (polyneuronal innervation) [14, 80]. The disappearance of polyneuronal innervation of DIAm fibers by the second postnatal week in rats coincides with the emergence of type IIx and/or IIb fibers [14, 20, 75, 80, 81], suggesting that some trophic relationship between larger PhMNs and type IIx and/or IIb DIAm fibers may exist. This is supported by studies showing that unilateral denervation of the DIAm in the first postnatal week in rats results in stunted growth of type IIx and/or IIb fibers on the affected side. It is important to note however that ventilation is not affected by unilateral DIAm denervation [82].

A loss of PhMNs may also occur in other disease conditions, most notably amyotrophic lateral sclerosis (ALS). Importantly ALS also appears to affect larger motor neurons [83–86], and this may explain the initial maintenance of ventilation in ALS patients. However, ALS patients are at increased risk for airway infections and pneumonias [87–89], perhaps reflecting their inability to effectively clear their airways. Other neural degenerative diseases may also differentially affect larger PhMNs, but no systematic analysis has been performed.

Neural Control of P_{di} in Different Motor Behaviors:

Due to the high duty cycle requirements to sustain ventilation, the inspiratory pump function of the DIAm must involve generation of non-fatiguing P_{di}. Although a positive P_{ab} is generated with each breath, expiration is typically passive driven by elastic recoil of the lungs and chest wall. Active recruitment of the DIAm and other abdominal wall muscles to generate higher force P_{di} is more infrequent (i.e., a lower duty cycle). These higher force expulsive behaviors often require near-maximal activation of the DIAm. Accordingly, the morphological and functional design of the DIAm involves two groups of motor units: one a lower force but fatigue resistant set of motor units (type S and FR comprising type I and IIa fibers) that are efficiently designed to generate adequate P_{di} to sustain breathing, and a second set of higher force but fatigable motor units (type FInt and FF comprising type IIx

and/or IIb fibers) that are optimally designed for short duration bursts of near-maximal P_{di} [14].

Neuromotor control of the DIAm to generate P_{di} during different motor behaviors is accomplished by recruitment and rate coding of motor units. The recruitment order of DIAm motor units is determined by the intrinsic size-dependent electrophysiological properties of PhMNs [16, 85, 90–92] described by the following equation:

$$dV_m/dt = I_{syn}/C_m$$

Where dV_m/dt is the change in membrane potential, I_{syn} is the synaptic current and C_m is membrane capacitance. Thus, for a given I_{syn}, smaller PhMNs with less surface area and lower C_m have a greater dV_m/dt compared to larger PhMNs with greater C_m. Smaller PhMNs are more excitable reaching a threshold for action potential generation sooner. It is widely assumed that smaller PhMNs innervate fatigue resistant type S and FR DIAm motor units, whereas larger PhMNs innervate type FInt and FF motor units, and that an orderly recruitment of motor units occurs across the range of forces required for different motor behaviors (Figure 2). This recruitment order is maintained even with increasing neural drive (I_{syn}), but there is a decrease in delay before recruiting motor units and an increase in discharge rate [93].

Based on the assumption of an orderly size-dependent recruitment of PhMNs, we developed a model for DIAm motor unit recruitment (Figure 2) [7, 12, 16, 79, 94, 95]. Our initial model for the cat DIAm, was based on direct measurements of motor unit force and estimates of the contribution of each motor unit type compared to $P_{di_{max}}$ generated by bilateral phrenic nerve stimulation [7, 16, 60, 79, 96, 97]. Subsequently, in rodent DIAm, the force contribution of different motor unit types was estimated based on measurements of Ca^{2+} activated specific force generated by different types of single permeabilized DIAm fibers [8, 51], their mean cross-sectional areas [47, 50, 65], the relative fiber type proportion [1, 7, 73] and the assumption of similar innervation ratios across motor unit types [12, 78, 79]. In each of these models, ventilatory behaviors (i.e., eupnea, hypoxia/hypercapnia, sighs and airway occlusion) were accomplished by the recruitment of only type S and FR motor units [12, 16, 79, 98, 99]. It was determined that during eupnea and hypoxia/hypercapnia the DIAm generates <15% of $P_{di_{max}}$ [1, 7, 16, 26, 59]. Sighs are spontaneous deep inspirations that have a P_{di} amplitude >2-fold tidal breaths. During airway occlusion, as occurs during obstructive sleep apnea, P_{di} approaches ~40–50% $P_{di_{max}}$, and this maximum ventilatory-related effort is largely accomplished by maximal recruitment of type S and FR motor units with some minimal recruitment of FInt motor units [23]. However, to perform higher force, expulsive behaviors (i.e., coughing, sneezing, defecation), the recruitment of additional type FInt and FF motor units is required [12, 16, 79, 98, 99].

