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Mathematical Modelling of Airway Smooth Muscle

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Abstract

The bronchial tree consists of thousands of airways, from the trachea down to the small terminal bronchioles. Surrounding each airway is a layer of Airway Smooth Muscle (ASM) tissue. It is thought that abnormalities in the properties of the muscle play a key role in the pathogenesis of asthma and airway hyper-responsiveness. Specifically, stiffening of the ASM tissue or a weakened sensitivity in its response to stretch can lead to sustained airway narrowing, one of the major characteristics of airway diseases. A long-term goal is to learn more about the effects of increased muscle mass or changes in the heterogeneity of the lung response on diseases such as asthma. This entails the formulation of a mathematical representation of the entire bronchial tree.

The first step towards this goal is to represent a single airway. By coupling a model of the airway radius with a model of its ASM lining, a description of the response of a single airway under various chemical and mechanical stimuli can be obtained. The force generated by the muscle will determine the extent to which the airway will narrow. A sophisticated ASM model developed previously is capable of reproducing many of the observed properties from tissue strip experiments but is difficult to analyse and computationally expensive to solve. Employing such a complex model to describe the ASM generated force would make a representation of the entire tree almost impossible. As such, our goal is to determine whether this model could be replaced with a vastly simplified model.

We demonstrate that, under some circumstances, complex ASM properties at the tissue level can have a profound impact on the airway response. Naturally, we want to further our understanding of these properties, particularly passive mechanisms such as cytoskeletal remodelling. Our interest in these processes stems from the roles they are likely to play in increasing the cell’s resistance, stiffening the muscle to prevent airway relaxation and modulating the sensitivity of the cell to stretch. We are particularly concerned with how this behaviour is regulated, its implications in airway disorders and how such passive dynamics interact with their active counterparts.

It is found that a simplified model can be employed to describe the ASM generated force for large airways. However, for a small range of parameter values, the small airway response to imposed pressure oscillations varies significantly depending upon the complexity of the model used to describe the ASM force.
In addition, we find that both crosslinkers and latchbridges are needed to provide a complete representation of ASM contraction. Specifically, latchbridges are required to explain the decrease in phosphorylation following muscle activation while crosslinkers are required to explain the independence of passive stiffness on myosin light chain kinase activity as well as the passive length-tension curves observed by Naghshin et. al. [93].

A constitutive formulation, typical of that found in the complex fluids literature, can be employed as a method of combining the active and passive sides of ASM contraction. In addition, this formulation improves upon the predicted force-length relationship observed experimentally by several groups.
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## Contents

Glossary ................................. v

1 Introduction .......................... 1
   1.1 Smooth Muscle Properties and Composition ....................... 2
   1.2 Active Airway Smooth Muscle ....................................... 4
   1.3 Passive Airway Smooth Muscle ..................................... 5

2 Literature Review: Biological and Mathematical Background .... 10
   2.1 Model Development ................................................... 10
   2.2 Asthma ................................................................. 14
   2.3 Shortening Velocity and the Latch State .......................... 16
      2.3.1 Plasticity ......................................................... 18
   2.4 Force-Length Loops .................................................. 20
   2.5 Deep Inspirations and Soft Glassy Materials ...................... 23
   2.6 Interactions Between the Parenchyma and ASM Tissue: Intact Airway Models .................................................. 27

3 Mathematical Modelling of Airway Smooth Muscle .......... 29
   3.1 The Crossbridge Model ............................................... 29
      3.1.1 The Effects of Varying Calcium and Agonist Concentrations on Airway Contraction ................................................. 32
   3.2 The Crosslinker Model ................................................. 34
   3.3 Coupling of the Crossbridge and Crosslinker Models (the Parallel Model) ................................................................. 37
   3.4 Numerical Method ....................................................... 38

