Length adaptation of airway smooth muscle

Ynuk Bossé[1,4], Apolinary Sobieszek[2], Peter D. Pare[1,3], and Chun Y. Seow[1,4]

[1]The James Hogg iCAPTURE Centre for Cardiovascular and Pulmonary Research, Providence Health Care/St. Paul's Hospital, University of British Columbia; [2]Institute for Biomedical Aging Research, Austrian Academy of Sciences; [3]Department of Medicine, University of British Columbia; [4]Department of Pathology and Laboratory Medicine, University of British Columbia

Corresponding author (also to whom request of reprints should be addressed):

Dr. Chun Y. Seow
iCAPTURE Centre/St. Paul's Hospital
Room 166, 1081 Burrard Street
Vancouver, BC, Canada, V6Z 1Y6
Phone: (604) 806-9268
Fax: (604) 806-9274
Email: cseow@mrl.ubc.ca

This work was supported by operating grants from Canadian Institutes of Health Research (MOP-13271, MOP-4725).

Running Title: Mechanical plasticity in smooth muscle

Word count: 3369

Abstract

Many types of smooth muscle, including airway smooth muscle (ASM), are capable of generating maximal force over a large length range due to length adaptation - a relatively rapid process in which smooth muscle regains contractility after experiencing a force decrease induced by length fluctuation. Although the underlying mechanism is unclear, it is believed that structural malleability of smooth muscle cells is essential for the adaptation to occur. The process is triggered by strain on the cell cytoskeleton that results in a series of yet-to-be-defined biochemical and biophysical events leading to restructuring of the cytoskeleton and contractile apparatus, and consequently optimization of the overlap between the myosin and actin filaments. Although length adaptability is an intrinsic property of smooth muscle, maladaptation of ASM could result in excessive constriction of the airways and inability of deep inspirations to dilate them. In this review, we describe the phenomenon of length adaptation in ASM and some possible underlying mechanisms that involve the myosin filament assembly and disassembly. We also discuss a possible role of maladaptation of ASM in the pathogenesis of asthma. We believe that length adaptation in ASM is mediated by specific proteins and their post-translational regulations involving covalent modifications, such as phosphorylation. The discovery of these molecules and the processes that regulate their activity will greatly enhance our understanding of the basic mechanisms of ASM contraction and will also suggest molecular targets to alleviate asthma exacerbation related to excessive constriction of the airways.

Key words: Cytoskeleton, contractile apparatus, contraction mechanism, mechanical plasticity, airway constriction, asthma.

(Word count: 244)

Although the physiological role played by airway smooth muscle (ASM) in regulating lung function is still being debated (1-5), the muscle's role in asthma exacerbation is unambiguous - exaggerated narrowing of the airways seen in acute asthma attacks is due to excessive shortening of the airway smooth muscle (1, 6). Asthma is a complex disease with multiple causes. However, the final common pathway that leads to the manifestation of symptoms is ASM contraction with an excessive amount of shortening (7). The mechanism underlying the abnormal ASM shortening and the resultant airway hyperresponsiveness seen in asthma is not known. It could be a result of increased ASM mass in the airways, an enhancement of contractility of individual cells, or both. It could also be a result of an altered airway environment that reduces the load imposed on the muscle, leading to unencumbered shortening and excessive constriction of the airways. Irrespective of the primary mechanism for airway hyperresponsiveness, length adaptation of the muscle to passively or actively shortened lengths has the capacity to further exacerbate the ASM shortening and narrowing of the airways (8-10). Understanding length adaptation is not only a crucial component for elucidating the basic mechanism of ASM contraction, it also aids identification of potential targets for intervention designed to prevent maladaptation and ASM dysfunction that cause asthma symptoms.

AIRWAY SMOOTH MUSCLE

In general, smooth muscle controls vital functions by regulating the physical dimension and mechanical property of hollow organs. Many of these organs (e.g., stomach and urinary bladder) regularly undergo large changes in volume, thus requiring the muscle cells lining the organ to have a large working length range. Although a large functional length range is not required for airway smooth muscle, it is known that this muscle can generate maximal force over at least a 3-fold length

range after length adaptation (11). Length adaptability (also known as mechanical plasticity) of airway smooth muscle therefore imparts instability to airways in terms of their ability to maintain patency, because of the possibility of airway closure due to smooth muscle being adapted to abnormally short lengths.

Evidence to date suggests that the ability of smooth muscle to generate force over a broad length range stems from the muscle's malleable myofilament lattice - a network of actin (thin), myosin (thick), intermediate filaments, and the anchoring proteins that bind the filaments together. This newly recognized dynamic filament organization is thought to allow the muscle to adapt by optimizing contractile-filament overlap, such that the maximal force is maintained over the range of lengths needed for its physiological function (1, 12-17). These recent conclusions suggest a completely new paradigm for smooth muscle contraction, which has the potential to alter totally our approach to understand excessive airway narrowing. They also suggest that it is imperative that ASM research must focus on the regulation of organization (assembly) of structural proteins that are associated with cell malleability. The function of many proteins (some of them highly abundant) in smooth muscle is currently unknown. The change in our view that emphasizes structural malleability and its regulation in smooth muscle cells may aid us in finding the real purposes for these proteins. Abnormal expression and/or function of these proteins may also represent novel therapeutic targets in diseases that involve altered structural and functional properties of smooth muscle cells.

THE PHENOMENON OF SMOOTH MUSCLE LENGTH ADAPTATION

The fixed filament lattice limits striated muscle to generate maximal force within a length range of 10-20% of its resting length (18). This range is not adequate for most smooth muscles to

carry out organ functions such as emptying a urinary bladder or pushing out a fetus from a uterus. In many types of smooth muscle, maximal force generation is associated with a very broad length range, and the range becomes even broader if the muscle is allowed to adapt at each of the lengths where force measurements are made (8, 11). Length adaptation in smooth muscle can be induced by a single contraction (19, 20), a series of brief activations (11, 21) or a continuous submaximal activation (10) over a period of tens of minutes. Adaptation can also occur in a relaxed muscle set at a fixed length over a period of hours (8, 22) or days (9, 23-24). Although acute and long-term length adaptation may have different mechanisms, results obtained with the various protocols appear to be the same: allowing the muscle to regain (at least partially) its ability to generate maximal force at the new length and causing a shift of the peak force of the bell-shaped "conventional" (non-adapted) active length-force curve towards the new length (Fig. 1). The passive length-force curve also shifts with the active curve (8-9). This mechanical plasticity extends the working length range of smooth muscle. Length adaptation is triggered by strain on the contractile apparatus and cytoskeleton (25), and is characterized by an initial phase during which partial disassembly of the contractile and cytoskeletal structures occurs, followed by a later phase during which reassembly of the structures occurs at the new cell length (8, 26). The initial phase is often associated with a decrease in the muscle's active force and passive stiffness, whereas the later phase is associated with force and stiffness recovery (8). Impairment of the initial phase or enhancement of the later phase will impart instability to airway smooth muscle in terms of its ability to regulate airway diameter. In the first case, failure of the muscle to respond to the initial trigger will result in an airway non-responsive to external strains (such as those induced by deep inspirations) that would otherwise lead to ASM relaxation. In the second case, because of the combined effects of the LaPlace Law (transmural pressure = wall tension/airway radius, i.e., a

