Dynamic Equilibration of Airway Smooth Muscle Length during Physiological Loading

By

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ABSTRACT

Airway smooth muscle shortening is of importance in the understanding of asthma. While previous study of this phenomenon has focused on static mechanical equilibrium of the muscle under static loading conditions, it has recently been proposed that muscle length is actually determined by a dynamically equilibrated state of the muscle. This is due to the effect of tidal stretches in perturbing the actin myosin binding in the smooth muscle. Previous studies have shown that force fluctuations around a constant mean force bias the muscle towards lengthening and oscillatory length changes cause a decrease in the active force generated by the muscle. In this thesis we have developed a method for applying a more physiological load that is representative of the actual load seen by the muscle in vivo. This method uses as its independent variable transpulmonary pressure fluctuations simulating tidal breathing. This method was implemented experimentally using a servo-controlled lever arm to load maximally activated airway smooth muscle strips with transpulmonary pressure fluctuations of increasing amplitude. This method shows results consistent with the theory of perturbed equilibrium of myosin binding and previous work using simpler loading conditions. The advantage to the method developed here is the incorporation of the airway physiology mathematically into the experimental setup. This allows for the study of many factors, such as airway wall thickening, loss of parenchymal support and breathing pattern, which were previously outside of experimental control.

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Chapter 1

Introduction

Asthma is a chronic inflammatory lung disease affecting over 17 million Americans, making it the 7th ranked chronic condition in the United States (1). The instances of asthma have increased dramatically over the past twenty years, particularly in children who make up over one third of reported cases and mortality rates for this disease have increased over 55% in the past twenty years. While many triggers for asthma attacks are known, such as cold air, exercise, and allergens, and medicines for the control of asthma are available, the cause of asthma and the mechanism behind its major symptoms remain uncertain (1).

The three major features of the disease are chronic inflammation of the airway, airway hyperresponsiveness, and reversible airway obstruction (2). Airway hyperresponsiveness is defined as excessive airway constriction after the application of a nonspecific stimulus such as histamine or methacholine. This increase in responsiveness can be either an increased sensitivity (hypersensitivity) corresponding to a leftward shift on the dose response curve, or an increased degree of response, indicated by an elevation or lack of the plateau seen in dose response curves compared with healthy subjects (3,4). In healthy individuals, deep inspirations, such as naturally occurring sighs, have the effect of immediate bronchodilation. In asthmatics, however, there is evidence that deep inspirations not only fail to dilate the airways, they can in fact worsen airway

obstruction (5, 6). While airway obstruction can be caused by many processes including airway wall thickening, loss of parenchymal support, and secretions into the lumen from inflammation (7), the key effector of airway narrowing is the airway smooth muscle (ASM). As the smooth muscle surrounding the airway shortens, the airway lumen narrows, limiting airflow. The amount that the airway smooth muscle shortens is determined not only by the force generated by the muscle during the contraction, but also the reaction force against which it is shortening. While it has been shown muscle at its optimal length can shorten to 30% of its original length if shortening against small isotonic loads, this does not normally occur in vivo, as it would cause airway closure (7,8). This indicates that the contraction of the muscle is not isotonic and that the surrounding lung parenchyma provides an elastic load upon the airway wall which decreases the amount of shortening.

While it is apparent that excessive airway smooth muscle shortening plays a pivotal role in the pathophysiology of asthma, the exact mechanism responsible for excessive shortening is unknown (9,10). The muscle length could be compromised by either an increase in the force generated by the muscle or a decrease in the load against which it acts. Unfortunately, studies have been able to show no conclusive evidence that airway hyperresponsiveness is due to either hypersensitivity or increased force generation of the muscle during isotonic or isometric smooth muscle contraction (9). Nor has any study been able to locate a specific cause for diminished loading of the muscle during inflammatory lung disease (10). However, these previous studies have focused on static conditions and static forces, namely, the isometric steady state force generated by the muscle and static loading conditions (11-15). While this approach will indicate the statically equilibrated length that muscle would tend towards, recent studies suggest that the

dynamically equilibrated state of the muscle achieved under fluctuating loading conditions must be further examined.

1.1 Dynamic Loading

Oscillatory stresses and strains are imposed continuously upon airway smooth muscle by the tidal action of breathing, and upon muscular arteries and arterioles by the pulsatile action of the heart. Smooth muscles in the urethra, urinary bladder and gut are also subjected to periodic stretches. Dynamic loading is an intrinsic part of smooth muscle physiology.

In the case of airway smooth muscle, the effects of oscillatory loads were first addressed by Sasaki and Hoppin (16) and later by Gunst and colleagues (8,17-19), who demonstrated that imposition of tidal changes in muscle length depresses the active force generated by the muscle. Subsequent studies showed that imposed fluctuations of muscle length about a fixed mean length caused graded depression of muscle force F and muscle stiffness E (averaged over the stretch), and augmentation of the specific rate of ATP utilization and the hysteresivity η (2,20). In 1999, Fredberg et al (2) performed experiments in which airway smooth muscle was subjected to sinusoidal fluctuations around a fixed mean muscle force simulating tidal breathing. Increasing the fluctuation amplitude was found to systematically biases the ASM toward lengthening. This phenomenon, called fluctuation-driven muscle lengthening, suggests a mechanism in which tidal muscle stretch acts as its own catalyst. The more the muscle stretches the easier it becomes to stretch more.

1.2 Perturbed Myosin Binding Theory

To explain how the tidal action of lung inflation modulates smooth muscle function the theory of perturbed myosin binding has recently been put forward (2). This theory holds that lung inflation strains airway smooth muscle with each breath. Cross bridges between the myosin head

and the actin filaments cycle naturally between the force generating attached state and the relaxed detached state (21-23). However the periodic mechanical strains imposed by tidal breathing, when transmitted to the myosin head can cause it to detach from the actin filament much sooner than it would during an isometric contraction. This premature detachment profoundly reduces the duty cycle of myosin, typically to less than 20% of its value in the isometric steady state, and depresses to a similar extent total numbers of bridges attached and active force (2). Of the full isometric force generating capacity of the muscle, therefore, only a modest fraction comes to bear on the airway narrowing, even when the muscle is activated maximally. At the macroscopic level the fully activated muscle is much less stiff and much more viscous than in an isometric contraction, and becomes in effect a gooey liquid. At the molecular level this liquid–like state corresponds to perturbed equilibrium of myosin binding, in which there are few cross links attached at any moment but they are cycling very rapidly, almost as if the muscle had 'melted'.