4 Coupling the Airway Wall with the Airway Smooth Muscle Tissue 40
   4.1 Introduction ............................................................. 40
   4.2 The Airway Model ....................................................... 41
4.2.1 Determining the Fixed Points of the System ........................................... 44
4.2.2 Constant Force ....................................................................................... 45
4.2.3 A Dynamically Determined Force .......................................................... 45
4.2.4 Force Predicted by the Parallel Model ..................................................... 50
4.3 Experiments on Large Airways ................................................................. 51
  4.3.1 Airway Response to Varying Degrees of Activation (Protocol A.) ....... 51
  4.3.2 Airway Response to Increasing Amplitudes of Pressure Oscillations
      (Protocol B.) ............................................................................................. 53
4.4 The Small Airway Response ....................................................................... 59
  4.4.1 The Constant Force Model ..................................................................... 59
  4.4.2 The Dynamically Determined Force Model ........................................... 67
  4.4.3 The Parallel Model ................................................................................ 70
4.5 Discussion .................................................................................................. 74

5 Properties of Airway Smooth Muscle ........................................................... 76
  5.1 Latch State Behaviour .............................................................................. 76
  5.2 Capturing Latch State Behaviour ............................................................... 78
    5.2.1 The Crossbridge Hypothesis ............................................................ 78
    5.2.2 The Crosslinker Hypothesis: An Alternative Approach to Capturing
        Latch State Behaviour ........................................................................... 82
    5.2.3 Different Length Change Scheme Leads to Very Different Model Re-
        sults: A Feature of the Crosslinker Model .............................................. 83
    5.2.4 Theories on the Link Between Active and Passive Smooth Muscle
        Dynamics ................................................................................................. 86
    5.2.5 Phosphorylation vs Velocity Curves .................................................. 88
    5.2.6 Conclusions ....................................................................................... 92
  5.3 Length Adaptation .................................................................................... 94
    5.3.1 Length-Tension Curves ....................................................................... 96
    5.3.2 Passive Stiffness Recovery .................................................................. 97
  5.4 The Regulatory Mechanisms of Passive Stiffness ..................................... 100
    5.4.1 The Latchbridge Hypothesis ............................................................. 105
    5.4.2 The Crosslinker Hypothesis ............................................................. 106
  5.5 Conclusions ............................................................................................ 109
6 A new way of combining active and passive sides of airway smooth muscle contraction

6.1 Introduction to complex fluids ......................................................... 111
6.2 Complex Fluids .............................................................................. 113
   6.2.1 Experimental Observations of Complex Fluid Behaviour ........ 114
6.3 Complex Fluid Model Formulation .................................................... 115
6.4 Passive ASM ................................................................................. 117
   6.4.1 The Importance of the Derivative of $\Phi$ in the Constitutive Formulation 120
6.5 Approximation for $\Phi$ in Isometric Stretch and Hold Procedures ........ 120
6.6 Activated ASM .............................................................................. 124
   6.6.1 The Isometric Case ................................................................. 124
6.7 The Isotonic Case ........................................................................... 131
6.8 Conclusions ................................................................................... 136

7 Discussion ....................................................................................... 138
7.1 Future Work .................................................................................. 142

Appendices ......................................................................................... 144

A List of Parameters ........................................................................... 145

B Convergence Tests ......................................................................... 148
   B.0.1 A Strictly Isometric Case ...................................................... 150
   B.0.2 A Stretch and Hold Procedure ............................................. 151
   B.0.3 The Non-Uniform Mesh Method .......................................... 153
Glossary

**ASM** Airway Smooth Muscle, the lining of smooth muscle encircling the airways..  1

**DI** Deep Inspiration, a voluntary deep breath, usually taken to relax airways..  112

**EFS** Electrical Field Stimulation, short bursts of current applied to stimulate the muscle as an alternative to the application of chemical stimuli..  94

**FAK** Focal Adhesion Kinase, an enzyme involved in chemical and mechanical signaling pathways that lead to actin polymerization and filament bundling..  87

**MLCK** Myosin Light Chain Kinase, the enzyme that assists in the phosphorylation of the myosin head to initiate crossbridge cycling..  4

**MLCP** Myosin Light Chain Phosphatase, the enzyme that assists in the dephosphorylation of the myosin head to arrest crossbridge cycling..  4
1

Introduction

Airway smooth muscle (ASM) encircles the airways of the lung and responds to various chemical and mechanical stimuli to modulate luminal airway diameter and regulate airway tone. While the precise role of ASM in the healthy lung remains unclear, it is believed that an impairment in ASM activity contributes to the pathogenesis of asthma and other lung diseases [56]. Specifically, airway wall thickening and airway inflammation are thought to be brought about through an imbalance of the factors which regulate ASM activity. Abnormalities in the remodelling of the cell’s cytoskeleton and changes in the behaviour of contractile filaments within the cell may lead to the excessive airway narrowing characteristic of airway hyper-responsiveness [85].