There are only a few studies that have assessed the increase in P_{di} during exercise as ventilatory or abdominal core requirements increase. During exercise intensities inducing maximum minute ventilation $\left(V_{E_{max}}\right)$, tidal volume can increase by ~5-fold from eupneic

levels, presumably requiring increased P_{di} generation. In previous studies, we examined maximum voluntary ventilation (MVV) under isocapneic conditions in human subjects, which ranged from ~180 to 230 L/min in healthy young subjects [100, 101]. However, MVV cannot be sustained and rapidly declines within ~2 min to a maximum sustainable ventilatory capacity (MSVC) of ~60% MVV (Figure 4). In addition, changes in DIAm EMG spectral content occur indicating DIAm fatigue. Thus, it appears that some more fatigable DIAm motor units can be recruited during more extreme ventilatory efforts. The $V_{E_{max}}$ in normal subjects does not exceed MSVC. However, there is some evidence that under conditions of intense exercise in highly trained individuals DIAm fatigue occurs [102], indicating that the recruitment of more fatigable DIAm motor units may limit exercise performance in some individuals. During non-ventilatory body core exercises (e.g., weight lifting, sit-ups), the DIAm is more active, and a level of ~50% $P_{di_{max}}$ has been reported [103], but this P_{di} would not necessarily require recruitment of more fatigable DIAm motor units.

As the superior wall of the abdominal cavity, the DIAm is indispensable in the generation of positive P_{ab} for a variety of motor behaviors. Indeed, many of the non-ventilatory behaviors of the DIAm are associated with the generation of extremely high forces and thus necessitate the extensive recruitment of PhMNs and DIAm motor units [14]. The highest P_{ab} are generated during straining behaviors (e.g., defecation, vomiting, and parturition) that consist of very high levels of DIAm activation with co-contraction of external intercostal and abdominal muscles, while simultaneously closing of the glottis by coordinated activation of the laryngeal lateral cricoarytenoid and transverse arytenoid muscles [104]. Synchronous co-contraction of the abdominal muscles and DIAm against a closed airway prevents their relative shortening [105, 106], ensuring that the optimal lengths of these muscles are maintained throughout the duration of activation. Preserved optimal lengths during voluntarily expulsive Valsalva maneuvers in humans explain why the P_{di} generated is so high (e.g., P_{di} up to 220 cmH_2O) [107–109]. Coughing and sneezing are expulsive airway clearance behaviors that require coordinated co-activation of the DIAm and abdominal muscles. During forceful coughing and sneezing the Pdi generated can approach $P_{di_{max}}$.

Even when the abdominal muscles are paralyzed in quadriplegic patients, DIAm activation is still preserved during coughing efforts [110]. It is important to recognize that while expulsive straining behaviors require the generation of very high DIAm forces/Pdi, these pressures do not need to be sustained for long-periods of time. Thus, DIAm fatigue is not a factor unless these behaviors are repeated, e.g., parturition, chronic coughing [14, 85]. Parturition is associated with very forceful co-activation of the DIAm and abdominal muscles, which is prolonged and repeated. Coordinated contractions of the DIAm and abdominal muscles can generate intra-uterine pressures of ~170–300 cmH_2O [111–113]. Thus, it is not surprising that DIAm fatigue may occur during labor [114]. In addition, DIAm injury may occur during high-force expulsive efforts associated with parturition [115–122] as well as coughing [123–127], vomiting [128] or defecation. In fact, non-traumatic DIAm herniation is commonly reported in association straining behaviors [129]. Conversely, patients who have severe DIAm weakness, report difficulties with coughing and defecation [130].