However this process would become compromised without the effect of the load fluctuations on the muscle. In asthma for example, the chronically inflamed airway and the peribronchial adventitia remodel in such a way that is thought to uncouple the muscle from the load fluctuations (24). Such an uncoupling would permit myosin to approach an unperturbed binding equilibrium, where the cross bridges would convert to slower cycling latch bridges. In this case the muscle would shorten, stiffen and virtually freeze in the latch state and the myosin duty cycle would tend toward 100% of its value in isometric contraction (2). At the macroscopic level the fully activated muscle is much more stiff and less viscous than in the melted state, and becomes in effect a solid-like substance that is characterized at the molecular level by a static equilibrium of myosin binding in which there are many cross links attached at any moment, but

they are cycling very slowly, almost as if the muscle had 'frozen'. Because there is no load fluctuations occurring the muscle in this state is statically equilibrated.

The melted or dynamically equilibrated state is thought to govern airway reactivity in the healthy individual and, thereby, to account for the normal airway's response. That is to say, the existence of the plateau on the dose response curve, as well as the modest level of that plateau, are attributable to the relatively small active force that is attainable by the muscle in these states (2). The potent bronchodilator response to a deep inspiration is attributable to the decreased muscle stiffness, which permits big muscle stretches. By contrast, the frozen (or latch) state is thought to govern airway reactivity in the asthmatic individual and, thereby, to account for excessive airway narrowing (hyperresponsiveness) (2). In that circumstance the elevated level of the plateau in the response or the disappearance of the plateau altogether, are attributable to the large active force that can be attained in muscle in the isometric steady state. (25). The impaired ability of deep inspirations to dilate the airway is attributable the fact that muscle becomes so stiff in the frozen state that it stretches little in response to the physiological forces that are imposed upon it during the deep inspiration.

The objective of this thesis is to examine these phenomena under the most physiologically accurate conditions possible. To this end, we have developed a mathematical model that simulates the actual physiologic load seen by airway smooth muscle during tidal breathing. This model was then applied experimentally to airway smooth muscle. The development of this muscle system will allow for the investigation of many aspects of airway physiology in conjunction with the effect of dynamic loading on the muscle.

Chapter 2

Physiological Muscle Loading Conditions

To further investigate the implications of perturbed equilibrium of myosin binding it is essential to study smooth muscle tissue under dynamic as well as static loading conditions. Previous studies into perturbed equilibria have simplified the ASM system by either loading the muscle with fluctuations around a constant mean force or oscillating it around a constant mean length. Neither of these conditions approaches the actual physiological loading the muscle experiences in vivo. In this thesis our goal is to develop a physiologically based load characteristic and to examine experimentally the effects on airway smooth muscle.

Using the known relationship between bronchial radius (and thus muscle length) and peribronchial stress, as well as the theories developed by Macklem (13) and Lambert et al. (24), which include the pressure-area relation of the airway and Lai-Fook's measurements of the parenchymal shear modulus and its dependence on transpulmonary pressure (26,27) a "virtual" load can be constructed, which is representative of the load presented to the airway smooth muscle by the airway wall and the surrounding parenchyma *in vivo*. This virtual load was then integrated into a servo-controlled lever system attached to maximally activated airway smooth muscle isolated in a muscle bath. The system was then made to "breathe" by varying the virtual transpulmonary pressure to simulate tidal breathing. In this apparatus the muscle is free to

accommodate its length according to the time-varying virtual load that is mechanically in series with it.

The strength of this method is that because it is physiologically based, factors such as breathing pattern, airway wall thickening and changes of parenchymal elastic support are represented mathematically within the setup, and are under experimental control. While the goal of this thesis is the development of this method and focuses on the effect of the nonlinearity of the elastic load and its changes in time, the eventual goal is to measure the extent to which these factors modulate the muscle state, including fluctuation driven lengthening, myosin dynamics, and plasticity of the contractile response.

2.1 Setting the Muscle Load:

Airway smooth muscle *in situ* is tethered elastically to the lung parenchymal tissues that surround the airway. The lung parenchyma is deformable, and this deformability comes into play in two ways. First, for a given fixed transpulmonary pressure, parenchymal deformability determines how peribronchial stress varies as airway smooth muscle shortens. Second, for a given fixed smooth muscle length, parenchymal deformability determines how peribronchial stress varies as the transpulmonary pressure increases (11,26,27). The implication of parenchymal deformability in real physiologic circumstances is that neither the length of the smooth muscle nor the peribronchial stress is the independent variable; rather, the transpulmonary pressure is the independent variable. From the point of view of the muscle, therefore, the deformability of the airway and the surrounding parenchyma may be thought of as being the source impedance, with changes of transpulmonary pressure being the source.

Experiments have previously been performed using length control, which corresponds to applying an infinite source impedance to airway smooth muscle (8,17-19), and force control,

which corresponds to applying a zero source impedance (2). The goal in this case however is to load the muscle using a realistic physiologic source impedance. This was done using the known relationship between bronchial radius, R (and thus muscle length, L) and peribronchial stress to create a virtual load impedance as presented to the airway smooth muscle by the airway wall and the surrounding parenchyma.