There is an abundance of properties that characterise ASM, making it interesting to study. Particular phenomena such as length adaptation and force maintenance have intrigued researchers for decades. The complex mechanisms underlying these properties provide us with a puzzle which scientists worldwide are trying to piece together, each aiming to contribute to advancing our knowledge of the causes of asthma.

Several milestones have so far been overcome. We know that sustained contraction, one of the major characteristics of asthma, is likely to be brought about through a restructuring of the cell’s contractile apparatus and the remodelling of its cytoskeleton to achieve a ‘frozen’ configuration of filaments inside the smooth muscle cells surrounding the airway [45, 47]. We want to know what sub-cellular filaments are involved in these processes, how they are regulated, how their behaviour affects airway diseases and how different events within the cell are likely to interact with each other.

We are particularly interested in how complex ASM properties at the tissue level affect the airway response. The long term goal is to formulate a mathematical representation
of the bronchial tree. This involves coupling a model of the ASM tissue with a model of the airway. Our current ASM model has the ability to capture several key aspects of ASM contraction but comes with the cost of being computationally expensive and difficult to analyse. Since the bronchial tree consists of thousands of airways, representing ASM with such a sophisticated model is almost impossible. To overcome this, we explore the possibility of employing simplified models to instead account for the force response of the tissue encircling the airway.

Our aim is to shed light on the answers to these questions through mathematical modelling of the processes involved. These models help to explain or strengthen belief in the pathways and structures that contribute to ASM properties in a relatively cheap and time efficient way: they have the ability to predict results of experiments which may be extremely difficult or even impossible to perform in a lab.

We want to use our models to capture certain aspects of ASM behaviour and mechanisms involved in regulating this behaviour. We explore alternative methods of modelling to determine whether different approaches may provide more accurate predictions of observed properties.

Perhaps most importantly, we want to know how essential it is to represent the complex properties, characteristic of ASM in tissue strip experiments, in a model of an intact airway. Of particular concern is the degree to which imposed pressure oscillations can prevent airway closure following stimulation with bronchoconstrictors. This is mainly a modelling question; data of this type does not exist for the small airways that we focus on. As such, the outcome will be an indication of the extent to which ASM properties, such as the latch state, affect airway closure.

1.1 Smooth Muscle Properties and Composition

Smooth muscle tissues line the organs and structures of many bodily systems including the digestive system, the reproductive system, the respiratory system, the urinary system and the circulatory system. Smooth muscle contraction is crucial to the function of these organs, for example, the movement of food through the digestive system. In contrast with smooth muscle, skeletal muscle cells are highly organised in terms of the structure of their intra-cellular filaments. These filaments are arranged into contractile units, or sarcomeres, which run in parallel throughout the cell. Although physiological function and biochemical activation mechanisms are very different, smooth muscle and skeletal muscle contraction is assumed to occur via similar processes, the sliding of the filaments over each other [116,
Smooth muscle cells are thick in the middle and tapered at the ends as in Figure 1.1. They are arranged longitudinally in sheets that line the airways and other organ structures.

![Smooth Muscle Cell Diagram](image)

**Figure 1.1:** A typical ASM cell found lining the airways in its relaxed and contracted states. Image from [118] with Open Source license number CC-BY-3.0.