constricted airway will require less wall tension to maintain a certain transmural pressure) and increased muscle force (wall tension) due to adaptation, the likelihood of airway closure (even when the muscle is not maximally activated) will increase dramatically. However, it should be pointed out that length adaptation can be both detrimental and beneficial in terms of maintenance of airway patency. Adaptation of airway smooth muscle to a longer length will cause a shift of the length-force relationship to the right, reducing the amount of shortening against a constant load (27) and prevent excessive narrowing of the airways. This mechanism may underlie the observation made by Xue et al (28) that airway responsiveness in rabbits *in vivo* is reduced by prolonged continuous positive airway pressure.

Although the maximal muscle force is largely independent of muscle length after adaptation, other properties such as shortening velocity and muscle compliance are highly length dependent (11, 26). A simple model that explains all these observations consists of contractile units arranged in series and in parallel (26, 27); adaptation of the muscle to different lengths involves adding or subtracting the units in series. (See Fig. 2 for a schematic illustration). Because a myosin filament is the central component of a contractile unit, any change in the number of contractile units inside a cell will be accompanied by a corresponding change in the number of myosin filaments, possibly through polymerization or depolymerization. Furthermore, the change has to occur sufficiently rapidly to account for the observed swift time-course of length adaptation (11, 25).

EVIDENCE FOR RAPID CONTRACTILE FILAMENT ASSEMBLY AND DISASSEMBLY IN INTACT SMOOTH MUSCLE

The underlying mechanism for the phenomenon of length adaptation in smooth muscle is not entirely clear. As mentioned above, available evidence suggests that structural malleability of

the network of contractile and cytoskeletal filaments is a key factor that gives the muscle the ability to adapt and function over a large length range (8, 11, 19, 25-27, 29-38). Studies reveal changes in the myosin-filament content in smooth muscle cells during contractile activation (39-41) and in its adaptation to different cell lengths (26, 32, 42). The content of actin filaments in smooth muscle has also been found to be variable - the filament content increases during contractile activation (32, 43). It appears therefore that the content of contractile filaments in smooth muscle can change rapidly, sometimes within seconds (42). The rapidity confers feasibility to the proposed models (Fig. 2) based on myosin filament evanescence (26-27) and suggests that rapid filament formation and dissolution in smooth muscle cells is governed by an equilibrium between non-filamentous and filamentous myosin. In ASM cells adapted to a stretched length, we have observed a substantial increase in myosin filament content (26). We have proposed that this increase is due to additional contractile units incorporated into the contractile apparatus during the process of length adaptation. As shown in Fig. 3, the observed increase in muscle power-output (26), rate of ATP consumption (26), and shifts in the length-force relationship (27) also agrees with this explanation. These observations suggest that the structural basis of smooth muscle contraction is not static akin to that of striated muscle but is dynamic and possesses many features of non-muscle motile cells (16-17).

POSSIBLE MECHANISMS REGULATING MYOFILAMENT ORGANIZATION AND LATTICE MALLEABILITY

It has been known for a long time that myosin filaments of smooth muscle are not stable and can be dissociated in solution at physiological levels of MgATP, ionic strength and pH, and that phosphorylation of the regulatory myosin light chain (ReLC) stabilizes the filaments (44). Assembly of non-phosphorylated myosin monomers into filaments in solution has been found to be

dependent on the monomer concentration; once a critical concentration is reached, self-assembly occurs (45). For a wide range of ionic strength, this critical concentration increases dramatically when ATP is present in the solution (45) and decreases when ReLC is phosphorylated (45-46). The relevance of these *in vitro* mechanisms to intact smooth muscle cells is still a question. Based on these observations, at physiological levels of MgATP, ionic strength, and pH, and in the absence of ReLC phosphorylation, there should be few (if any) thick filaments formed in solution (47). Unfacilitated self-assembly of non-phosphorylated monomers into thick filaments *in vivo* is therefore not likely. The fact that thick filaments are found in relaxed smooth muscle cells suggests that there are other factors contributing to the thick filament formation *in vivo*. In intact airway smooth muscle, we tested the idea that phosphorylation of ReLC may facilitate thick filament formation. By applying large amplitude length oscillation to relaxed trachealis muscle strips, we observed that thick filaments could be partially disassembled (25) (Fig. 4). Reassembly of the filaments occurred when the muscle was repeatedly activated and relaxed in the absence of mechanical perturbation (25). However, this thick filament reassembly was prevented when phosphorylation of ReLC was inhibited (48). The results suggest that phosphorylation of ReLC is just as important for thick filament formation *in vivo* as it is *in vitro* (47).

A critical question is why are there so many thick filaments present in intact smooth muscle in the relaxed state when there is no ReLC phosphorylation (48). A possible answer is that the actin filament network plays a crucial role in guiding and facilitating thick filament formation. *In vitro* experiments (49-50) suggest that this is possible; however it needs to be validated *in vivo*. Caldesmon (an actin filament associated protein) has also been shown to promote myosin filament formation *in vitro* (51); again, this needs to be verified by *in vivo* experiments. Telokin that modulates myosin phosphorylation rate (52) is very likely involved in the filament assembly

process (53-54), but the mechanisms need to be elucidated. Other proteins such as 38k protein (55) could also contribute to the maintenance of thick filaments in the relaxed smooth muscle. These findings suggest that many proteins present in smooth muscle have the potential to preserve thick filaments *in vivo*, a role that was not considered important or necessary, until recently.

For a contractile apparatus to function properly and generate force, thick filaments have to be placed in a lattice of thin filaments where they can interact with thin filaments possessing the appropriate polarities. Perhaps it is for this reason that many thick filament-stabilizing proteins are part of the thin filament network. These proteins are likely placed in specific areas of the thin filament network, and their locations determine where the thick filaments are formed. Caldesmon, for example, is found interspersed in the thin filament network where myosin filaments are also found (56-57).