2.2 Mathematical Formulation of Load

The law of Laplace describes the force that the muscle must generate, Fmus(R), in order for the airway to attain a given radius R. The forces developed by passive structures within the airway wall are Fwall(R), and the transmural pressure Ptm is the difference between the internal pressure and the peribronchial distending stress. Then,

$$Fwall(R) - Fmus = PtmR \tag{1}$$

We consider first Fwall (R). For the passive structures of the isolated airway, Lambert (28) developed a mathematical formulation of the relationship between airway radius (R) and transmural pressure Ptm_o, where Fwall(R) = R Ptm_o,

$$\begin{aligned} R &= \sqrt{\alpha_o (1 - Ptm_o / P_1)^{-n_1}} && Ptm_o \le 0 \\ R &= \sqrt{1 - (1-\alpha_o)(1 - Ptm_o / P_2)^{-n_2}} && Ptm_o \ge 0 \end{aligned} \tag{2}$$

$$\begin{aligned} P_1 &= \alpha_o n_1 / \alpha_o' \\ P_2 &= -n_2 (1 - \alpha_o) / \alpha_o' \end{aligned}$$

The values of the constants are determined to be α_o'=0.1125, P_1=0.1, n_1=0.045, P_2=-20, and n_2=3. It is also necessary to use the inverse of these functions.

$$Ptm_o = P_1\left[1-\left(\frac{R^2}{\alpha_o}\right)^{-1/n_1}\right] \qquad R \le \sqrt{\alpha_o}$$
$$Ptm_o = P_2\left[1-\left(\frac{1-R^2}{1-\alpha_o}\right)^{-1/n_2}\right] \qquad R \ge \sqrt{\alpha_o} \tag{3}$$

We consider next the transmural pressure (Ptm), which can be computed from the work of Lai-Fook (27) as,

$$Ptm(R, P_L) = P_L - 2\mu\frac{(Ri - R)}{Ri} \tag{4}$$

where

$$\mu = 0.7P_L$$

The second term on the right hand side of equation 4 gives the component of the distending stress attributable to shear deformation of the lung parenchyma when the airway radius R departs from its undeformed reference radius Ri.

Returning to the force balance equation (equation 1) we can now compute the force that the muscle must generate, Fmus, in order for the airway to attain a given radius R, solely in terms of the transpulmonary pressure,

$$Fmus(R, P_L) = R\left[P_L + 2\mu\frac{(Ri - R)}{Ri}\right] - Fwall(R) \tag{5}$$

This is the muscle load. This load characteristic is shown in Figure 1 for a series of different transpulmonary pressures. Since this load is purely elastic, if the transpulmonary pressure P_L changes in time, the load will change in time as dictated by Equation 5.

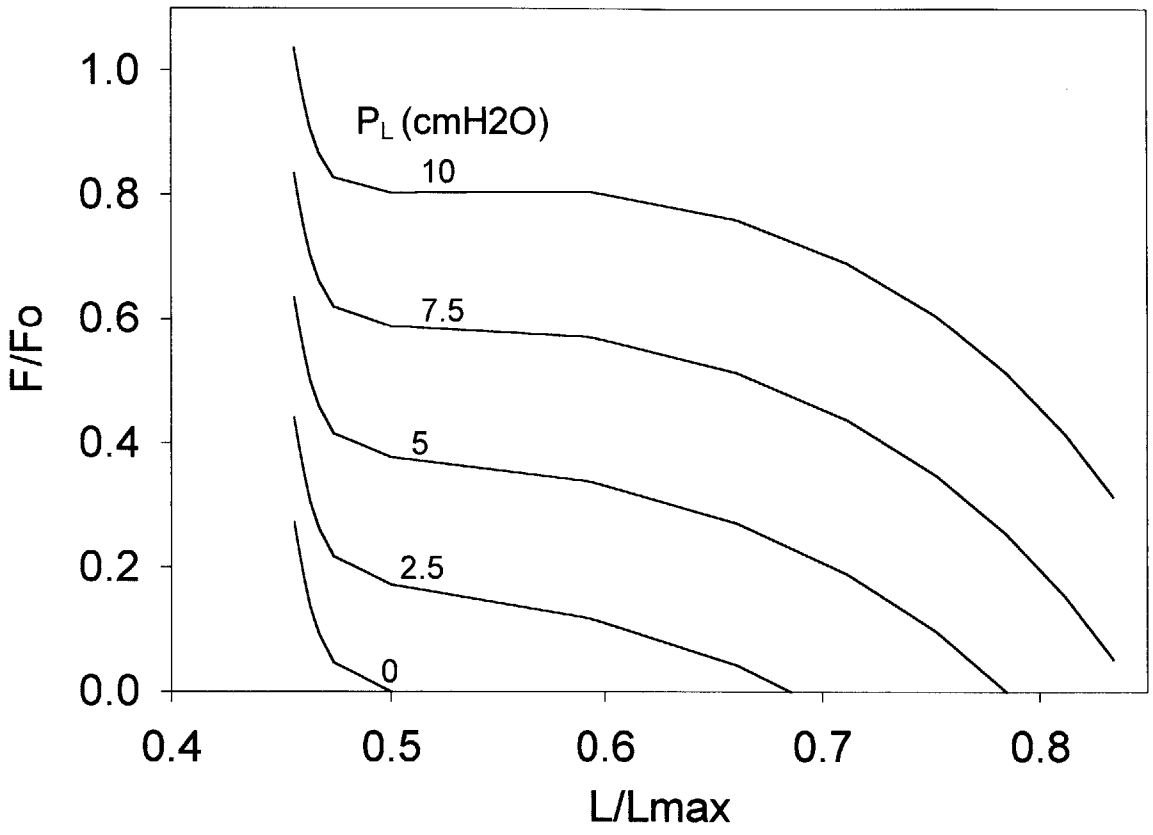


Figure 1. The force- length load characteristic for transpulmonary pressures of 0, 2.5, 5, 7.5, and 10cmH2O, dictated by Equation 5.

