MECHANICAL PROPERTIES OF ASTHMATIC AIRWAY SMOOTH MUSCLE
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Rationale: Airway smooth muscle (ASM) is the major effector of excessive airway narrowing in asthma. Changes in some of the mechanical properties of ASM could contribute to excessive narrowing and have not been systematically studied in human ASM from non-asthmatic and asthmatic subjects.

Methods: Human ASM strips (8 asthmatic and 6 non-asthmatic) were studied at in situ length ($L_{ret}$) and force was normalized to maximal force ($F_{max}$) induced by electric field stimulation (EFS). Measurements included: passive and active force versus length before and after length adaptation, the force-velocity relationship, maximal shortening and force recovery after length oscillation. Force was converted to stress by dividing by cross-sectional area of muscle.

Results: The only functional differences were that the asthmatic tissue was stiffer at longer lengths ($P < 0.05$) and oscillatory strain reduced isometric force in response to EFS by 19% as opposed to 36% in non-asthmatics ($p < 0.01$).

Conclusion: The mechanical properties of human ASM from asthmatic and non-asthmatic subjects are comparable except for increased passive stiffness and attenuated decline in force generation after an oscillatory perturbation. These data may relate to reduced bronchodilation induced by a deep inspiration in asthmatic subjects.
INTRODUCTION

Asthma is characterized by exaggerated airway narrowing caused by airway smooth muscle (ASM) shortening. However, it is unclear whether there is a fundamental phenotypic change in the ASM itself or if the non-muscle components of the airway wall or surrounding lung parenchyma are primary contributors to this airway hyperresponsiveness (AHR). [1,2] A major hurdle to a clear understanding of ASM contractile function in disease has been the limited data. Of the twelve studies in which ASM mechanical properties have been compared in asthmatic and non-asthmatic tissue, seven have demonstrated no differences [3-10], while five have shown increases in force, shortening, or agonist sensitivity [11-14].

We have previously demonstrated that ASM cell bundles carefully dissected from the tracheas of non-asthmatic subjects whose lungs were donated for medical research provide a valuable, high quality tissue preparation for study of the mechanical properties of ASM [15]. We showed that the mechanical properties of non-asthmatic ASM were similar to those measured in other mammalian models. This is in contrast to previous studies which suggested that human ASM produced less force per unit area and shortened less than the ASM of other mammals [16].

The purpose of this study was to reevaluate a series of hypotheses related to ASM mechanics which have been suggested as possible defects in asthmatic ASM function and potential contributors to AHR. These include determining whether asthmatic ASM produces more stress (force per unit cross-sectional area of muscle) than non-asthmatic ASM [17, 18]; whether the length-tension relationship of asthmatic ASM or its ability to undergo length adaptation are altered [19, 20]; whether the muscle shortens faster or more extensively in asthmatics than non-asthmatics at different loads [21-24]; and whether asthmatic ASM responds differently to a mechanical perturbation than non-asthmatic ASM [25, 26].

We found that passive stiffness was greater and the force reduction following a length perturbation was less in the asthmatic ASM preparations compared to non-asthmatic ASM. These abnormalities could contribute to altered in vivo airway function and increased airway responsiveness.

MATERIALS AND METHODS

Tissue Preparation and Equilibration: The lungs of 12 asthmatic and 9 non-asthmatic subjects were used for these studies. The human lungs were donated for research through the International Institute for the Advancement of Medicine (IIAM: Edison, NJ - http://www.iiam.org/). Donor deaths were primarily because of head trauma in the non-asthmatics, while 8 of 12 asthmatics died during exacerbations of their asthma. The subject demographics and clinical details are shown in Table 1.

The study was approved by the UBC-St. Paul’s Hospital Ethics Committee. The preparation of the tissue and details of the mechanical and morphometric measurements were as previously described [15, 27] and detailed in the online supplement. Airway dimensions were measured on intraparenchymal airways, ASM mechanics on trachealis muscle strips. The measurements included: 1) airway dimensions on intraparenchymal airways, 2) maximal trachealis muscle isometric force (Fmax) at in situ length (Lref), 3)
passive and active force-length relationships (lengths of 0.5, 0.75, 1.0, 1.25, and 1.5 $L_{\text{ref}}$),
4) force-velocity curves at two time points (the peak of tetanic force, and midway to the
peak [15, 28]), 5) maximal isotonic shortening and 6) force recovery following a 10
minute 0.2 Hz oscillation of the relaxed muscle (60% $L_{\text{ref}}$ peak-to-peak amplitude – ie
30% lengthening – Our lever system was not able to apply half sine waves (stretch only).
When the compliance of the lever system was taken into account, the actual stretch
applied to the muscle was ~ 25%). The maximal stress produced by the trachealis
muscle was determined by dividing $F_{\text{max}}$ (mN) by the cross-sectional area (mm$^2$) of
muscle present in the preparation.