The structure and polarity of the actin filaments in smooth muscle has been well described (see review by Hodgkinson (58)), the same cannot be said for myosin filaments. An important feature of the thick filaments assembled *in vitro* is the absence of a central cross-bridge free (bare) zone seen in thick filaments from skeletal muscle (59). Instead, the thick filaments of smooth muscle showed asymmetrical cross-bridge free edges at their two ends (60). On the basis of these observations, a bipolar building unit made up of anti-parallel myosin dimers was proposed. A more detailed model of the assembly of such dimers into a helical filament was subsequently described (61), emphasizing a major future of mixed polarity of the cross-bridges. In this model, myosin heads with the same polarity are lined up in a row extending helically from one end of the filament to the other, with the neighboring rows having the opposite polarity. The bipolar dimeric building unit has been incorporated into another model of smooth muscle myosin filaments (face polar model) that is based on observations of flat filaments with myosin heads possessing the same

polarity on one side of the filament and those with opposite polarity on the other side (62). Both types of filament model will work (in theory) in contractile units such as those shown in Fig. 2, but the frequent observation of myosin filaments surrounded by rosettes of actin filaments in smooth muscle favors the helical thick filament model.

Taken together, it appears that *lability* of myosin filaments in smooth muscle, especially in the relaxed (non-phosphorylated) state, is a crucial property that allows the muscle to adapt to changes in configuration of the thin filament lattice, brought about by external and internal strains that change the cell dimension. Somehow, the mechanical strains that shape the filament lattice also results in disassembly of the unphosphorylated thick filaments. Unraveling the molecular processes of the thick filament disassembly and subsequent reassembly during the course of length adaptation is critical to our understanding of normal smooth muscle function and its alteration in disease.

MALADAPTATION OF AIRWAY SMOOTH MUSCLE AS A MAJOR CAUSE FOR ASTHMA EXACERBATION - A HYPOTHESIS

Before the discovery of the adaptation phenomenon in smooth muscle, shortening of the muscle from its "optimal" length was thought to be always associated with a diminished ability for the muscle to generate force. Now we know that adaptation allows smooth muscle to recover part (if not all) of its maximal force producing capacity after a length change, our view has changed regarding how airway calibre is controlled by the balance of muscle force and load. Because adaptation occurs most effectively under static conditions, the mechanically dynamic lung environment is probably the most important factor that prevents length adaptation of airway smooth muscle from occurring and the ensuing excessive narrowing of the airways. If, for any reason, the airways are uncoupled from the lung parenchyma due to a loss of effective tethering or overly stiff

airways (the latter could be due to an alteration in the mass or intrinsic properties of airway smooth muscle, or a change in the properties of the airway wall), a pernicious cycle could set in and lead to excessive narrowing or even closure of the airways. As illustrated in Fig. 5, adaptation of airway smooth muscle will lead to continued narrowing of the airways if there is no intervention (such as a length perturbation on the smooth muscle induced by a big breath in a healthy lung) to break up the cycle of length adaptation.

Summary

Although length adaptation in smooth muscle is essential for physiological function of many hollow organs that undergo large changes in volume, it could be a source of instability for the airways in terms of maintenance of airway patency. *In vitro* experiments have clearly demonstrated the phenomenon of length adaptation in airway smooth muscle and its ability to shorten to such a length that would result in complete airway closure. However, *in vivo* observations in healthy lungs suggest that such harmful adaptation does not occur and that what prevents such adaptation from occurring is perhaps the constant agitation on airway smooth muscle by the dynamic lung environment. A loss of this mechanical perturbation coupled with length adaptation could underlie the pathogenesis of many obstructive airway diseases such as asthma.

References

1. Seow CY, Fredberg JJ. Historical Perspective on Airway Smooth Muscle: The saga of a frustrated cell. *J. Appl. Physiol* 2001; 91: 938-952.

2. Mitzner W. Airway smooth muscle: the appendix of the lung. *Am J Respir Crit Care Med* 2004; 169(7): 787-790.

3. Mead J. Point: Airway smooth muscle is useful. *J Appl Physiol* 2007; 102(4):1708-1709.

4. Fredberg JJ. Counterpoint: airway smooth muscle is not useful. *J Appl Physiol* 2007; 102(4): 1709-1710.

5. Seow CY, Mitzner W, Irvin CG, Dubois AB, Ameredes BT, Panettieri RA Jr, Amrani Y, Permutt S. Point:counterpoint comments. *J Appl Physiol* 2007; 102(4): 1712-1714.

6. King GG, PD Pare, CY Seow. The mechanics of exaggerated airway narrowing in asthma: the role of smooth muscle. *Respiration Physiology* 1999; 118: 1-13.

7. An SS, Bai TR, Bates JHT, Black JL, Brown RH, Brusasco V, Chitano P, Deng L, Dowell M, Eidelman DH, Fabry B, Ford LE, Fredberg JJ, Gerthoffer WT, Gilbert SH, Gosens R, Gunst SJ, Halayko AJ, Ingram RH, Irvin CG, James AL, Janssen LJ, King GG, Knight DA, Lauzon AM, Lakser OJ, Ludwig MS, Lutchen KR, Maksym GN, Martin JG, Mauad T, McParland BE, Mijailovich SM, Mitchell HW, Mitchell RW, Mitzner W, Murphy TM, Paré PD, Pellegrino R, Sanderson MJ, Schellenberg RR, Seow CY, Smith PG, Solway J, Silveira PSP, Stephens NL, Sterk PJ, Stewart AG, Tang DD, Tepper RS, Tran T, Wang L. Airway smooth muscle dynamics: a final common pathway of airway obstruction in asthma. *Eur Respir J* (In press).

8. Wang L, Pare PD, Seow CY. Selected contribution: effect of chronic passive length change on airway smooth muscle length-tension relationship. *J Appl Physiol* 2001; 90(2):734-740.

9. Naghshin J, Wang L, Pare PD, Seow CY. Adaptation to chronic length change in explanted airway smooth muscle. *J Appl Physiol* 2003; 95(1): 448-453.

   McParland BE, Tait RR, Paré PD, Seow CY. The role of airway smooth muscle during an attack of asthma simulated *in vitro*. *Am J Respir Cell Mol Biol*. 2005; 33(5): 500-504..

11. Pratusevich VR, Seow CY, Ford LE. Plasticity in canine airway smooth muscle. *J Gen Physiol* 1995; 105(1): 73-94.

12. Gunst SJ, Fredberg JJ. The first three minutes: smooth muscle contraction, cytoskeletal events, and soft glasses. *J Appl Physiol* 2003; 95(1): 413-425.