Chapter 3

Experimental Setup and Protocol

3.1 Servo-Controlled Lever Arm System

This load characteristic was next integrated mathematically into a servo-controlled lever system to create, in the muscle bath, dynamic loading conditions that closely approximate those expected *in vivo*. The muscle is attached to a servo controlled lever arm (Model 305B; Cambridge Technology) via a force transducer. The force and length output signals were low pass filtered with a cutoff at 25Hz then sent through and analog to digital converter to a computer. A program was written using National Instruments LabVIEW software, version 4, which samples the signals from the lever arm at 50Hz and stores the data to file. The length signal (normalized by the optimal length, Lo) and the independently determined transpulmonary pressure were then used to calculate the load (from equation 5) to be applied to the muscle. Because the lever arm system works under length, not force, control it was necessary to use a second PID controller to convert the desired force signal generated by the LabVIEW program to a length signal. The PID controller operates on a digital switch, which switches it from length to force control as the muscle becomes activated. When set to force control, the PID controller calculates the desired length signal to send to the lever arm from the force signal output by the computer and the measured force. The control switch must be set to length control and the initial length signal to

zero before the beginning of the experiment to prevent damage to the muscle from unexpected signals. This was done by including code to initialize the systems at the end of the LabVIEW program and allowing it to run for one minute without connecting the length output to the lever arm. In order to make the muscle "breathe" a transpulmonary pressure signal was created which varied sinusoidally at a rate of 0.2 Hz, allowing 250 samples per cycle. At the end of the experiment the LabVIEW program sends a signal to the lever arm to hold its position until stopped by the user to prevent damage to the muscle strip. The program then reinitializes the system.

3.2 Tissue Preparation

For these experiments smooth muscle from bovine tracheas were obtained from a local slaughterhouse. A section of 4-5 rings in the caudal to central region of each trachea is removed and stored in a cold Krebs-Henseleit solution (in mM: 118 NaCl, 4.59 KCl, 1.0 $KH2PO_4$, 0.050 $MgSO_4$, 0.18 $CaCl_2$, 11.1 glucose, 23.8 $NaHC0_3$; pH at 7.4) for up to 24 hours before use. Before the experiment the inner layer of connective tissue, adjoining cartilage and outer connective tissue were carefully removed and muscle strips measuring approximately 2mm by 10 mm dissected out. Each end of the tissue strip was glued (cyanoacrylate) to small brass clips. The strip was then suspended in a glass tissue bath using a fine steel rod (0.10mm diameter). The top of the rod was attached to the servo-controlled lever arm via a miniature force transducer. The lower clip was latched onto an adjustable glass hook positioned near the bottom of the bath. The bath is then filled with a Krebs solution aerated with 95% O_2-5% CO_2 and maintained at 37°C by a surrounding water jacket.

3.3 Experimental Protocol

After being placed in the bath the muscle strip was allowed to equilibrate for 1hour. The strip was then brought to optimal length (Lo) using electric field stimulation (EFS) adjusted for optimal response, beginning in the neighborhood of 20V at 40Hz, 1.5 ms pulse duration, for approximately 30s. The passive force on the muscle before stimulation and the peak force achieved during were recorded and the difference was used to determine the active force. Between the stimulations, which were performed at five minute intervals, the muscle was allowed to relax for 1 minute then stretched in increments of 1mm. When the percent error between successive active force values reached 1% or below the muscle was determined to be at optimal length which was then measured with calipers. The maximal tension (To) was then determined by activating the muscle with a dose of Ach (10^{-4} M) and allowing it to contract for 15 minutes. The bath at that time was flushed 5 times with fresh Krebs solution until the muscle force returned to the passive level. At the same time the LabVIEW program was run for one minute to set the initial length signal to zero and the switch in the external PID controller to length control. After entering the values for Lo and To (in volts) and the duration in minutes for each part of the protocol into the front panel of the LabVIEW program and connecting the length output to the lever arm, the program was started. The muscle strip was immediately loaded at a fixed (virtual) P_L of either 2.5 or 5 cm H_2O (delpl=0), while the muscle was again activated with a high constant concentration of Ach (10^{-4} M), and allowed to shorten 120 minutes to its static equilibrium length (LSE). As soon as the muscle was activated, the PID controller was switched from force to length control. In order to maintain a constant concentration of Ach in the bath a solution of 10^{-4}M Ach in Krebs was pumped through the bath throughout the experiment. The muscle strip was given two hours to equilibrate to the static conditions, then sinusoidal transpulmonary pressure

fluctuations with amplitude δP_L and frequency of 0.2Hz were imposed on the muscle. After 60-minute intervals to allow the muscle to become dynamically equilibrated at a new length, δP_L was increased from 10% to 25%, 50% and 100% of the initial fixed P_L. After completing the largest fluctuation amplitude ($100\%P_L$), the fluctuation amplitude was returned to $25\%P_L$ and the muscle allowed to reshorten. When the protocol was finished the LabVIEW program held the muscle in position until directed by the user. In order to avoid damage to the muscle it was necessary to remove the strip from the lever arm before allowing the program to reset the length signal to zero and the switch to length control. The muscle was returned to the bath after the signals were reset and again flushed 5 times with fresh Krebs solution. Once the muscle had returned to the passive state it was again activated with Ach and allowed to contract 15 min to determine if the force generating capacity of the muscle has been affected.

Chapter 4

Results

Figure 2a depicts a representative tracing of muscle force versus muscle length. The initial force and length of the relaxed muscle is depicted by the closed circle. When the transpulmonary pressure (P_L) was held constant at 5 cmH_2O and acetylcholine was added to the bath, the muscle force increased and the muscle length decreased. The muscle shortened along the corresponding load characteristic until it stopped shortening at the point indicated by the open circle. This point represents the statically equilibrated state of the muscle.

After two hours the muscle had become statically equilibrated at a transpulmonary pressure of 5 cmH_2O; sinusoidal fluctuations of transpulmonary pressure were then initiated with an amplitude of 5cmH_2O at a frequency of 0.20 Hz. Upon applying these fluctuations, force-length loops arose and spiraled slowly down and to the right until a steady-state loop was observed (shown in gray). This loop corresponds to the dynamically equilibrated state of the muscle for the proscribed loading conditions. Imposed fluctuations of transpulmonary pressure drove the muscle to progressively greater lengths and smaller forces. A series of dynamically equilibrated loops for increasing amplitude of transpulmonary pressure fluctuations is shown in Figure 2b.