Smooth muscle contraction is typically slow, strong and enduring [95]. In contrast with skeletal or cardiac muscle, smooth muscle has the ability to generate force over a wide range of lengths and its capacity to generate force can often be far greater [105]. The ability of smooth muscle to generate force over a wide range of lengths is important in allowing it to adapt to large changes in the volume of hollow organs such as the intestine or urinary bladder. Some smooth muscles (e.g. the sphincters) are in a constant or nearly constant state of contraction but this is always completely involuntary. In fact, childbirth is among the few occasions in life when some humans consciously experience smooth muscle contraction.

Clearly the ability of smooth muscle to adapt in such ways is useful in many circumstances. However, this property in ASM only serves to cause trouble: excessive airway narrowing may ensue once a shortened airway has adapted to its new length.

Fundamental to the behaviour of smooth muscle is the latch state. This phenomenon, unique to smooth muscle, enables it to generate a contractile force for prolonged periods of time with minimal energy expenditure [107]. This is crucial to the function of some organs, for example, the epiglottis (one of the sphincter muscles) is used to seal off the windpipe when swallowing so as to ensure no food or liquid enters the lungs. However, the prevalence of the latch state in ASM can have serious consequences leading to asthma and airway hyper-responsiveness. When cells surrounding the airway enter the latch state, they may remain in a contracted state for prolonged periods, leading to sustained airway narrowing.
1.2 Active Airway Smooth Muscle

Mechanisms that characterise ASM contraction are divided into passive and active events. The crossbridge cycle, described below, gives rise to ASM contraction and categorises the active events. Passive events, such as cytoskeletal remodelling, can take place independently of muscle activation and crossbridge cycling. Cytoskeletal remodelling, brought about by the reconfiguration of the cell’s actin filament network, may be implicated in reducing the intracellular load of the cell thereby sustaining contraction [115].

Much of the cytoplasm of smooth muscle cells is made up of actin and myosin filaments. Smooth muscle contraction is caused by the sliding of these filaments over each other. There are many more actin filaments than myosin filaments (10:1 in smooth muscle and 2:1 in skeletal muscle) but many actin filaments do not interact with a myosin filament (at least over part of its length) [99]. Actin filaments form the majority of the cell’s cytoskeleton. The structure and stability of the cell depends upon filaments such as alpha-actinin and dense bodies which connect actin filaments to each other or to the walls of the cell. Actin filaments are relatively thin and can span many microns in length [66].

Myosin filaments are much larger than actin filaments in diameter but much shorter in length. They are composed of two heavy chains and four light chains: two regulatory light chains and two essential light chain. The heavy chains coil around one another to form the tail of the myosin filament while the four light chains form two large heads that protrude from the end of the myosin tail. The light chains associated with each myosin head play a critical role in ASM contraction. They contain domains to which adenosine triphosphate molecules attach, leading to a ‘power stroke’ in which the myosin heads move to push nearby actin filaments forward. When the cytoplasmic calcium concentration increases within the cell, the enzyme myosin light chain kinase (MLCK) becomes activated. This enzyme promotes the attachment of the adenosine triphosphate molecules to the myosin heads. MLCK attaches to the myosin head, changing its configuration and enabling adenosine triphosphate to bind. In this process the myosin head becomes phosphorylated [99].

The power stroke mentioned previously is initiated by the dephosphorylation of adenosine triphosphate. The energy released from this reaction causes the myosin head to bend and form a strong bond between it and actin. The subsequent dissociation of adenosine diphosphate causes the myosin head to push the actin filament forward.

The phosphorylation of myosin can be reversed by the enzyme myosin light chain phosphatase (MLCP). This enzyme is activated over a longer time scale by calcium relative to MLCK hence the initial influx of calcium causes contraction to ensue as myosin phosphoryla-
tion by MLCK is the dominant mechanism. Contraction ceases as MLCP dephosphorylates the myosin head. In this dephosphorylated state, myosin has a low affinity for actin and can no longer bind [108]. These events are illustrated in Figure 1.2.

However, dephosphorylation of myosin may take place while the head is attached to the actin filament. In this case, a latchbridge is formed and detaches at a very slow rate. It is thought that force maintenance is achieved in this way [63]. This has been the most elegant and well accepted mechanisms underlying the sustained airway narrowing seen in asthma. It provides a clear demonstration of how shortening may persist for longer periods in asthmatic airways.