13. Gunst SJ, Tang DD, Opazo Saez A. Cytoskeletal remodeling of the airway smooth muscle cell: a mechanism for adaptation to mechanical forces in the lung. *Respir Physiol Neurobiol* 2003; 137(2-3): 151-168.

14. Gunst SJ. Actions by actin: reciprocal regulation of cortactin activity by tyrosine kinases and F-actin. *Biochem J* 2004; 380(Pt 2): e7-8.

15. Lenormand G, Millet E, Fabry B, Butler JP, Fredberg JJ. Linearity and time-scale invariance of the creep function in living cells. *J R Soc Interface* 2004; 1(1): 91-97.

16. Seow CY. Biophysics: Fashionable cells. *Nature* 2005; 435(7046):1172-1173.

17. Seow CY. Invited perspective in cell physiology: Myosin filament assembly in an ever-changing myofilament lattice of smooth muscle. *Am J Physiol Cell Physiol* 2005; 289(6):C1363-C1368.

18. Gordon AM, Huxley AF, Julian FJ. The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J Physiol 1966;* 184(1): 170-192.

19. Seow CY, Pratusevich VR, Ford LE. Series-to-parallel transition in the filament lattice of airway smooth muscle. *J Appl Physiol* 2000; 89(3):869-876.

20. Gunst SJ, Wu MF. Selected contribution: plasticity of airway smooth muscle stiffness and extensibility: role of length-adaptive mechanisms. *J Appl Physiol* 2001; 90(2): 741-749.

21. Silberstein J, Hai CM. Dynamics of length-force relations in airway smooth muscle. *Respir Physiol Neurobiol* 2002; 132(2): 205-221.

22. Martinez-Lemus LA, Hill MA, Bolz SS, Pohl U, Meininger GA. Acute mechanoadaptation of vascular smooth muscle cells in response to continuous arteriolar vasoconstriction: implications for functional remodeling. *FASEB J* 2004; 18(6): 708-710.

23. Arner A, Malmqvist U, Uvelius B. Structural and mechanical adaptations in rat aorta in response to sustained changes in arterial pressure. *Acta Physiol Scand* 1984; 122(2): 119-126.

24. Zeidan A, Nordstrom I, Dreja K, Malmqvist U, Hellstrand P. Stretch-dependent modulation of contractility and growth in smooth muscle of rat portal vein. *Circ Res* 2000; 87(3): 228-234.

25. Kuo KH, Wang L, Pare PD, Ford LE, Seow CY. Myosin thick filament lability induced by mechanical strain in airway smooth muscle. *J Appl Physiol* 2001; 90(5):1811-1816.

26. Kuo KH, Herrera AM, Wang L, Pare PD, Ford LE, Stephens NL, Seow CY. Structure-function correlation in airway smooth muscle adapted to different lengths. *Am J Physiol Cell Physiol* 2003; 285(2): C384-C390.

27. Herrera AM, McParland BE, Bienkowska A, Tait R, Pare PD, Seow CY. 'Sarcomeres' of smooth muscle: functional characteristics and ultrastructural evidence. *J Cell Sci* 2005; 118(Pt 11): 2381-2392.

28. Xue Z, Zhang L, Ramchandani R, Liu Y, Antony VB, Gunst SJ, Tepper RS. Respiratory system responsiveness in rabbits in vivo is reduced by prolonged continuous positive airway pressure. *J Appl Physiol* 2005; 99(2): 677-682.

29. Gunst SJ, Meiss RA, Wu MF, Rowe M. Mechanisms for the mechanical plasticity of tracheal smooth muscle. *Am J Physiol* 1995; 268(5 Pt 1): C1267-C1276.

30. Wang L, Pare PD, Seow CY. Effects of length oscillation on the subsequent force development in swine tracheal smooth muscle. *J Appl Physiol* 2000; 88(6): 2246-2250.

31. Fabry B, Maksym GN, Butler JP, Glogauer M, Navajas D, Fredberg JJ. Scaling the microrheology of living cells. *Phys Rev Lett* 2001; 87(14): 148102.

32. Herrera AM, Martinez EC, Seow CY. Electron microscopic study of actin polymerization in airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2004; 286(6): L1161-L1168.

33. Dowell ML, Lakser OJ, Gerthoffer WT, Fredberg JJ, Stelmack GL, Halayko AJ, Solway J, Mitchell RW. Latrunculin B increases force fluctuation-induced relengthening of ACh-contracted, isotonically shortened canine tracheal smooth muscle. *J Appl Physiol* 2005; 98(2): 489-497.

34. Tang DD, Zhang W, Gunst SJ. The adapter protein CrkII regulates neuronal Wiskott-Aldrich syndrome protein, actin polymerization, and tension development during contractile stimulation of smooth muscle. *J Biol Chem* 2005; 280(24): 23380-23389.

35. Zhang W, Gunst SJ. Dynamic association between alpha-actinin and beta-integrin regulates contraction of canine tracheal smooth muscle. *J Physiol* 2006; 572(Pt 3): 659-676.

36. Bursac P, Lenormand G, Fabry B, Oliver M, Weitz DA, Viasnoff V, Butler JP, Fredberg JJ. Cytoskeletal remodelling and slow dynamics in the living cell. *Nat Mater* 2005; 4(7): 557-561.

37. Bursac P, Fabry B, Trepat X, Lenormand G, Butler JP, Wang N, Fredberg JJ, An SS. Cytoskeleton dynamics: fluctuations within the network. *Biochem Biophys Res Commun* 2007; 355(2): 324-330.

38. Deng L, Trepat X, Butler JP, Millet E, Morgan KG, Weitz DA, Fredberg JJ. Fast and slow dynamics of the cytoskeleton. *Nat Mater* 2006; 5(8): 636-640.

39. Gillis JM, Cao ML, and Godfraind-De Becker A. Density of myosin filaments in the rat anococcygeus muscle, at rest and in contraction. II. *J Muscle Res Cell Motil* 1988; 9(1): 18-29.

40. Xu JQ, Gillis JM, Craig R. Polymerization of myosin on activation of rat anococcygeus smooth muscle. *J Muscle Res Cell Motil* 1997; 18(3): 381-393.

41. Herrera AM, Kuo KH, Seow CY. Influence of calcium on myosin thick filament formation in intact airway smooth muscle. *Am J Physiol Cell Physiol* 2002; 282(2): C310-C316.

42. Smolensky AV, Ragozzino J, Gilbert SH, Seow CY, Ford LE. Length-dependent filament formation assessed from birefringence increases during activation of porcine tracheal muscle. *J Physiol* 2005; 563(Pt 2): 517-527.