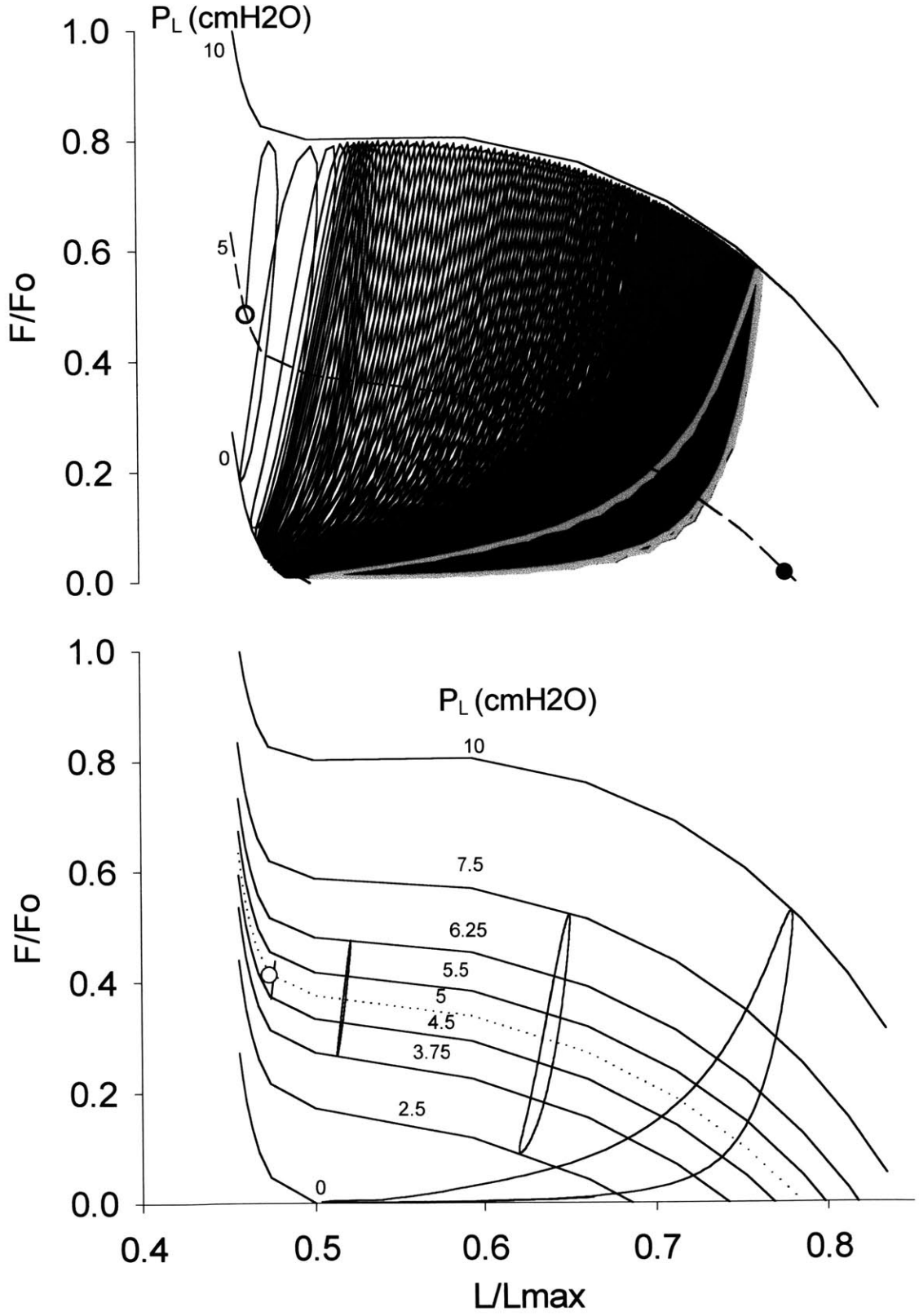


Figure 2A. Transpulmonary pressure fluctuations with amplitude of 5cmH2O around a mean of 5cmH2O cause the muscle to move from the statically equilibrated state (open circle) to the dynamically equilibrated loop indicated in gray. The closed circle shows the passive state of the muscle. Figure 2B. The statically equilibrated state of the muscle (open circles) and the steady state loops for ASM dynamically equilibrated to pressure fluctuations of 0.5, 1.25, 2.5 and 5cmH2O show that increasing fluctuations drives the muscle to greater mean lengths and lower mean forces.

The evolution of the force, length, stiffness, hysteresivity, and tidal strain (averaged over the tidal loop) is shown in figure 3 for a representative muscle strip. With the onset of the contractile stimulus the force increased and the muscle length decreased. Although the initial shortening was quite rapid, approximately two hours were required for the muscle to reach its statically equilibrated length. This time span stands in contrast to the much shorter interval that is required for muscle held in isometric conditions to reach a force plateau. Although the muscle length equilibrated, the muscle force did not establish a plateau. As the fluctuations in transpulmonary pressure were increased in a stepwise manner, the muscle force and stiffness fell, and the muscle length and hysteresivity increased. The pooled steady state data for a mean transpulmonary pressure of 5cmH_2O is shown in figure 4.

Figures 2b, 3 and 4, taken together indicate that when δP_L amplitude was increased from 0 to 0.5 cm H_2O, the instantaneous muscle state simply oscillated about the static equilibrium state. The mean muscle length and force over the cycle remained close to their static equilibrium values (point B). But when δP_L was 2.5 cmH_2O or more, the force fell, the muscle lengthened, and became progressively less stiff and more hysteretic (figures 3 and 4). Although the muscle was at all times supporting the same mean transpulmonary pressure (5cm H_2O), the force and length to which the muscle equilibrated systematically exceeded the static equilibrium force and length. Fluctuations of the transpulmonary pressure systematically biased airway smooth muscle length.