**Statistical Analysis:** For the analysis of airway dimensions we compared groups and
calculated individual data for comparison with the trachealis muscle physiology. A linear
mixed-effects model was used to compare the area measurements of epithelium, lamina
propria, smooth muscle, adventitia and total wall all referenced to basement membrane
perimeter (see online supplement for details). A similar analysis was applied to the
relationships between the square root of the airway compartment areas and the basement
membrane perimeter. Using this relationship the airway smooth muscle area for an
airway with a diameter of 1 mm was calculated for each subject for comparison with the
physiological results from their trachealis muscle.

Force and length measurements were normalized to $F_{\text{max}}$ or $L_{\text{ref}}$ respectively. Velocity of
shortening was expressed as $\Delta L_{\text{ref}}$/sec. Aggregate data were expressed as mean ± SEM.
One and two way ANOVA and regression analyses were accomplished using GraphPad
Prism 5 (GraphPad Software, Inc.: La Jolla, CA). $p \leq 0.05$ was considered to be sufficient
to reject the null hypothesis.

**RESULTS:**
Trachealis muscle mechanics were determined on 8 of 12 and 6 of 9 of the asthmatic and
non-asthmatic subjects respectively; 6 of the asthmatics on whom trachealis mechanics
were performed had died from an asthmatic attack. For seven samples on which muscle
mechanics were attempted, little or no force could be measured in response to electrical
field stimulation (EFS). The average ages of the non-asthmatics and asthmatics were
25.3±8.1 years and 15.7±2.3 years respectively (t-test: $p=0.247$).

**Trachealis and Intra-parenchymal airway morphology:** The mean % ASM in the
trachealis preparations of the non-asthmatic subjects was 25.5 ± 9.0% while in the
asthmatics it was 28.8 ± 8.7% ($p=0.53$). The mean % connective tissue in muscle bundles
in the non-asthmatic subjects was 39.8 ± 5.7% and in the asthmatics it was 31.9 ± 11.2%
($p=0.12$). The airway wall dimensions of 207 airways from 12 asthmatic and 9 non
asthmatic donor lungs were analyzed. (Table 2) The ratios of area to basement
membrane perimeter (Pbm) for the smooth muscle ($p<0.01$), lamina propria ($P=0.013$),
adventitia ($P=0.020$), and total wall ($P=0.024$) were greater in the asthmatic than the non-
asthmatic subjects, while the epithelial area was not significant ($P=0.053$). A similar
analysis showed that the slope of square root of ASM area versus Pbm was steeper
($P>0.001$) in the asthmatic subjects compared with the non asthmatic subjects (Figure E2
in online supplement). The rationale for using the actual wall areas in the statistical
analysis and the square root of the wall area in the graphical analysis is described in the online supplement.

**Trachealis muscle mechanics:** Muscle stress generated with maximal EFS was 152.9±107.7 kPa in the asthmatic and 161.0±50.7 kPa in non-asthmatic preparations (p=0.858 - Figure 1).

Force-length properties are shown in Figure 2A. Immediately following a length change the active force produced by the muscle declined and gradually recovered over the 20-minute period in which the muscle length was held constant and EFS was applied at 5 min intervals (Figure 2B). After each length change to a shorter or longer length than L<sub>ref</sub>, and the determination of immediate force and adapted force at that length, the muscle was returned to L<sub>ref</sub> to allow for a period of adaptation. Except for the asthmatic group at 1.25 L<sub>ref</sub>, force did not adapt back to the level of F<sub>max</sub> at any of the length steps. The changes in passive force following length changes and repeated stimulations are shown in Figure 2C. The passive force increased dramatically when length was changed to longer lengths and decreased immediately following a change to a shorter length. While the passive force at longer lengths declined over the 20-minute period, it remained elevated in both groups when compared to the passive force at L<sub>ref</sub>.

The extent of length adaptation and a comparison between groups is illustrated in Figure 3. To quantify length adaptation, the first contraction after a length change was compared to the last contraction at that length. For both the non-asthmatics and the asthmatics, significant length adaptation occurred (ANOVA: p<0.01). For the non-asthmatic subjects Bonferroni post-tests revealed significantly greater force following adaptation at 0.5 L<sub>ref</sub> (P<0.05) and 1.5 L<sub>ref</sub> (P<0.01) but not at 0.75 and 1.25 L<sub>ref</sub>. The asthmatics demonstrated greater active force following adaptation at 1.25 and 1.50 L<sub>ref</sub> (p<0.01) but not at 0.75 or 0.5 L<sub>ref</sub>. Before adaptation the active force at 1.50 L<sub>ref</sub> was significantly lower in asthmatics compared to non-asthmatics (p=0.05) and after the adaptation period the asthmatics produced less force than the adapted non-asthmatics at 0.5 L<sub>ref</sub> (p<0.01).