43. Mehta D, Gunst SJ. Actin polymerization stimulated by contractile activation regulates force development in canine tracheal smooth muscle. *J Physiol* 1999; 519: 829-840.

44. Suzuki H, Onishi H, Takahashi K, Watanabe S. Structure and function of chicken gizzard myosin. *J Biochem (Tokyo)* 1978; 84(6):1529-1542.

45. Kendrick-Jones J, Smith RC, Craig R, Citi S. Polymerization of vertebrate non-muscle and smooth muscle myosins. *J Mol Biol* 1987; 198(2): 241-252.

46. Trybus KM, Lowey S. Assembly of smooth muscle myosin minifilaments: effects of phosphorylation and nucleotide binding. *J Cell Biol* 1987; 105(6 Pt 2): 3007-3019.

47. Craig R, Smith R, Kendrick-Jones J. Light-chain phosphorylation controls the conformation of vertebrate non-muscle and smooth muscle myosin molecules. *Nature* 1983; 302(5907):436-439.

48. Qi D, Mitchell RW, Burdyga T, Ford LE, Kuo KH, Seow CY. Myosin light chain phosphorylation facilitates *in vivo* myosin filament reassembly after mechanical perturbation. *Am J Physiol Cell Physiol* 2002; 282(6):C1298-C1305.

49. Mahajan RK, Vaughan KT, Johns JA, Pardee JD. Actin filaments mediate Dictyostelium myosin assembly *in vitro*. *Proc Natl Acad Sci U S A* 1989; 86(16): 6161-6165.

50. Applegate D, Pardee JD. Actin-facilitated assembly of smooth muscle myosin induces formation of actomyosin fibrils. *J Cell Biol* 1992; 117(6): 1223-1230.

51. Katayama E, Scott-Woo G, Ikebe M. Effect of caldesmon on the assembly of smooth muscle myosin. *J Biol Chem* 1995; 270(8): 3919-3925.

52. Nieznanski K, Sobieszek A. Telokin (kinase-related protein) modulates the oligomeric state of smooth-muscle myosin light-chain kinase and its interaction with myosin filaments. *Biochem J* 1995; 322 (1): 65-71.

53. Kudryashov DS, Vorotnikov AV, Dudnakova TV, Stepanova OV, Lukas TJ, Sellers JR, Watterson DM, Shirinsky VP. Smooth muscle myosin filament assembly under control of a kinase-related protein (KRP) and caldesmon. *J Muscle Res Cell Motil* 2002; 23(4): 341-351.

54. Sobieszek A, Andruchov OY, Grabarek Z, Kulikova N, Liebetrau C, Matusovsky OS. Modulation of myosin filament activation by telokin in smooth muscle Liberation of myosin kinase and phosphatase from supramolecular complexes. *Biophys Chem* 2005; 113(1): 25-40.

55. Okagaki T, Nakamura A, Suzuki T, Ohmi K, Kohama K. Assembly of smooth muscle myosin by the 38k protein, a homologue of a subunit of pre-mRNA splicing factor-2. *J Cell Biol* 2000; 148(4): 653-663.

56. Mabuchi K, Li Y, Tao T, Wang CL. Immunocytochemical localization of caldesmon and calponin in chicken gizzard smooth muscle. *J Muscle Res Cell Motil* 1996; 17(2): 243-260.

57. Wang CL. Caldesmon and smooth-muscle regulation. *Cell Biochem Biophys* 2001; 35(3): 275-288.

58. Hodgkinson JL. Actin and the smooth muscle regulatory proteins: a structural perspective. *J Muscle Res Cell Motil* 2000; 21(2): 115-130.

59. Huxley HE. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J Mol Biol* 1963; 7:281-308.

60. Small JV, Sobieszek A. The contractile apparatus of smooth muscle. *Inter Rev Cytol* 1980; 64:241-306.

61. Hinssen H, D'Haese J, Small JV, Sobieszek A. Mode of filament assembly of myosins from muscle and nonmuscle cells. *J Ultrastruct Res* 1978; 64:282-302.

62. Craig R, Megerman J. Assembly of smooth muscle myosin into side-polar filaments. *J Cell Biol* 1977; 75:990-996.

Figure legends

Figure 1: Schematic illustration of a shift of active and passive length-force relationships based on data from Wang et al (2001). A smooth muscle adapted at an arbitrary reference length (L_{ref}) will produce a maximal isometric force at L_{ref} (labeled "A"). The active and passive length-force relationships obtained without allowing the muscle to adapt to any length except L_{ref} are illustrated by the solid curves. An acute shortening of the muscle by the amount of X (from L_{ref}) will reduce the force to that indicated by "B". If the muscle is then allowed to adapt at the shortened length (L_{ref} - X), isometric force will recover and eventually reach the same maximum before the shortening ("C"). The corresponding (non-adapted) active and passive length-force relationships (dotted curves) will also be shifted by the same amount (X) as the maximal force.

Figure 2: Schematic illustration of length adaptation in smooth muscle. The model assumes that the number of contractile units in series is a linear function of the adapted muscle cell length.

Figure 3: Mechanical and metabolic properties of smooth muscle as functions of adapted muscle length in airway smooth muscle. Based on the conceptual model shown in Fig. 2, the shortening velocity and power output of the muscle is predicted (and verified by Pratusevich et al (1995) and Kuo et al (2003)) to be a linear function of the adapted muscle length, as indicated by the solid line. The rate of ATP utilization (solid circle) and myosin filament content (open circle) at different adapted muscle cell lengths are found to obey the same linear relationship (Kuo et al, 2003), thus supporting the basic assumption upon which the model (Fig. 2) is based.

Figure 4: Time course of recovery (adaptation) of isometric force and myosin filament content in airway smooth muscle after length perturbation (a 5-minute sinusoidal length oscillation with frequency of 0.5 Hz and 30% strain amplitude). The arrow indicates cessation of length perturbation. Isometric force was measured from brief tetani (12-s duration) induced by electrical field stimulation. Thick filament density was measured from cell cross-sections using electronmicroscopy after fixing the muscle at the indicated time points. Reference values were those obtained before length perturbation. (Modified from Kuo et al, 2001, with permission).