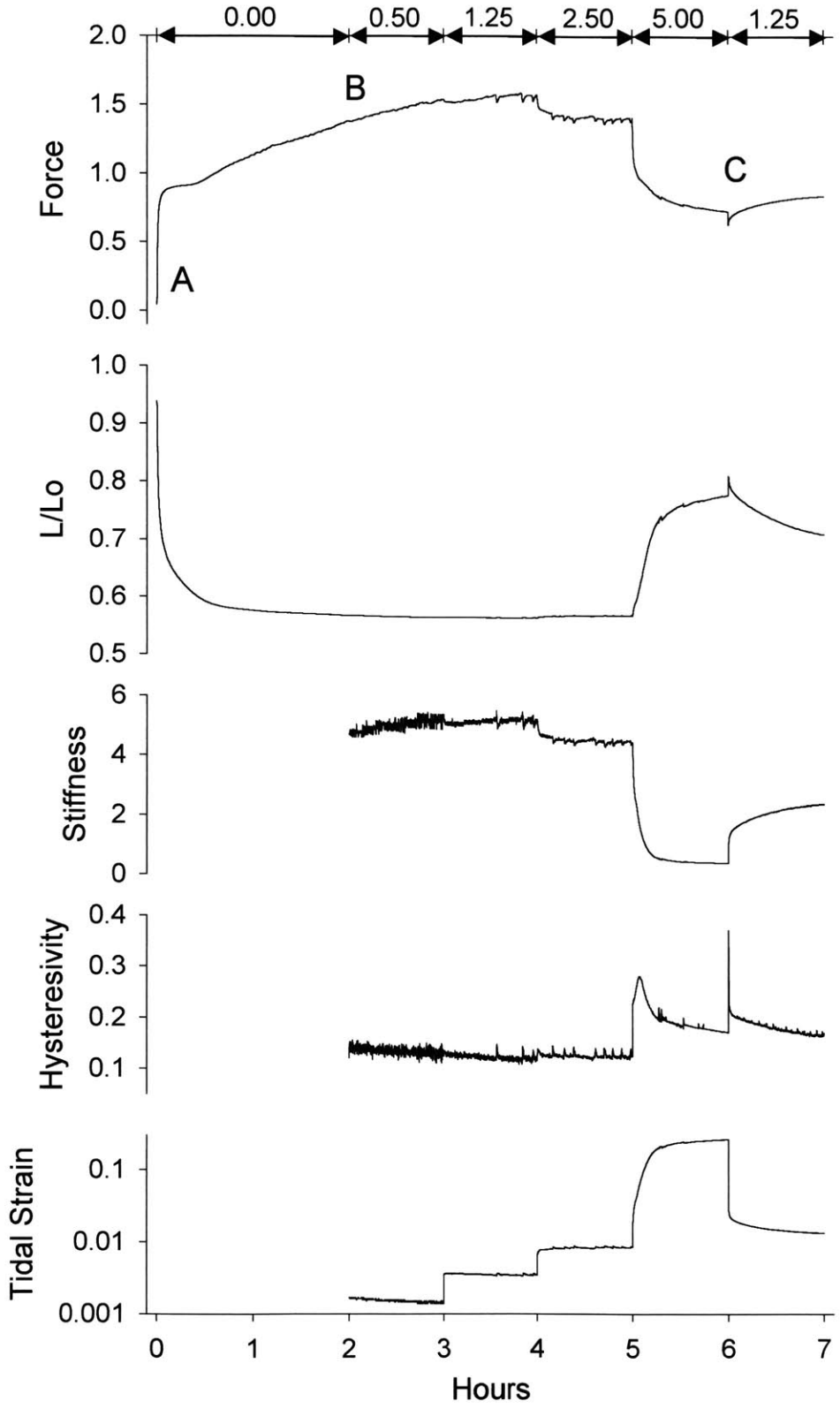


Figure 3. Evolution of mechanical properties of a representative muscle during contraction against a steady mean transpulmonary pressure of 5cmH2O upon which is superimposed pressure fluctuations (0.2Hz) of graded amplitude δP_L. The mechanical properties shown are mean force and length averaged over each cycle, Loop stiffness, hysteresivity (dimensionless) and tidal strain. Pressure fluctuations are seen to drive the contractile state away from static equilibrium conditions.