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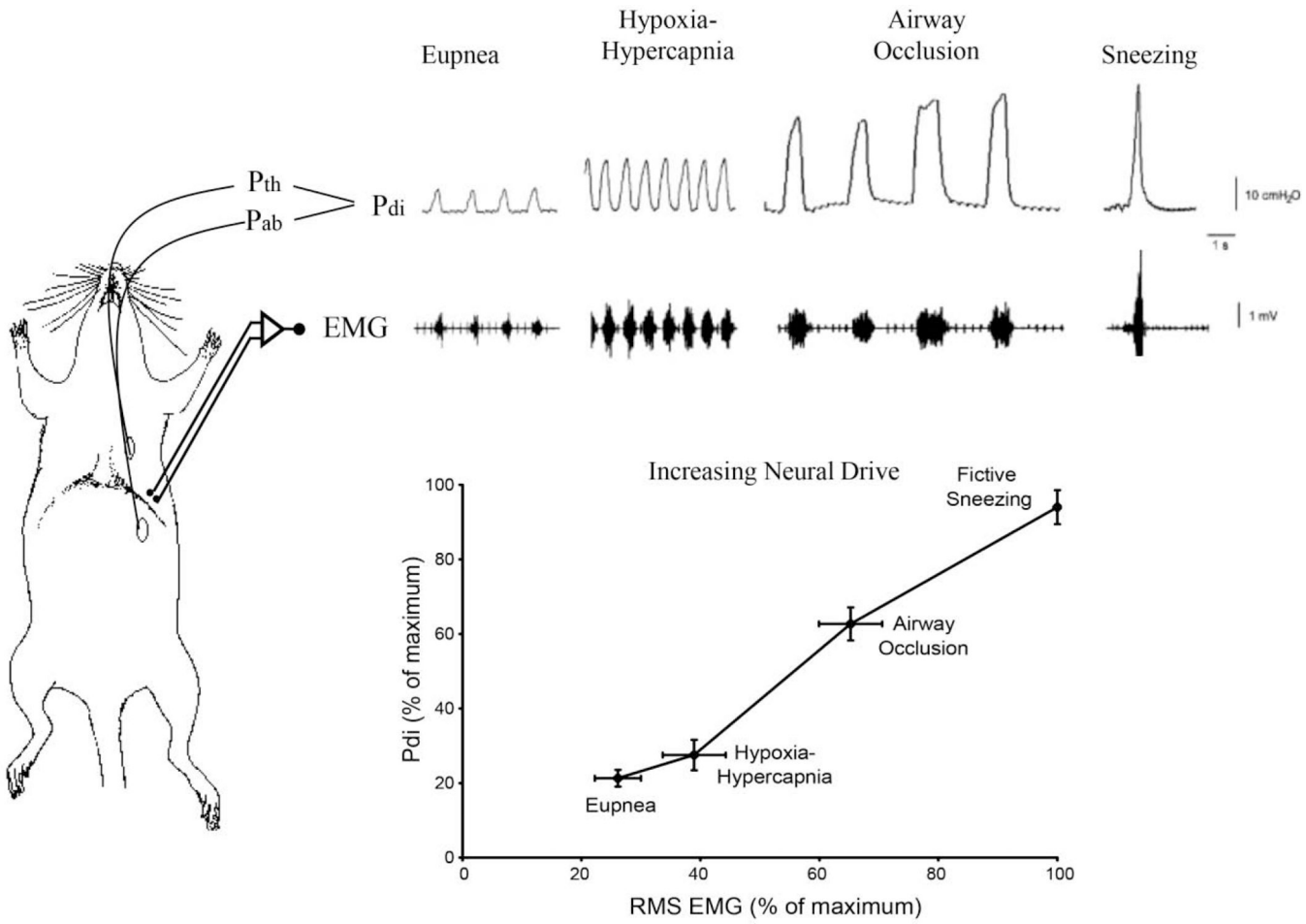

Figure 1:

Anatomical schematic illustrating oesophageal and gastric placement of the solid-state pressure catheters measuring the intra-thoracic (P_{th}) and intra-abdominal (P_{ab}) pressures in rodents. Negative P_{th} during DIAm contraction causes inspiratory airflow. Caudal motion of DIAm during contraction also produces a positive P_{ab}. The resulting transdiaphragmatic pressure ($P_{di} = P_{ab} - P_{th}$) reflects the magnitude of DIAm force generation. Traces show representative P_{di} and raw electromyographic (EMG) measurements obtained in a rat across different ventilatory (eupnea, hypoxia/hypercapnia and occlusion) and non-ventilatory behaviors ('sneezing' induced by airway irritation with capsaicin). The graph depicts the relationship between DIAm EMG activity (maximum RMS EMG amplitude) and P_{di} measurements across different behaviors in adult rats. All values are normalized to P_{dimax}, achieved with bilateral phrenic nerve stimulation. Both RMS EMG and P_{di} increased progressively from eupnea to hypoxia-hypercapnia to airway occlusion to sneezing. The P_{di} and RMS EMG generated during occlusion and fictive sneezing was significantly greater than that generated during eupnea or hypoxia-hypercapnia.

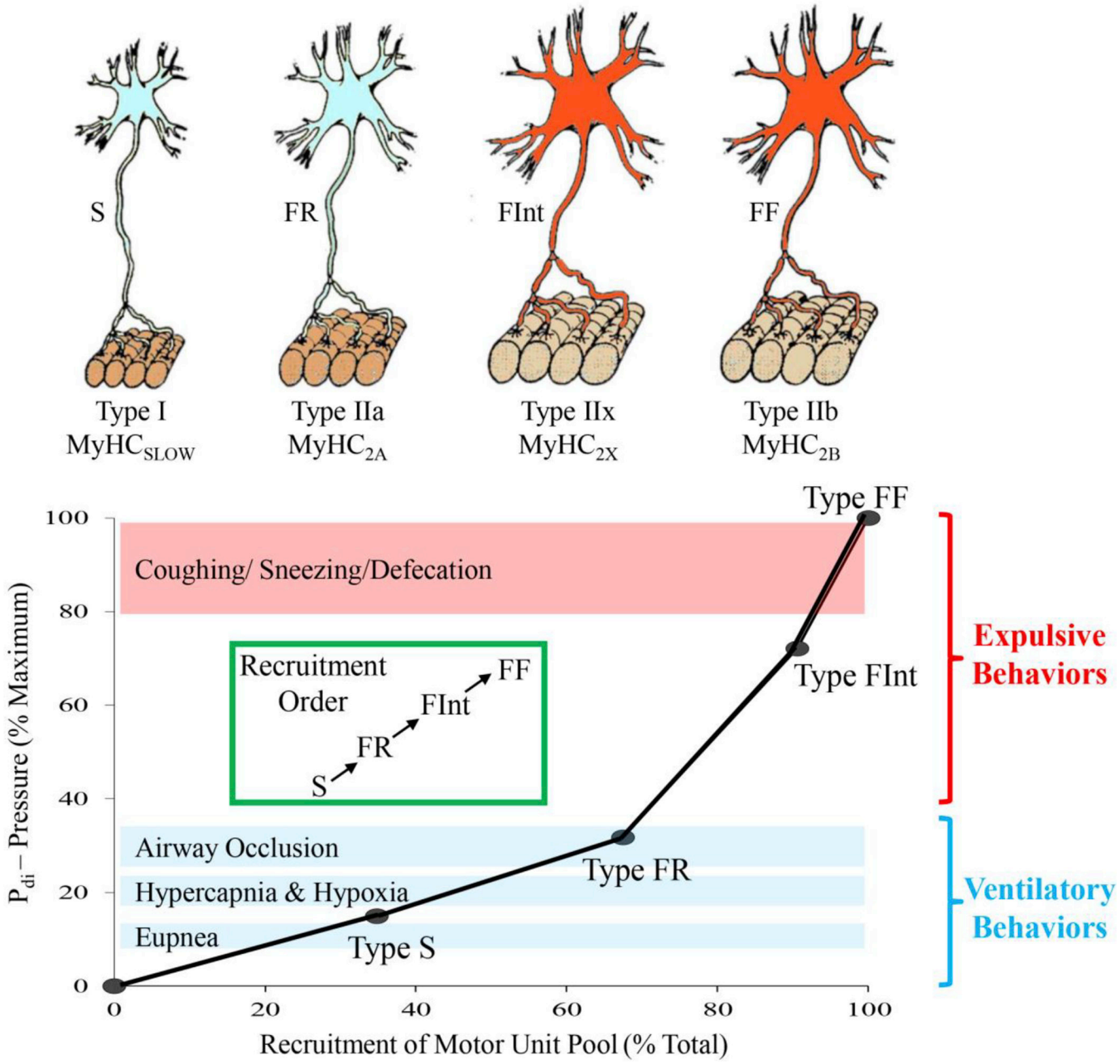

Figure 2:

Different DIAm motor unit types are distinguished by their intrinsic, mechanical, and fatigue properties, and are classified as type S, FR, FInt, and FF. Within an individual motor unit, all constituent muscle fibers exhibit homogeneous myosin heavy chain (MyHC) expression. In the DIAm of most species, type I and IIa muscle fibers have smaller cross-sectional areas than those of type IIx and/or IIb fibers. Differences in specific force between different fiber types is related to the different MyHC content per half sarcomere and the differing unitary forces produced by different MyHC isoforms, such that forces produced by type I fibers are less than forces produced by type IIa fibers that are less than forces produced by IIx and/or IIb fibers. Recruitment of DIAm motor units is in an orderly fashion, necessary to

accomplish a range of motor behaviors. Ventilation (eupnea, hypoxia/hypercapnia and breathing against occlusin) is accomplished by recruitment type S and FR motor units, whereas higher-force airway clearance behaviors and straining/expulsive manoeuvres require recruitment of more fatigable type FInt and FF motor units. Adapted from elements within [14].

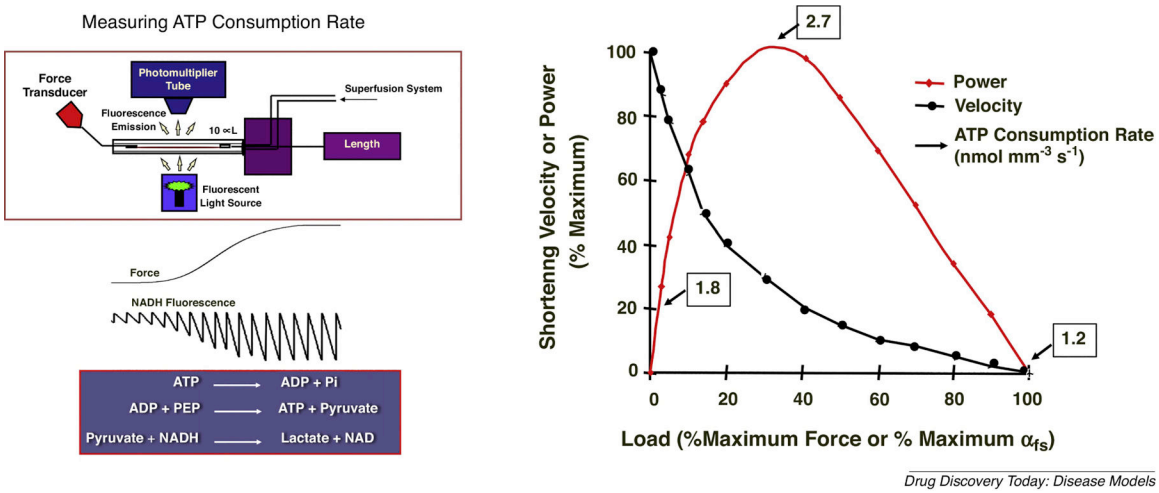

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Figure 3:

Simultaneous measurement of ATP consumption rate and force production in single permeabilized DIAm fibers is achieved by use of a perfusion cuvette. In the presence of activating or relaxing solutions, DIAm fiber isometric force is measured by a force transducer. ATPase activity was measured at 15°C by using a fluorescence-coupled enzyme assay, which involves the following reactions: i) ATP is hydrolyzed by the actomyosin ATPase to ADP and inorganic phosphate (Pi); ii) ATP is regenerated from ADP and PEP by PK; and iii) the resulting pyruvate is converted to lactate by LDH, which results in stoichiometric conversion of fluorescent NADH to nonfluorescent NAD^+. For each 1 mole of ADP produced by actomyosin ATPase, 1 mole of NADH is converted to NAD^+. Thus, the amount of ADP production by is determined by measuring the decrease in NADH fluorescence after stopping the flow in the cuvette. In single DIAm fibers, peak ATP consumption rates (2.7 nmol mm^{-3} s^{-1}) occur at peak power output. The shortening velocity is reduced with loading.