It has been shown that when a load is applied to a muscle tissue strip, the resulting velocity decreases as the time following muscle activation increases. Dillon et al. [31] hypothesized that this was due to the formation of latchbridges. The relatively high levels of latchbridges present with a long activation period are capable of generating a strong force to resist the applied load. Hence the velocity obtained when a given load is applied at this point in time will be lower than any previous point for the same applied load.

However, there is also a large body of evidence indicating that latchbridges cannot be the only source of passive stiffness within ASM cells [106]. The latch state and its regulator mechanisms will be discussed in greater detail in Chapters 3 and 5.

1.3 Passive Airway Smooth Muscle

During smooth muscle contraction, passive events take place alongside the active events described above. It is thought that passive elements present within the cell may work to slow down contraction or relaxation. However, the nature of these passive elements is unclear. In contrast to active dynamics, much less is understood about their passive counterparts such as the extent to which behaviour attributed to the latch state can be explained by passive mechanics, or the nature of the interaction between passive and active mechanics. Because the retardation of airway relaxation plays a key role in asthma, a better understanding of the source of ASM passive stiffness is crucial.

It is known that crosslinking proteins exist in the smooth muscle cell’s cytoskeletal framework and form connections between its actin filaments. ‘Crosslinkers’ are filaments which connect actin filaments and have the ability to redistribute themselves from high to low strain configurations upon changes in cell length. When contraction is initiated, the actin filament configuration is disrupted, causing a disruption in the configuration of crosslinkers. The rigid movements of newly bound crosslinkers may retard deformations in
Figure 1.2: An increase in the concentration of intracellular calcium leads to MLCK activation. Once activated, MLCK phosphorylates the myosin head, enabling it to attach to a nearby actin filament. Once attached, energy is released causing the myosin head to push the actin filament forward. The overlap of actin filaments resulting from these actions leads to smooth muscle contraction. Image from [43] with Open Stax College licence number CC-BY-3.0.
cell length leading to slower relaxation. Along with the formation of latchbridges, these events are believed to contribute to the latch state and sustained airway contraction characteristic of asthma [7, 47].

Furthering our knowledge of how these processes are regulated and how they affect crossbridge cycling is critical to the progress of research into asthma and airway hyperresponsiveness. During an attack, people with asthma have difficulty exhaling, stale air fills and becomes trapped in the lungs. The muscles surrounding the airways tighten (bronchospasm) and the lining of the airways becomes inflamed, see Figure 1.3. These two events narrow the opening of the airways.

![Figure 1.3: A normal and asthmatic airway. This image has been reproduced from [15] with Open Source licence number CC-BY-3.0.](image)

Many of the observed ASM properties such as the latch state come from experiments performed on strips of ASM tissue as opposed to intact airways. These experiments are valuable in improving our modelling approaches to capturing ASM contraction and provide a wealth of information in relation to the possible pathogenesis of asthma. However, in-vivo conditions may greatly affect the behaviour of ASM. For example, it is unclear whether the length adaptation observed in tissue strip experiments occurs in intact airways [93]. For these reasons it is also crucial to consider the correct morphology of the airways.
In Chapter 4 we will couple our model of ASM tissue with a model of an intact airway to determine how much of the observed behaviour at the tissue level is needed to accurately represent the airway. Using highly simplified models to replace the parallel model described in Chapter 3, we will determine the small airway response to imposed pressure oscillations. Comparing the model predictions will be an indication of the degree to which complex ASM properties, reproduced with a model of ASM tissue, affect the airway response.

In Chapter 5, we will explore some of these properties and their regulatory mechanisms. For instance, it is widely believed that slower airway relaxation, characteristic of asthma, is associated with passive ASM stiffness. We will show that it is likely that both latchbridges and crosslinkers contribute to force maintenance through their return to a low strain distribution following contraction. We will investigate the regulation of passive stiffness and active force within ASM cells and examine the recovery in passive stiffness following stretch. The ability of ASM to adapt to large changes in length will be explored, with particular emphasis on the passive length-tension relationship.