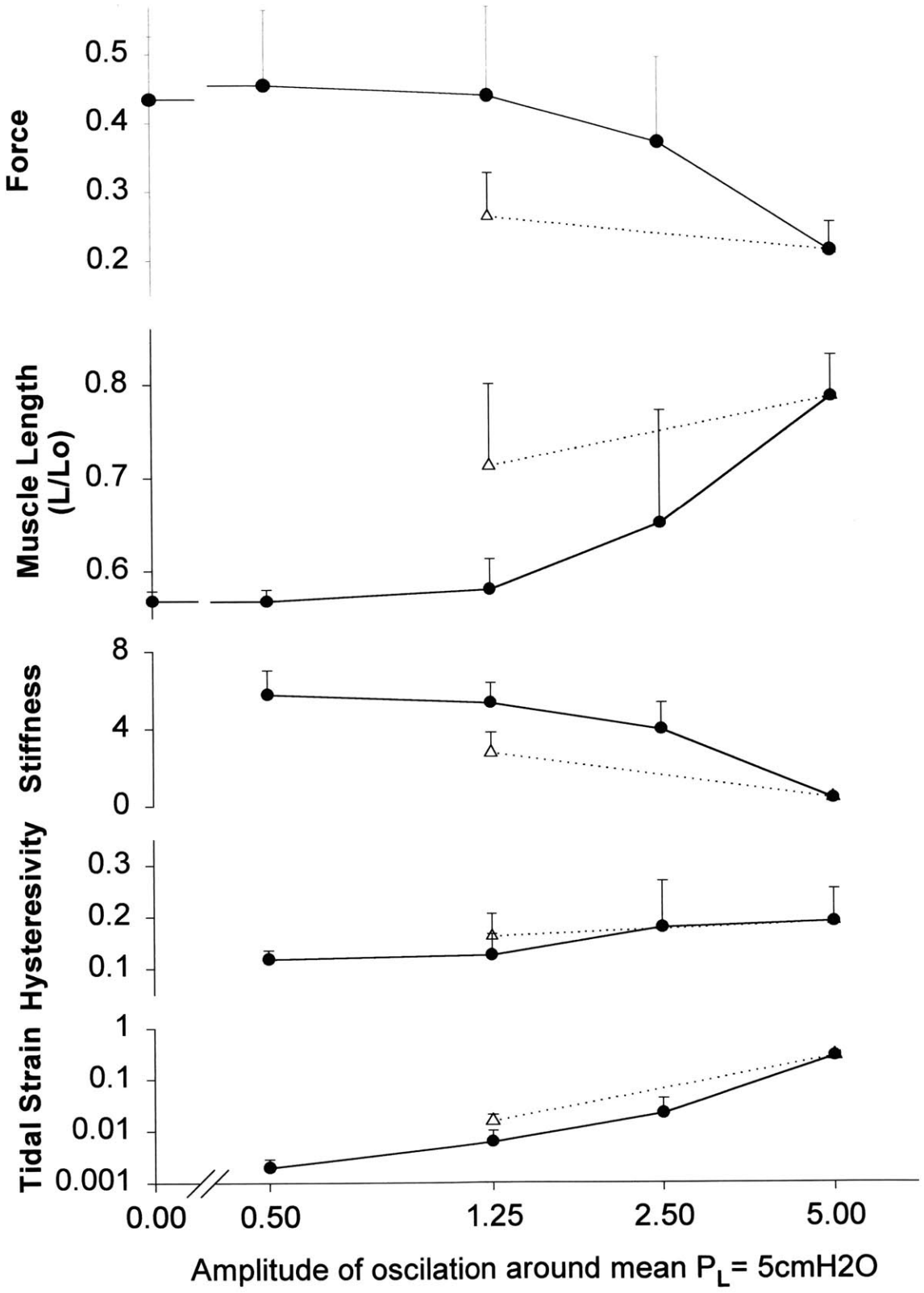
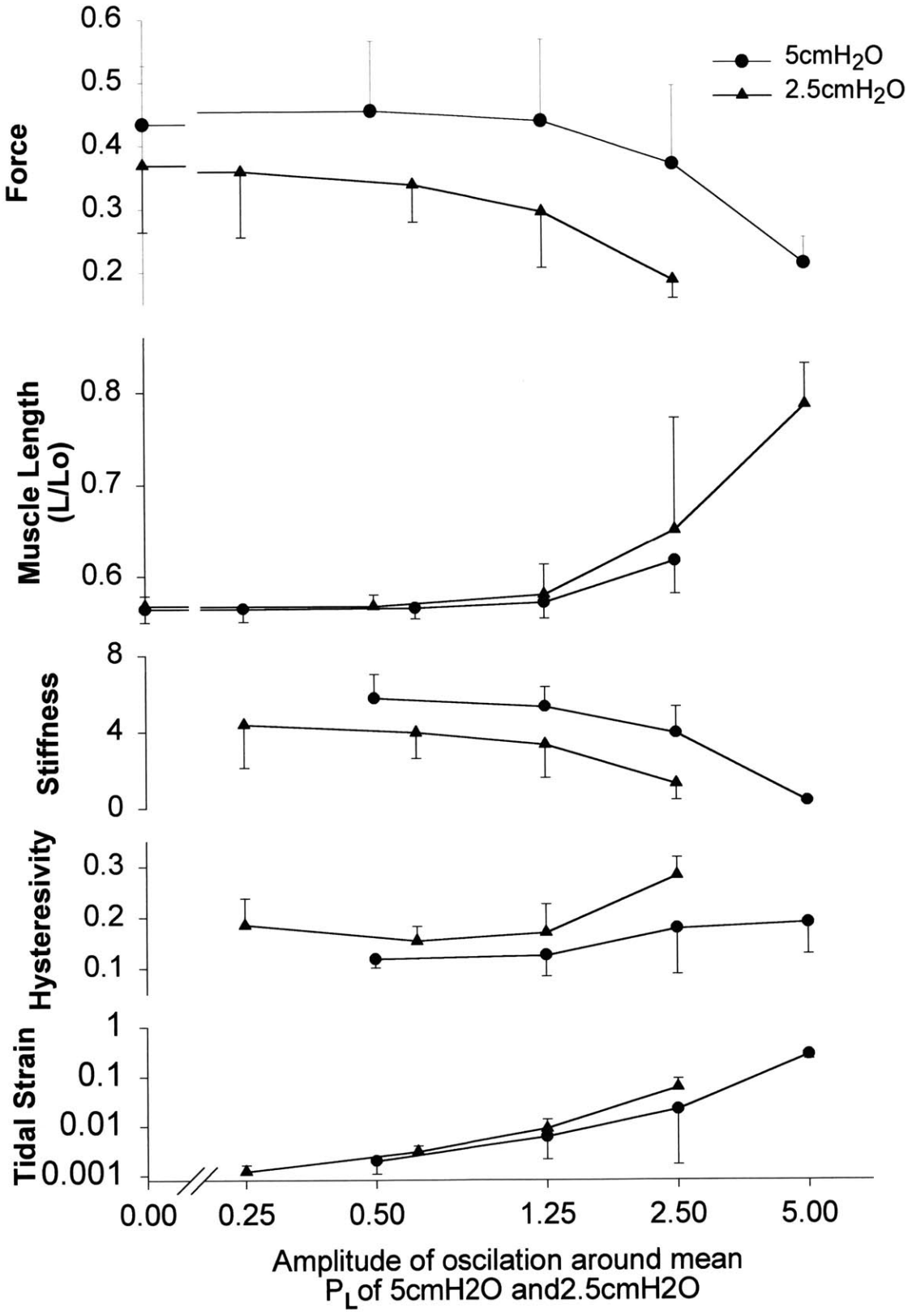


Figure 4. Pooled observations for muscle strips with mean transpulmonary pressure of 5cmH2O(n=5; error bars denote SD between muscle strips drawn in only one direction for clarity) for dynamically equilibrated force and length, loop stiffness, hysteresivity, and tidal length change versus pressure fluctuation amplitude. Open triangles denote state after pressure fluctuations were reduced from 5cmH2O back to 1.25cmH2O.

