Marfan syndrome (MFS) is caused by mutation of the FBN1 gene encoding the extracellular matrix (ECM) protein fibrillin-1 that forms elastic microfibrils. This study aims to characterize the micromechanics of aortic and lung tissues from wild type (WT) and FBN1-deficient mutant (MT) mice to identify biochemical determinants of cardiopulmonary disease in MFS. For testing by atomic force microscopy (AFM), full-thickness sections of ascending aorta were dissected from 4-month-old野生 mutant (0.5–5-mo) WT and MT mice, mounted flat with the lumens facing up, and indented with a 15-μm spherical probe at 3-5 sites per sample from 1-4 animals per condition. Indentation force–depth curves were analyzed using a hybrid theoretical and finite element analysis to determine the elastic modulus (Emean) and media (Emean) layers. The Kolmogorov–Smirnov test was performed for pairwise statistical analysis; p<0.008 was considered significant based on the Bonferroni correction. Emean at 0.5-mo showed no difference between WT (10.1±2.7kPa, n=8; mean±SEM for n-curves) and MT (7.5±0.6kPa, n=80). Emean increased with age (p<0.0006), but was significantly softer in MT (21.3±1.4kPa, n=112) vs WT (37.4±2.2kPa, n=65, p=0.0001). Emean also increased with age (p<0.003) from ~2kPa to ~5-10kPa, but showed no significant difference between WT and MT at matched ages. Similar nano-indentation studies, using a pyramidal tip, on the parenchyma and adventitia isolated from 2-mo WT and MT mice showed Emean for MT (1.7±0.1 kPa, n=92) was significantly softer than WT (4.8±0.3kPa, n=91, p=0.0001). In conclusion, AFM revealed age-dependent softening of micro-elastic modulus in elastin-rich tissues including lung parenchyma and aortic media, but not in the aortic intima. In contrast to macro-scale measurements of aortic stiffening in MFS, these micro-scale AFM findings appear consistent with histological observations of local disruption of ECM microstructure in MFS.

Bronchial but not Tracheal Smooth Muscle is Hypercontractile in an Equine Model of Severe Asthma
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Asthma is an inflammatory disease of the airways characterized by airway hyperresponsiveness, an exaggerated bronchoconstrictive response of airway smooth muscle (ASM) to stimuli. However, whether asthmatic ASM is mechanically different from non-asthmatic ASM is still unclear. Most studies so far have been performed on the trachealis muscle but it might not be representative of smooth muscle in the more peripheral airways. Thus, we addressed this question in an equine model of asthma (horse with heaves) and compared the trachealis (TSM) and peripheral bronchial (BSM) airway smooth muscle mechanically.

Muscle strips from de-epithelialized TSM and BSM (less than 10 mm in diameter) were mounted horizontally in an experimental chamber between a length transducer (Aurora Scientific Inc.). Maximal velocity of shortening (Vmax), methacholine dose response and stress were measured.

We found no difference in Vmax between TSM from control horses and horses with heaves (0.124±0.01 vs. 0.119±0.01; control vs. heaves; P=0.8), whereas the BSM exhibited a significantly higher Vmax (0.114±0.01 vs. 0.239±0.02; P=0.03). Western blot analysis showed a significant increase of the (+) insert fast smooth muscle myosin heavy chain (SMMHC) isofrom in BSM of heaves-affected horses (0.78±0.04 vs. 1.0±0.05, P=0.0092), while no such difference was found in TSM (0.91±0.05 vs.0.97±0.08; P=0.54). The expression of calponin was significantly increased in BSM of heaves-affected horses (0.124±0.01 vs. 0.49±0.18; P=0.62). There were no differences detected in the expression of MLCK, total SMMHC and transgelin. Peripheral airways may have a greater exposure to inflammatory cells than the trachealis which may explain their differences in mechanics. Further studies await to determine the mechanism underlying the observed effect.

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Defining the Latch-State in Human Airway Smooth Muscle
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Rationale: The latch-state describes the ability of tonic smooth muscle to maintain force at reduced ATP consumption and crossbridge cycling rate. In animal models this is evidenced by reduced unloaded shortening velocity (Vmax), presumably indicative of crossbridge cycling rate, and reduced myosin light chain phosphorylation (pMLC). In this study we aim to assess this phenomenon in human airway smooth muscle (hASM).

Methods: hASM tissues were dissected from trachea procured from the International Institute for the Advancement of Medicine. Force-velocity curves and pMLC measurements were performed at several time points in multiple prolonged methacholine 10-6M contractions. Rat ASM strips were tested with the same protocol. A mathematical model was developed to explain the findings.

Results: Despite a 40% drop in pMLC between the 1st to the 20th min after the peak contraction, Hasm Vmax did not show any change. Conversely, rat ASM Vmax decreased by 50%. A mathematical model of the cross-bridge cycle, combining strain-dependent ADP affinity of myosin with actin regulated binding of dephosphorylated myosin heads, was capable of reproducing our findings.

Conclusion: As our results contradict the classical latch-bridge theory, we suggest an alternative model. We propose that Vmax, as calculated from standard force-velocity curves, is not indicative of the crossbridge cycling rate during latch. Instead, Vmax is reduced due to drag caused by dephosphorylated myosin heads binding to actin, with their binding force regulated by actin regulatory proteins and their activation state. The increase in dephosphorylated myosin late in contraction decreases the measured Vmax, unless its binding force is down-regulated by regulatory proteins. Our modelling further shows that force maintenance can be explained by phosphorylated myosin heads remaining attached to actin in a locked configuration, unable to release ADP because of strain dependence of the ADP affinity of myosin.

Kinetic Characterization of Stabilized Smooth Muscle Myofilaments
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We have measured kinetic parameters of smooth muscle myosin (SMM) filament interactions with actin, ADP, and ATP using stopped-flow fluorometry. One of the key issues was to determine which parameters, if any, are different from those of monomeric myosins, S1 and HMM, using buffers at physiologic ionic strength. Previously we measured the ATP-dependence of SMM filament velocities (using EDC-cross-linked filaments that are stable to ATP-induced depolymerization) in an in vitro motility assay with myosin filaments moving over surface-attached actin. We compared this to the ATP-dependence of solution actin-activated ATPase measurements. The two relationships are very similar, suggesting that they are both attachment-limited and regulated by the same kinetic step, presumably phosphate release (Haldeman et al JBC 2014). This is in contrast to the kinetics of actin moving over a surface of monomeric SMM (standard vitro motility assay), known to be predominately detachment-limited, limited by ATP release. We hypothesized that heads within moving filaments are attached to unhindered thin bodies 2 regions putting minimal strain on heads put in the drag position. In contrast, the S2 region of monomeric myosin is bound to the surface and not as flexible allowing the relative stiff head domains to become strained, which affects the underlying kinetics of acto-SMM interactions. All kinetic parameters measured so far in solution for myosin or acto-myosin, ADP association, ADP dissociation, ATP binding, ATP-induced dissociation, and acto-myosin affinity, are very similar to those for S1 and HMM. Measurements of Pi release kinetics by limited turnover are planned. If Pi release kinetics is also similar, this will suggest that the attachment-limited kinetics that is unique to filaments is not due to alterations in any fundamental kinetic parameters, but instead is due to differences in the effective head or head2 stiffness during the assays.