Figure 4 shows the changes in passive force with changes in length and during the adaptation process. Both the non-asthmatics and asthmatics demonstrated significant passive force adaptation - i.e. the passive force decreased significantly after the period of adaptation in both groups (ANOVA: p=0.024 for non-asthmatics and p=0.018 for asthmatics). Bonferroni post-tests demonstrate that the passive force at 1.50 L<sub>ref</sub> for the asthmatics was significantly greater than that of the non-asthmatics before adaptation (p<0.05). In addition the passive force at 1.50 L<sub>ref</sub> was significantly lower in the asthmatics after adaptation than before adaptation (p<0.05).

Two sets of force-velocity relationships were determined: one during the early-phase of contraction and one at the peak of tetanic contraction, as previously described [15,28]. The force-velocity curves for individual muscle strips varied as shown in Figures E3A and B. Force-velocity data for each muscle strip were initially fitted with Hill’s hyperbolic equation [29] before averaging the curves (Figures. 5A and B). There were no differences in the shape or position of the force-velocity relationships between non-asthmatic and asthmatic subjects.

Four non-asthmatic and four asthmatic muscle strips were analyzed to determine maximal isotonic shortening (Figure 6). The extent of shortening was recorded at preloads of 10% and 20% of EFS induced F<sub>max</sub>. Maximal shortening, as determined by extrapolation to no load, was 72.2±4.9% for the non-asthmatics and 70.5±5.6% for
asthmatics (p=0.946). With no difference between the groups, all muscle strips were combined into one linear regression (central dashed line, Figure 6) and maximal shortening was calculated as 71.4±3.4% (p<0.01, r²=0.708). In the unloaded condition, the 95% confidence intervals were 64.0-78.8% shortening.

Active force recovery following a ten-minute length oscillation was followed for 30 minutes in both non-asthmatic and asthmatic tracheal strips (Figure 7). The response to oscillation was significantly different between asthmatics and non-asthmatics (p<0.01 for two way ANOVA with time and group as variables). Initially following oscillation the non-asthmatics produced an average force of 0.63±0.03 F_max compared to 0.81±0.04 F_max in asthmatics (Bonferroni post-tests; p<0.01). The recovery of force was greatest over the first five minutes in the non-asthmatics, however, the average force recovery was below F_max at 0.95±0.03 F_max. The asthmatics demonstrated slower force recovery but recovered beyond F_max after 30-minutes (1.05±0.03 F_max).

Since ASM area, normalized to Pbm, was the morphologic variable that best separated asthmatic from non-asthmatic airways we calculated the smooth muscle area at a Pbm of 3140 µm (which conforms to an airway with a diameter of 1000 µm) to use as a continuous variable to compare with trachealis muscle function. Although this variable separated asthmatics from non-asthmatics (0.27 +/- 0.15 um² versus 0.20 +/- 0.09 um² P = 0.07) there were no significant relationships between this variable and any of the functional variables when we included all subjects in the analysis or when we limited the analysis to asthmatics. Figure E4 shows the relationship of trachealis muscle stress versus the ASM area in an idealized airway with a diameter of 1000 µm. Similar results were obtained with use of the slope of the relationship between the square root of ASM area and Pbm as the morphological estimate of airway remodeling (data not shown).

DISCUSSION

Exaggerated airway narrowing in response to bronchoconstricting stimuli is a defining feature of asthma. Airway hyperresponsiveness (AHR) is characterized by both an increase in sensitivity to such stimuli as well as an increase in the maximal airway narrowing that can be achieved [30]. Despite its importance the mechanism(s) leading to AHR remain unclear. It has been suggested that changes in ASM phenotype could be responsible but non-muscle properties of the airways have also been implicated [2]. A number of investigators have attempted to determine whether asthmatic ASM is mechanically different than non-asthmatic ASM [3-14] but the results thus far have been equivocal.

The present study provides evidence that there may be intrinsic differences in ASM behavior in asthma, changes that could contribute to airway hyperresponsiveness. Although we found no differences in the stress produced by EFS, the velocity of shortening or the extent of shortening in the asthmatic muscle compared to the non-asthmatic muscle there was a substantial difference in the relaxed muscle’s response to a length perturbation. Although the active force generated by EFS was decreased following the length oscillation in both non-asthmatic and asthmatic tissues the decrease was significantly less in the asthmatic tissue.