Figure 5: Illustration of how length adaptation could lead to excessive shortening of airway smooth muscle. Panel **A** outlines a hypothetical load (against which the airway smooth muscle shortens) as a function airway radius. R_o is the radius before airway constriction. The curve labeled "α" represents the load stemming from the transmural pressure, according to the LaPlace Law (assuming a constant pressure). The curve labeled "β" represents the load stemming from parenchymal tethering and resistance from folding of lamina propria-submucosal layer as a function of airway radius. The total load "seen" by the muscle is ($\alpha + \beta$). In Panel **B**, the total load ($\alpha + \beta$) is duplicated. The two solid lines represent length-force relationships of smooth muscle adapted to two lengths (from Herrera et al, 2005). Point "a" represents maximal isometric force of muscle adapted at R_o. When the muscle is stimulated to contract, it shortens following the instantaneous length-force curve (indicated by arrows) until the load equals the force at Point "b". If the muscle is allowed to adapt at the shortened length, its ability to generate force will increase with time and eventually reach Point "c". If the muscle is then stimulated again, it will shorten, following a new instantaneous length-force curve to reach Point "d". The process can repeat itself and lead to excessive constriction of the airway.

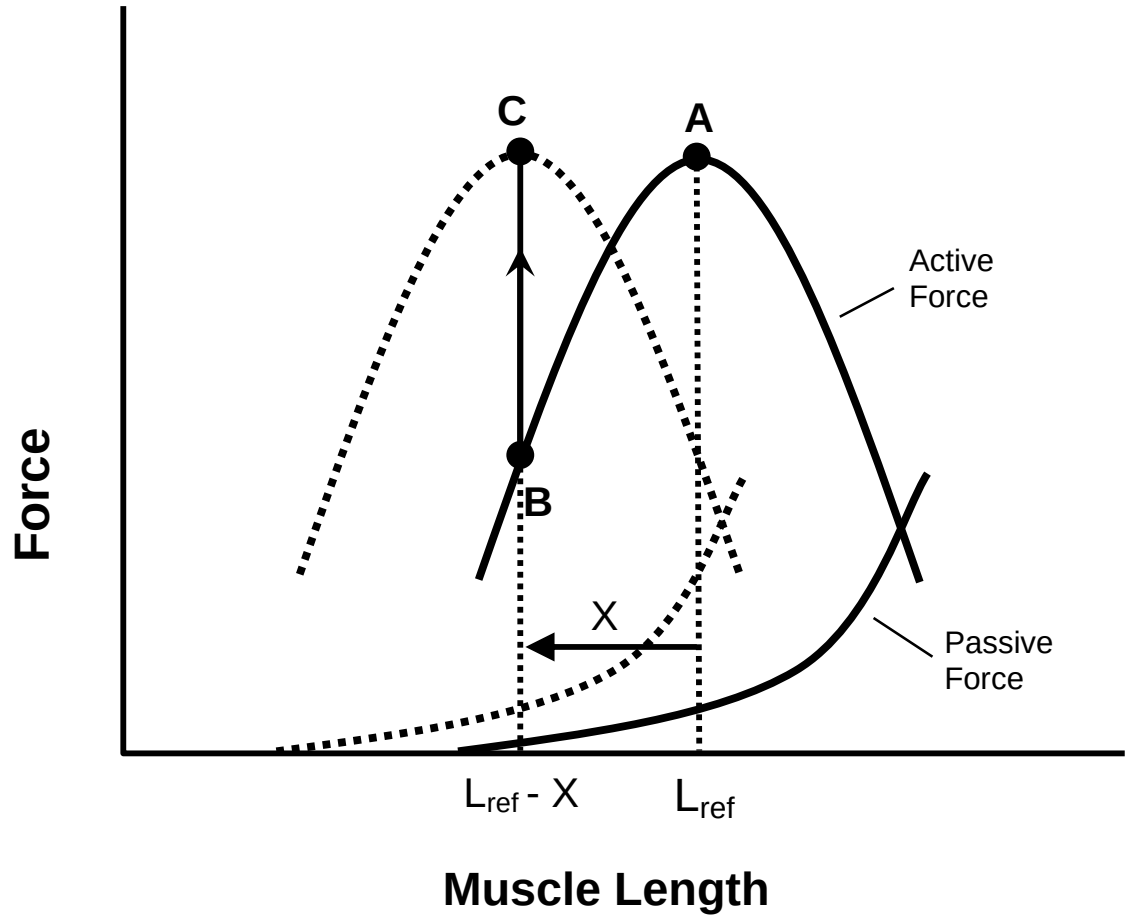

Figure 1: Schematic illustration of a shift of active and passive length-force relationships based on data from Wang et al (2001). A smooth muscle adapted at an arbitrary reference length (L_{ref}) will produce a maximal isometric force at L_{ref} (labeled "A"). The active and passive length-force relationships obtained without allowing the muscle to adapt to any length except L_{ref} are illustrated by the solid curves. An acute shortening of the muscle by the amount of X (from L_{ref}) will reduce the force to that indicated by "B". If the muscle is then allowed to adapt at the shortened length (L_{ref} - X), isometric force will recover and eventually reach the same maximum before the shortening ("C"). The corresponding (non-adapted) active and passive length-force relationships (dotted curves) will also be shifted by the same amount (X) as the maximal force.

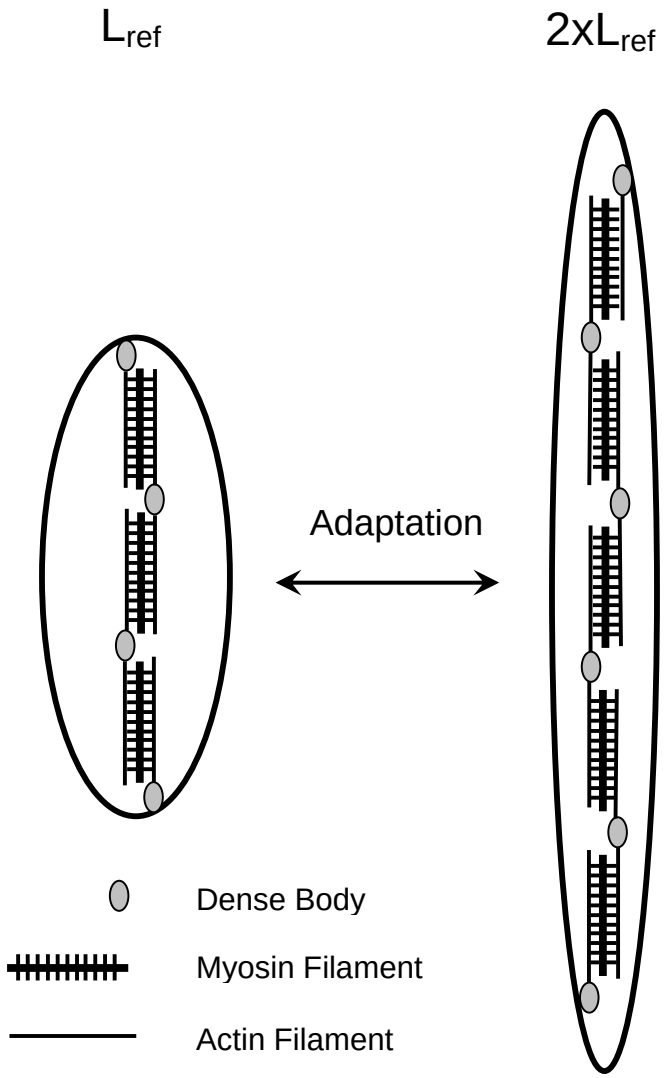

Figure 2: Schematic illustration of length adaptation in smooth muscle. The model assumes that the number of contractile units in series is a linear function of the adapted muscle cell length.