To deepen our understanding of the force-length relationship of ASM in response to tidal oscillations and deep inspirations, in Chapter 6 we will take an alternative modelling approach. We hope to use this to explain the enigma surrounding deep inspirations in asthmatics: why does taking a deep breath sometimes exacerbate airway narrowing in asthmatics rather than relaxing the airways as expected [46]?

The properties of ASM mentioned above will be discussed in depth in the following chapter and an overview of how mathematical models have been developed to capture the characteristic behaviour of ASM will be given.
2

Literature Review: Biological and Mathematical Background

2.1 Model Development

Mechanisms behind the excessive airway contraction found in asthma are not yet fully understood. While experimentalists have devised protocols to investigate smooth muscle properties on isolated tissue strips, mathematicians have formulated models to account for the observed properties. Both groups have the sole ambition to further our understanding of how ASM contraction takes place in the hope that this will provide insight into abnormalities in contraction that could lead to asthma.

The phosphorylation, dephosphorylation and actin binding events mentioned in Chapter 1 have been extensively documented and approaches to modelling this behaviour have been developed considerably over the past few decades.

One of the first approaches to modelling muscle contraction was made by A.F. Huxley [60] in 1957 for striated or skeletal muscle. Huxley based his model upon the sliding of actin filaments over one another as the muscle contracts. Prior to this, many had accepted the notion that the muscle contracts due to the shortening of the muscle filaments. However, through the use of electron microscopy and advances in x-ray imaging, Huxley was able to show that the filaments overlap as the muscle contracts and their length remains constant throughout the process.

The model describes this behaviour by assuming that, once myosin heads are phosphorylated, they can bind to an actin filament and exert a power stroke to push it forward. The main assumption of the model is that these phosphorylated myosin heads can only
exist in one of two states: attached or unattached to the actin filament. A schematic of this approach is as follows:

\[
\begin{array}{c}
A + M_p \\
g_p(x) \quad f_p(x) \\
AM_p
\end{array}
\]

where \(f_p(x)\) and \(g_p(x)\) represent the binding rates. \(A\) represents the actin thin filament and \(M\) represents the myosin head. The rate constants depend upon the position of the myosin head relative to the nearest binding site on the actin filament.

Huxley assumes that the muscle is either fully activated or deactivated. In the activated state, crossbridges are either attached or unattached, hence the following partial differential equation was formulated

\[
\frac{\partial n(x, t)}{\partial t} - v \frac{\partial n(x, t)}{\partial x} = (1 - n(x, t))f_p(x) - n(x, t)g_p(x),
\]

where \(n(x, t)\) represents the level of bound crossbridges and \(v\) is the velocity of shortening of half of a sarcomere (the fundamental units of skeletal muscle contraction consisting of a highly organised configuration of thick and thin filaments).

Skeletal muscle cells are highly organised with their contractile units arranged in parallel. While smooth muscle is very different to skeletal muscle in terms of filament arrangement and mechanisms of activation [57], contraction is assumed to occur via similar processes i.e., the sliding of filaments over one another. As such, the model can adequately account for the force-velocity relationship of ASM and for relations between load and rate of energy liberation described by Hill in 1938 [125].

The partial differential equation formulated by Huxley can be computationally expensive, can require stiff numerics and is difficult to analyse. To overcome this, Zahalak [138] uses a distribution moment approximation which reduces the partial differential equation to a system of ordinary differential equations by assuming that the strain distribution of the bound and unbound populations is Gaussian in form. However, this approach may not be valid for length perturbations away from isometric equilibrium or for large stretch velocities.

While the model of Huxley cannot capture the dynamic features of varying levels of myosin phosphorylation or produce the separation of the force-velocity curves obtained at
Model Development

various times following activation, the model provided a solid foundation for a series of subsequent approaches to smooth muscle modelling.