That an impaired response to length oscillation could contribute to AHR is based on evidence that the stretching of ASM as occurs in vivo during tidal breathing and deep
inspirations (DIs) is sufficient to reduce ASM contractility, as demonstrated by in vitro experiments where ASM is subject to length oscillation (31, 32). If this stretch-induced decrease in contractility were impaired the response to any contractile stimulus could be enhanced (ie hyperresponsiveness).

However, it has recently been shown that oscillation amplitudes comparable to those produced by tidal breath (~5 cm H₂O) do not affect the response of airway segments to a contractile agonist, although with amplitudes greater than 10 cm H₂O the airways do respond with a transient dilation (33, 34, 35, 36). Moreover, the transient airway dilation observed in airway segments is short lived, compared to the longer lasting effect of DI induced bronchodilation observed in healthy subjects (25, 37). These data support the idea that the reduced airway response following deep inspiration is likely more complicated than a simple stretch of ASM.

We have previously shown that the reduction in force which follows a length oscillation is associated with a reduction in myosin thick filament density and we have suggested that it is this evanescence of myosin filaments that is responsible for the plasticity of the ASM length tension relationship [38]. A similar change in smooth muscle function after deep inspiration in vivo could explain the broncho-protective effect of DI. Our results support the possibility that the myosin thick filaments are altered in asthma making them less prone to disruption following strain. Such an effect could prevent the decrease in force-generation that accompanies repeated sighs and other forms of deep inspiration and could make the muscle more susceptible to exaggerated contraction.

The functional changes in smooth muscle that could contribute to AHR are an increase in force/stress generating capacity, increased shortening velocity, increased ability to shorten, the ability to adapt excessively at short lengths and/or the inability of length perturbations to allow plastic rearrangement of the contractile apparatus so as to decrease force production and shortening.

Our data suggests that asthmatic ASM does not produce more stress (force per unit muscle area) than non-asthmatic ASM. However there was wide variation of stress produced in each group and a post-hoc power analysis determined that ~38 donors per group would be required to detect a 10% difference in mean stress between groups given this variation. While statistical significance may be possible with a larger sample size it is not certain whether this level of difference in stress production would be physiologically relevant.

Our data suggest that a difference in shortening velocity is not an important contributor to AHR. A limitation of this conclusion is that we were technically unable to measure shortening velocity at very low loads. Using our apparatus we were not able to determine velocities at forces below 2 mN which was approximately 5% Fmax . The study of larger strips of ASM may circumvent this limitation; ie Fmax would be large enough to avoid loads below 2 mN while working in the 5% Fmax range.

Using in situ length as reference length (Lref), both the asthmatics and the non-asthmatics had similar length-force relationships. Although the asthmatic ASM seemed to produce less force at the shorter and longer lengths than the non-asthmatics these differences were not significant. However following length adaptation, which was exhibited in both groups, the asthmatic ASM produced less force than the non-asthmatic ASM at the shortest length we examined (0.50 Lref). This observation suggests that asthmatic ASM is not more capable of length adaptation to short lengths than non-asthmatic ASM.
The different response to strain in the asthmatic ASM strips is the most intriguing aspect of this study (Fig. 7). It is well known that asthmatics’ airways respond differently than those of non-asthmatics to the stretch which accompanies deep inspiration [25, 39-42]. In vivo the effects of deep inspiration can be assessed by taking big breaths before or after the administration of a broncho-constricting stimulus. When applied after bronchoconstriction, DI produces a bronchodilating effect while taken before the administration of a constrictor it causes a broncho-protective effect, i.e., less constriction of the bronchi when stimulated. Asthmatics may have a reduced bronchodilating effect of deep inspiration, especially during spontaneous attacks of asthma [43], but more consistently show a defective broncho-protective effect such that prior deep inspiration fails to attenuate subsequent constriction [44-46].

Although it is tempting to speculate that the difference in attenuation in force following the length perturbation observed between asthmatic and non-asthmatic tissue is at the basis for the reduced bronchoprotective response to DI in asthma the picture is far from clear. While prior DI differentially modifies the methacholine-induced decline in FEV1 in asthmatics and non-asthmatics, it has no differential effect on the changes in FEV1/FVC ratio (47), partial expiratory flow (48) or airway resistance assessed using the forced oscillation technique (FOT) (49). It is argued by some that prior DI may not alter the initial airway narrowing produced by a constrictor but instead make the airway tree more responsive to a subsequent DI as is required to perform an FEV1 maneuver. Thus the different response to stretch we observed is unlikely to the sole mechanism to explain deficient bronchoprotection in asthma.