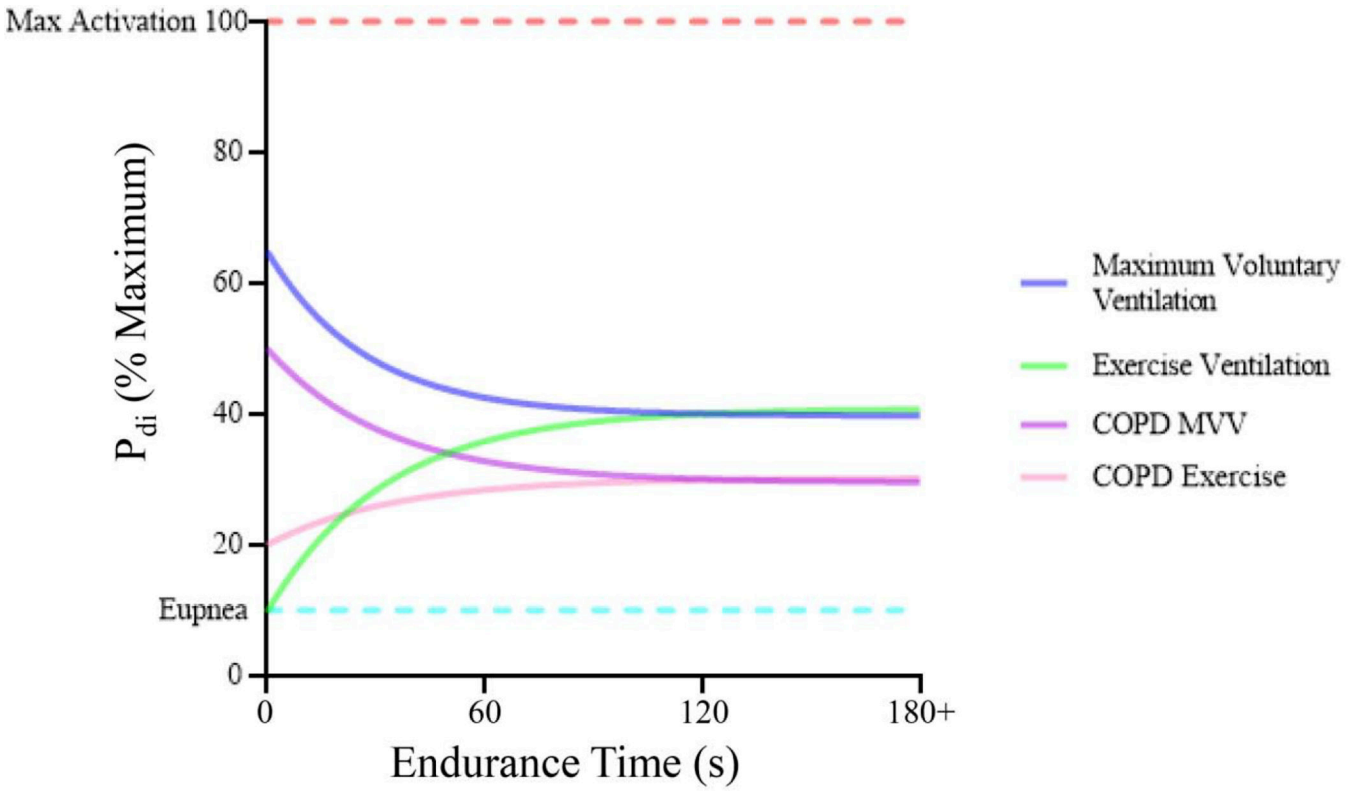

Figure 4:

Maximum voluntary ventilation (MVV) efforts approach ~60–70% of the maximum activation (P_{di}) of the DIAm. These efforts cannot be maintained and fatigue occurs at a level of ~60% of this effort, or ~40% of the maximum activation. By contrast, exercise increases activation until all fatigue resistant (type S and type FR) motor units are recruited. This level of activation, ~40% of maximum P_{di} can be maintained indefinitely. In scenarios where there are altered chest mechanics (eg. COPD), both MVV and exercise ventilation are reduced. Sadly, systematic assessments of P_{di} during disease conditions or exercise are lacking, particularly in relation to maximum P_{di}.