If the amplitude of the tidal fluctuation of transpulmonary pressure was subsequently reduced from 5 back to 1.25 cmH_2O (point C), the muscle reshortened to a new length that was substantially greater than the prior length in identical loading conditions (Figures 3 and 4[triangles]). Therefore, the fluctuation amplitude necessary to maintain the muscle at that new length was smaller than that required to break through initially and attain that length. Conversely, if the fluctuations were kept at 1.25 cmH_2O or less throughout the contractile event, the muscle remained close to the static equilibrium length, as if it were stuck at that length. The dynamic equilibrium length was determined by the tidal loading dynamics and, importantly, by the history of the tidal loading dynamics.

Following these tidal loading maneuvers, the isometric force generation capacity of the muscle was not compromised, retaining 85% or more of the initial capacity. Therefore, fluctuation-driven lengthening was not accounted for by muscle injury or fatigue. Rather, the muscle attained different modes of steady state operation, and could be made to switch between these modes by alteration of the history of the dynamic loading. As such, the contractile state was conditionally stable.

When these experiments were repeated at a smaller mean transpulmonary pressure (2.5cmH_2O), the dynamically equilibrated force and stiffness of the muscle were smaller and the hysteresivity was large than at the higher mean transpulmonary pressure (Figure 5). The smaller mean transpulmonary pressure had little effect on muscle length, however, probably due to the nature of the load characteristic, which was extremely steep at small muscle length.



'igure 5. Comparison of pooled observations for muscle strips with mean transpulmonary pressure of cmH2O(circles) and 2.5cmH2O (triangles) for dynamically equilibrated force and length, loop stiffness, ysteresivity, and tidal length change versus pressure fluctuation amplitude.

Chapter 5

Discussion

We have found that when activated airway smooth muscle is loaded under dynamic conditions that mimic as closely as possible those expected *in vivo*, muscle length becomes equilibrated at lengths that substantially exceed the static equilibrium length. The equilibration process is quite slow compared with the duration of one breathing cycle, and depends upon the magnitude of the pressure fluctuations.

The results reported here build on previous experiments corresponding to somewhat simpler loading conditions, namely, force control that was length independent (2). In the force control method, muscle force is specified and length must adapt accordingly. The method and results presented here have the advantage that both force and length are allowed to adapt, as is the case for muscle *in vivo*. The extent of the fluctuation driven muscle lengthening was smaller in the data reported here than in the force control method because the loading force decreased as muscle length increased as can be seen in figure 1. Nonetheless, the dynamically equilibrated muscle length substantially exceeded the statically equilibrated muscle length when the tidal variations in transpulmonary pressure exceeded 2.5 cmH_2O (figures 4 and 5). This suggests that even in the case of quiet tidal breathing the effects of tidal respiration are important in maintaining airway caliber; this conclusion is consistent with the findings in human subjects by Molfino et al

(29). These results are consistent with the theory of perturbed myosin binding (2) put forth to explain how the tidal action of lung inflations modulates smooth muscle function.

The dynamic equilibration process that results from a sudden change in the amplitude of the fluctuations of transpulmonary pressure had a time constant in the range of 20 minutes or more, which is slow compared with the duration of one breathing cycle (figure 3). On the other hand, it has been reported previously (20) that when length control is used to impose fluctuations of muscle length, the muscle becomes dynamically equilibrated very quickly, within one to three breaths. These seemingly disparate time scales required for the muscle to become dynamically equilibrate are reconciled by differences in the source impedance driving the muscle. In the case of length control, the servo-controller exerts whatever force that is required to attain the assigned length fluctuation, and in doing so disrupts many attached actin myosin cross bridges with the very first stretch. The myosin bond length distributions then adjust over time scales governed by bridge attachment and detachment rates, the slowest of which are on the order of seconds (30). By contrast, activated muscle that has become statically equilibrated muscle is relatively stiff compared to the source impedance driving the changes in muscle load in vivo and modeled here. It approximates a frozen state (2). Because it is so stiff, the muscle stretches relatively little when tidal changes of transpulmonary pressure are initiated, and very few bridges are disrupted with the first breath. After many such breaths, however, the muscle gradually works loose, so to speak, and the binding of myosin to actin can become strongly perturbed.

Although perturbed equilibria of myosin binding seem to be able to explain fluctuation driven muscle lengthening and its time course, this theory cannot explain the failure of the muscle to reshorten completely when the load fluctuations are removed (figure 4). This plasticity of the response must be attributable to other mechanisms (31-34).

Chapter 6

Conclusions

In this thesis we have developed and implemented experimentally a mathematical model for the non-linear dynamic load, representative of the load seen by airway smooth muscle in vivo and incorporated that load into an experimental apparatus using servo-controllers. This method provides a more physiologically accurate system for studying the effect of dynamic loading on smooth muscle and its implications in asthma. The results shown are consistent with the theory of perturbed myosin binding, which predicts that imposed load fluctuations will drive materials from solid to liquid like states. This simple idea of the activated muscle working in the frozen vs. the melted state seems to offer a unifying explanation for many features of the pathophysiology of asthma that were previously considered to be unconnected and inexplicable (2).

The key advantage of the experimental loading method reported in this thesis is that we can now simulate in the muscle bath many physiological factors that were previously outside of experimental control. The mathematical model for the source impedance lends itself readily to the incorporation of variables such as airway wall thickening, loss of parenchymal support, and changes of breathing pattern. It is known that asthmatics exhibit significant airway wall thickening from airway remodeling due to inflammation. The investigation of the effect of these factors on dynamically equilibrated muscle length is the natural next step and will contribute

important results towards the understanding the primary pathophsyiology of airway narrowing asthma.

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