In the present study the length oscillation was applied to the muscle before it was activated; this was to mimic the bronchoprotective effect of DI that has been shown to be more potent than the bronchodilating effect of DI in healthy human subjects (50). Although in isolated ASM preparations length oscillation has been clearly shown to have a large effect on the muscle's subsequent ability to generate force (51), in airway segments pressure oscillation does not lead to reduced airway narrowing on subsequent stimulation (52). The cause for the discrepancy between the results with ASM strips and airway segments is not clear but it could be because mid-sized airways (used in the pressure oscillation experiments) are less distensible than the smaller airways. DI has been shown to reduce peripheral airway closure (47). A recent study (53) has shown that a reduction in airway distensibility is one of the reasons that severe asthmatics do not benefit from the bronchodilating effect of DI. The reduced response of asthmatic ASM to length oscillation and the significantly higher passive tension at length >L_ref observed in the present study could contribute to reduced airway distensibility.

In this study we applied a length oscillation of 60% peak to peak which means a 30% lengthening strain. Accounting for the compliance of the apparatus, this translates into a ~25% lengthening of the smooth muscle preparation. This value of strain was selected because it resulted in significant force loss and decreased myosin thick filament density in previous studies (51). However this is more strain than is experienced by most of the ASM in the lung during a deep inspiration. If the airways dilate isotropically with the lung parenchyma the circumferential strain is about 20% with an inhalation from FRC to TLC since FRC is about 50% TLC. Since most airways are less distensible than the lung the strain that most of the smooth muscle experiences is even less than 20%. We also applied a longer period of length oscillation than is used in vivo to detect
bronchoprotection. Again this was chosen based on a previous protocol which was designed to maximize the effect of length oscillation on subsequent force development [51].

The difference in the response to DI in asthmatic subjects in vivo has been variously attributed to a failure of DI to translate into the same ASM strain in stiffened asthmatic airways or to an intrinsic difference in ASM mechanics. In vivo, deep inspiration applies a stress to the smooth muscle and the resultant strain is dependent on the stiffness of the airway wall as well as the elastic recoil of the lung (i.e. the same volume change produces less strain if lung recoil is reduced as it is in emphysema). Brown et al [54] used CT to estimate the strain (airway dilatation) produced by DI in normal and mild-moderate asthmatic subjects and found no difference. They suggested the different response to strain in asthma was at the basis of the defective response to DI. Our results support the contention that there is an intrinsic difference in smooth muscle behavior in asthmatic tissue. We applied a length oscillation rather than a force oscillation. While this is non-physiological, since strain is a dependent variable in vivo, it allows us to suggest that differences in the muscle response to strain, rather than attenuated strain due to stiff airways, is at the basis of the differential response.

On the other hand, a similar strain experienced by an airway strip in vitro or by an airway in vivo could result in a different strain being applied to the contractile apparatus of the muscle. An airway strip or circumferentially stretched airway can be simplistically thought of as a contractile element in series with an elastic element. The relative strains experienced by the contractile and series elastic elements could be different for any overall strain.

Although we observed a lesser response to length oscillation in the asthmatic tissue, the active length tension curve before adaption indicated that at high and low lengths force was lower in the asthmatic group (not consistent with AHR) and importantly there was no difference in adaptation. It seems paradoxical that the response to length oscillation differs but not the adaptive response. However the difference in force recoveries (adaptation) after length oscillation and after a step change in length could be due to the fact that the recovery occurred at different lengths in the latter protocol. We observed that the asthmatic ASM tended to recover less at lengths <Lref, and more at lengths >Lref. The reason for this difference is not clear. One can speculate that the structural disruption within the ASM associated with a 10-min length oscillation is different from that resulting from a step change in length, even though they both lead to some degree of force loss post perturbation.

A possible explanation for the reduced response to stretch is that there could be persistent low-grade activation of the asthmatic muscle making it less responsive to stretch. However such activation, if it did exist, was not manifested as an increased "passive" tension, as we did not observe an elevated resting tension in the asthmatic ASM (Fig. 4). It could, however, manifest itself as an increased passive stiffness (which we did not measure) and that could also explain the higher passive tension (at 1.5 Lref) observed in the asthmatic group.