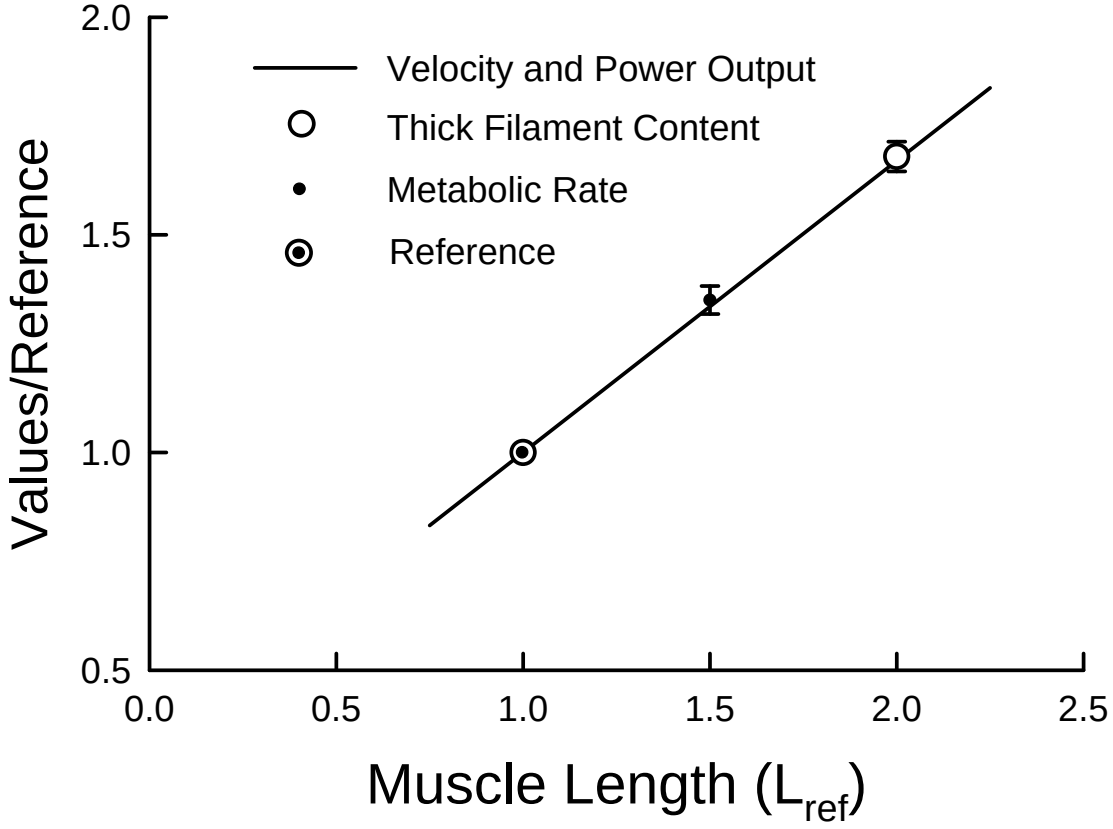

Figure 3: Mechanical and metabolic properties of smooth muscle as functions of adapted muscle length in airway smooth muscle. Based on the conceptual model shown in Fig. 2, the shortening velocity and power output of the muscle is predicted (and verified by Pratusevich et al (1995) and Kuo et al (2003)) to be a linear function of the adapted muscle length, as indicated by the solid line. The rate of ATP utilization (solid circle) and myosin filament content (open circle) at different adapted muscle cell lengths are found to obey the same linear relationship (Kuo et al, 2003), thus supporting the basic assumption upon which the model (Fig. 2) is based.