A strength of the present study is the well preserved tissue from asthmatic and non-asthmatic individuals. Some previous studies have been done on airway tissue recovered
at autopsy while others have examined specimens obtained at surgery. The study has limitations. 1) Little history was available on the subjects beyond that related to their terminal event. Because of this we undertook a detailed morphological examination of the airways to quantify the extent of pathological changes which are typically found in asthma. The results showing that the fatal asthmatics, as well as those asthmatics who died of other causes, had features of airway wall remodeling is an independent confirmation of the clinical phenotyping of the subjects. 2) The majority of the subjects had severe (fatal) asthma and thus the results may not apply to more mild disease. 3) The time from death to the time of study of the ASM was quite long which could result in the loss of mediators present in life that could cause increased ASM contractibility. 4) Although the histological features of asthma were present in the intraparenchymal airways the mechanics were done only on the trachealis; the assumption that the properties of the ASM are uniform may not be valid. 5) The patient group is small and varied. Treatment before death for the asthmatic group ranged from none to prednisone. This variation in severity may have affected our ability to detect differences between the groups but is unlikely to be responsible for the significant differences that we did find.

In conclusion, this study is the first to systematically examine the complete array of mechanical properties of ASM from asthmatic and non-asthmatic individuals. While the sample size was relatively small and the variability of some variables was wide we found no substantial difference in the stress produced by the asthmatic muscle preparations; indeed at lengths longer and shorter than L_ref maximal stress tended to be somewhat less in asthmatic airway smooth muscle preparations. There was no difference in the velocity or extent of shortening.

On the other hand, the results show, for the first time, that there is a difference in the ASM response to stretch in asthma. Following a 25% length oscillation there was less attenuation in ASM force in the asthmatic tissue. These results suggest that there is an intrinsic difference in the ASM’s response to strain in asthma. The increased passive stiffness of the asthmatic preparations may, in part, contribute to this difference, which is supported by in vivo data showing less airway dilation induced by a DI in asthmatics [55]. Another possibility is that the myosin thick filaments in asthmatic ASM are less prone to dissolution and rearrangement.
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### Table 1. Subject demographics and clinical details.

<table>
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<tr>
<th>Non-Asthmatic Subjects with Trachealis Mechanics</th>
<th>Sex</th>
<th>Age</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>Ethnicity</th>
<th>Cause of Death</th>
<th>Patient Medical History</th>
<th>Known Meds</th>
<th>Terminal Meds</th>
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<tr>
<td>1</td>
<td>M</td>
<td>22</td>
<td>112</td>
<td>193</td>
<td>Caucasian</td>
<td>Head trauma</td>
<td>Occasional marijuana (once a year)</td>
<td>None</td>
<td>Vasopressors</td>
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<tr>
<td>2</td>
<td>F</td>
<td>63</td>
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<td>159</td>
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<td>Gastrointestinal bleed</td>
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<td>Dopamine</td>
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<td>3</td>
<td>M</td>
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<td>175</td>
<td>Hispanic</td>
<td>Head trauma</td>
<td>None</td>
<td>None</td>
<td>Vasopressors</td>
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<td>4</td>
<td>F</td>
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<td>87</td>
<td>165</td>
<td>Caucasian</td>
<td>Head Trauma MVA</td>
<td>Beer/Hard Liquor 1-2X month for 2 years</td>
<td>None</td>
<td>Epinephrine, Dopamine, Dopamine, Vasopressors, Heparin, Mannitol and Crystalloids</td>
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<td>F</td>
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<td>Smoked cigarettes &lt; 1 PPD for 2 years. Marijuana smoked unknown frequency.</td>
<td>Pain medications and inhalants</td>
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<th>Height (cm)</th>
<th>Ethnicity</th>
<th>Cause of Death</th>
<th>Patient Medical History</th>
<th>Known Meds</th>
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<td>M</td>
<td>20</td>
<td>86</td>
<td>185</td>
<td>Caucasian</td>
<td>Head Trauma MVA</td>
<td>Cigarettes 2 per week, vodka ½ bottle per week, Occasional beer since age 18, Marijuana weekly</td>
<td>n/a</td>
<td>Ancef, Mannitol, Vitamin K, Protonix, Magnesium Sulfate, Morphine, Reglan, K-Phos, Hydralazine Lasix, Levothyroxine, Vasopressin, Norepinephrine, Desmopressin, Heparin</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>14</td>
<td>50</td>
<td>165</td>
<td>Caucasian</td>
<td>Head Trauma MVA</td>
<td>None</td>
<td>None</td>
<td>Nitroprusside</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Asthmatic Subjects with Trachealis Mechanics</th>
<th>Sex</th>
<th>Age</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>Ethnicity</th>
<th>Cause of Death</th>
<th>Patient Medical History</th>
<th>Known Meds</th>
<th>Terminal Meds</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>M</td>
<td>23</td>
<td>52.6</td>
<td>152.4</td>
<td>Hispanic</td>
<td>Asthma attack</td>
<td>Seizures related to asthma</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>10</td>
<td>43</td>
<td>157.5</td>
<td>Caucasian</td>
<td>Asthma attack</td>
<td>Asthmatic diagnosed at age 4</td>
<td>Advair, Allegra, Albuterol</td>
<td>Dopamine</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>10</td>
<td>15</td>
<td>149</td>
<td>African American</td>
<td>Asthma Attack</td>
<td>Asthma</td>
<td>Concerta, Lexapro</td>
<td>Vasopressin</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>25</td>
<td>103</td>
<td>185</td>
<td>Hispanic</td>
<td>Anoxia, suicide</td>
<td>Asthmatic diagnosed at</td>
<td>Albuterol, Advair, Steroids and vasopressors</td>
<td></td>
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<tr>
<td>14</td>
<td>11</td>
<td>69</td>
<td>170</td>
<td>Caucasian</td>
<td>Anoxia, probable asthma attack</td>
<td>Asthmatic diagnosed at age 2</td>
<td>Albuterol</td>
<td>Dopamine and vasopressors</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>11</td>
<td>65.3</td>
<td>152.5</td>
<td>Caucasian</td>
<td>Head trauma</td>
<td>Asthmatic diagnosed at age 6</td>
<td>None</td>
<td>n/a</td>
<td></td>
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<tr>
<td>16</td>
<td>15</td>
<td>67</td>
<td>177.8</td>
<td>Caucasian</td>
<td>Asthma attack</td>
<td>Asthma diagnosed at birth</td>
<td>Prednisone, Albuterol</td>
<td>Dopamine, Dextrose</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>15</td>
<td>56</td>
<td>162.5</td>
<td>Caucasian</td>
<td>Anoxia, probable asthma attack</td>
<td>Environmental allergies and asthma; smoked cigarettes &lt; 1 PPD† for 1 year</td>
<td>Singulair, Advair, Albuterol, Flovent, Montelukast, Prednisone</td>
<td>Vasopressors</td>
<td></td>
</tr>
</tbody>
</table>

**Asthmatic Subjects with no Trachealis Mechanics**

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<table>
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</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>F</td>
<td>8</td>
<td>30.5</td>
<td>117</td>
<td>Hispanic</td>
<td>Asthma Attack</td>
<td>Asthmatic diagnosed age 3, Respiratory Syncytial Virus Age 2</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>26</td>
<td>91.6</td>
<td>168</td>
<td>Caucasian</td>
<td>Anoxia, probable asthma attack</td>
<td>Asthma since childhood, Seizure in 5th grade, Frequent urinary tract infections, cigarettes 1 P/week since age 16, hard liquor and heavy drinker</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>21</td>
<td>55</td>
<td>170</td>
<td>Caucasian</td>
<td>Drug Overdose (Tylenol)</td>
<td>Asthma, Cervical Cancer (free for 1 year), Cigarettes (2PPDx6 years),</td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>36</td>
<td>91</td>
<td>173</td>
<td>Caucasian</td>
<td>Head Trauma</td>
<td>Asthma, Chewing Tobacco daily</td>
</tr>
</tbody>
</table>

Pack per day, n/a not available, MVA – motor vehicle accident
Table 2. Airway characteristics

<table>
<thead>
<tr>
<th></th>
<th>Asthmatic</th>
<th>Non-Asthmatic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td># Airways</td>
<td>10.8 ± 5.7£</td>
<td>8.1 ± 3.2</td>
<td>0.28</td>
</tr>
<tr>
<td>Average PBm</td>
<td>3691 ± 1713</td>
<td>3813 ± 1445</td>
<td>0.37</td>
</tr>
<tr>
<td>Median PBm</td>
<td>2479 (1373-7850)†</td>
<td>2886 (1286-8072)</td>
<td>0.47</td>
</tr>
<tr>
<td>Smooth muscle area/Pbm</td>
<td>0.0693 (0.0638-0.0749)*</td>
<td>0.0511 (0.0451-0.0572)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Epithelial area/Pbm</td>
<td>0.1026 (0.0907-0.1144)</td>
<td>0.0856 (0.0730-0.0981)</td>
<td>0.053</td>
</tr>
<tr>
<td>Lamina propria area/Pbm</td>
<td>0.1393 (0.1274-0.1511)</td>
<td>0.1167 (0.1041-0.1293)</td>
<td>0.013</td>
</tr>
<tr>
<td>Adventitial area/Pbm</td>
<td>0.2170 (0.1963-0.2377)</td>
<td>0.1804 (0.1584-0.2025)</td>
<td>0.020</td>
</tr>
<tr>
<td>Total area/Pbm</td>
<td>0.4112 (0.3730-0.4494)</td>
<td>0.3455 (0.3048-0.3863)</td>
<td>0.024</td>
</tr>
</tbody>
</table>