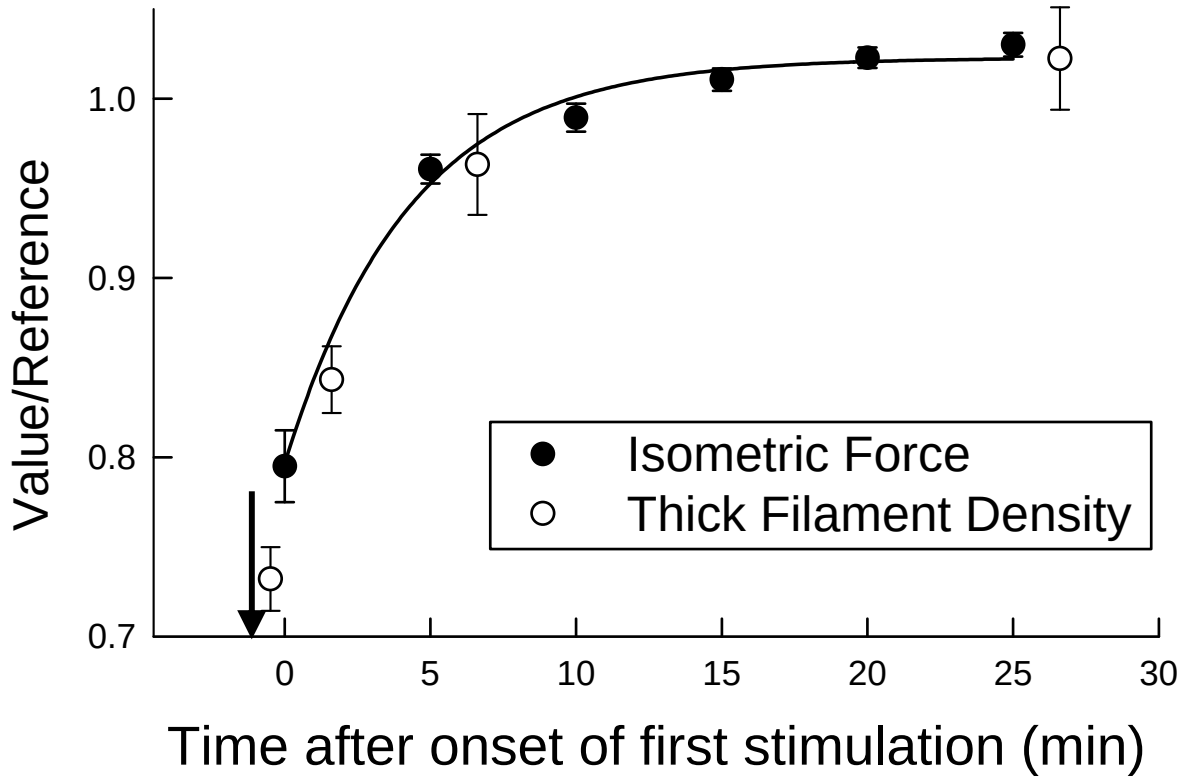

Figure 4: Time course of recovery (adaptation) of isometric force and myosin filament content in airway smooth muscle after length perturbation (a 5-minute sinusoidal length oscillation with frequency of 0.5 Hz and 30% strain amplitude). The arrow indicates cessation of length perturbation. Isometric force was measured from brief tetani (12-s duration) induced by electrical field stimulation. Thick filament density was measured from cell cross-sections using electronmicroscopy after fixing the muscle at the indicated time points. Reference values were those obtained before length perturbation. (Modified from Kuo et al, 2001, with permission).

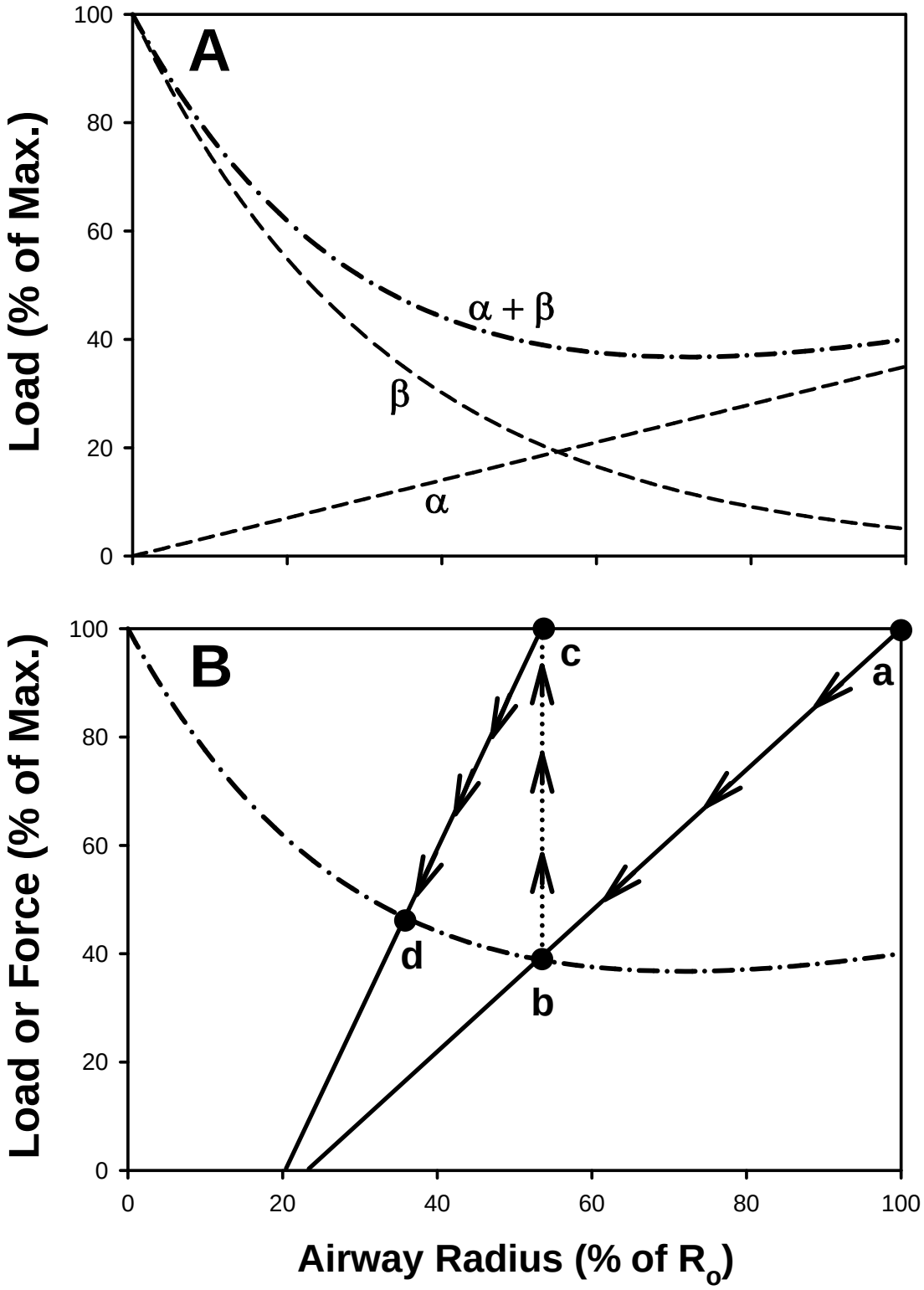
A
B
Load (% of Max.)
Load or Force (% of Max.)
α + β
β
α
a
b
c
d
0
20
40
60
80
100
Airway Radius (% of R_o)

Figure 5: Illustration of how length adaptation could lead to excessive shortening of airway smooth muscle. Panel **A** outlines a hypothetical load (against which the airway smooth muscle shortens) as a function airway radius. R_o is the radius before airway constriction. The curve labeled "α" represents the load stemming from the transmural pressure, according to the LaPlace Law (assuming a constant pressure). The curve labeled "β" represents the load stemming from parenchymal tethering and resistance from folding of lamina propria-submucosal layer as a function of airway radius. The total load "seen" by the muscle is (α + β). In Panel **B**, the total load (α + β) is duplicated. The two solid lines represent length-force relationships of smooth muscle adapted to two lengths (from Herrera et al, 2005). Point "a" represents maximal isometric force of muscle adapted at R_o. When the muscle is stimulated to contract, it shortens following the instantaneous length-force curve (indicated by arrows) until the load equals the force at Point "b". If the muscle is allowed to adapt at the shortened length, its ability to generate force will increase with time and eventually reach Point "c". If the muscle is then stimulated again, it will shorten, following a new instantaneous length-force curve to reach Point "d". The process can repeat itself and lead to excessive constriction of the airway.