£ = +/- SD, † = range, * = 95% confidence intervals

REFERENCES:


15. Chin LY, Bosse Y, Jiao Y, Solomon D, Hacket TL, Pare PD, Seow CY. Human airway smooth muscle is structurally and mechanically similar to that of other species. Eur Respir J. 2010;36:170-7


Figure Legends:

Figure 1  Comparison of maximal stress generation. Stress in response to EFS, calculated as $F_{\text{max}}$ divided by muscle cross-sectional area (kPa), between non-asthmatics and asthmatics was compared. Error bars indicate SEM, n=6 for non-asthmatics, n=8 for asthmatics.
Figure 2  Time course of length changes and the resulting active and passive force. ASM strips were either shortened or lengthened in the relaxed state to 0.50, 0.75, 1.25 or 1.50 L_{ref}. The muscle was stimulated to contract 5 times with EFS at 5-minute intervals after every length change. Between shortening and lengthening steps, the muscle was returned to L_{ref} and readapted. Grey hexagons indicate the time points where EFS occurred (A). The active force (B) and passive force (C) were recorded at every contraction. Both were normalized to F_{max}. Non-asthmatics are represented as black circles, while asthmatics are represented as grey squares. Error bars indicate SEM, n=6 for each group.
Figure 3  Active length-force relationship before and after length adaptation. From $L_{ref}$, the ASM strips were either shortened or lengthened to 0.50, 0.75, 1.25, and 1.50 $L_{ref}$ (refer to Fig. 4 for sequence and time course of length changes and stimulation). Active force before (solid lines and closed symbols) and after (dashed lines and open symbols)
length adaptation are shown. Non-asthmatics are represented by black circles and asthmatics by gray squares. Error bars indicate SEM, n=6 for each group. *: p<0.05, ***: p<0.01; compared to the non-asthmatic before length adaptation at the given length. †††: p<0.01; compared to the asthmatic before length adaptation at the given length. ‡: p<0.05; compared to the non-asthmatic after length adaptation at the given length.

Figure 4  Passive length-force relationship before and after length adaptation. From L_{ref}, the ASM strips were either shortened or lengthened to 0.50, 0.75, 1.25, and 1.50 L_{ref}. Passive force both before (solid lines and closed symbols) and after (dashed lines and open symbols) length adaptation are shown. Non-asthmatics are represented by black circles and asthmatics by gray squares. Error bars indicate SEM, n=6 for each group. * =: p<0.05 - passive force was greater in asthmatics compared to the non-asthmatic before length adaptation at 1.50 L_{ref}. † = p<0.05 - passive force was less following adaptation in the asthmatic tissue compared to before adaptation at 1.50 L_{ref}. 

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Figure 5. A. Averaged isotonic shortening velocities to various loads, early phase release. Hill’s hyperbolic equation was fitted to the data from each subject and the solid lines represent the average of these curves. B. Averaged isotonic shortening velocities to various loads, late phase release. Hill’s hyperbolic equation was fitted to the data from each subject and the solid lines represent the average of these curves. The dashed lines indicate SEM.
Figure 6  Maximal isotonic shortening. ASM strips were stimulated to contract with EFS and the total amount of isotonic shortening against small loads was measured. Using the amount of shortening recorded at loads representing 10% and 20% of F_max, maximal shortening was extrapolated. Shortening was recorded at both loads per donor, n=4 for each group. Non-asthmatics are represented as solid circles and asthmatics as solid squares. A combined (both groups) linear regression is represented as a dashed central line. The upper and lower dashed lines represents the 95% confidence interval for all data points.

Figure 7. Response to mechanical perturbation. Recovery of isometric force following a ten-minute, 0.2Hz, 30% L_ref length oscillations. ASM strips were adapted to L_ref prior to oscillation. The force produced by seven EFS-induced contractions was recorded at 5-minute intervals following the oscillation. ANOVA: p<0.01. Error bars indicate SEM, n=6 per group. In addition to the difference in the initial decline in force, the asthmatics demonstrated slower force recovery but recovered beyond F_max after 30-minutes (1.05±0.03 F_max). Non-linear regression analysis of the non-asthmatic and asthmatic data demonstrated these differences in recovery rate. The regressions were fit using a one-phase exponential equation. For the non-asthmatics: force = 0.947 – 0.296e^{-0.139t} (r^2=0.73); for asthmatics: force = 1.053 – 0.243e^{-0.073t} (r^2=0.48) where force was relative to F_max and t was time in minutes.
Normalized Force (F_max) vs. Time (mins)

- Non-asthmatics, n=6
- Asthmatics, n=6

p<0.0001

10 min Oscillation