Among the first of such models was that of Hai-Murphy [54]. By the time this paper was published, there was strong evidence of the existence of a slowly cycling dephosphorylated crossbridge, termed a latchbridge, within smooth muscle cells. Latchbridges have a much slower detachment rate relative to crossbridges and it is believed that they give rise to the separation of the force velocity curves observed in experiments in isolated smooth muscle strips. The slower detachment rates associated with latchbridges may lead to slower airway relaxation, exacerbating symptoms of asthma.

Hai-Murphy incorporated latchbridges into the Huxley model by assuming that both latchbridges and crossbridges contribute equally to smooth muscle active stress, allowing them to lump both populations into a single attached crossbridge pool. Like Huxley, they assume that attachment and detachment rates depend on the equilibrium position of the myosin head relative to the nearest binding site on the actin filament. They further assume that these rates also depend on the level of phosphorylation and dephosphorylation taking place within the cell. The schematic of this approach looks similar to that of Huxley but now the rates $f_p$ and $g_p$ also increase with increasing phosphorylation, reaching a maximum value beyond a certain level of phosphorylation.

The partial differential equation used to describe this system is

$$\frac{\partial n}{\partial t} - v \frac{\partial n(x,t)}{\partial x} = (1 - n)f_p(K,x) - ng_p(K,x),$$

where $n(x,t)$ is the level of attached crossbridges in both phosphorylation states and $K$ is the rate of phosphorylation.

Hai-Murphy considered the case in which the shortening velocity is constant and reduced their partial differential equation to the following ordinary differential equation:

$$\frac{sV}{2} \frac{dn}{dx} = (1 - n)f_p(K,x) - ng_p(K,x),$$

where $s$ and $V$ represent the sarcomere length and the shortening velocity respectively.

Using this model, Hai-Murphy were able to examine the force-velocity relationship of ASM. For constant velocity, the ordinary differential equation was solved analytically to find the crossbridge generated stress. The stress was found to be a function of shortening velocity and dependent upon the phosphorylation rate constants. Taking into account the phosphorylation data from Kamm et al. [65] and Dillon et al. [31], Hai-Murphy changed
the phosphorylation rates to reflect the decrease in phosphorylation during isometric contraction. They found the stress for a given velocity and rate of phosphorylation, resulting in the family of force-velocity curves produced experimentally by Dillon et al. [31].

However, it has been accepted that these classical concepts cannot capture the properties of ASM in physiological circumstances [50]. Phenomena associated with airway-hyper-responsiveness are mainly dynamic and are therefore unaccounted for by this and other models which consider only static forces [134, 74, 82]. The model also does not predict the contributions from each of the crossbridge populations to the total stress and Haim Murphy subsequently took a different approach by developing a four state model [53], again based on that of Huxley. This model comprised of myosin in its phosphorylated and dephosphorylated states, crossbridges and latchbridges. Four ordinary differential equations were formulated to model the system, shown schematically below:

\[
\begin{align*}
M & \xrightarrow{k_1} M_p \\
M & \xleftarrow{k_2} M_p \\
AM & \xrightarrow{k_3} AM_p \\
AM & \xleftarrow{k_4} AM_p
\end{align*}
\]

In this model, it is assumed that the phosphorylation of myosin is obligatory for cross-bridge attachment and the only regulatory mechanism that needs to be considered is the phosphorylation and dephosphorylation of myosin. They further assume that the dephosphorylation of an attached crossbridge results in the formation of a slowly detaching latchbridge. In an effort to describe, in a minimal fashion, the kinetics of myosin phosphorylation and stress development and the steady state dependence of stress on phosphorylation, spatial dependencies were omitted. Phosphorylation can be predicted directly from the model by summing the level of crossbridges and phosphorylated myosin, \( M_p + AM_p \). Contributions to the total stress from latchbridges and crossbridges is assumed to be equal and is given by the sum \( AM_p + AM \).

The model predicts that, as the rate of phosphorylation increases, the stress rapidly reaches a maximum value. Latchbridges are the dominant contributors to the stress at low levels of phosphorylation while crossbridges contribute increasingly as phosphorylation rises.

Regulatory mechanisms for the rate constants were not considered, meaning that in order to simulate varying levels of activation, the phosphorylation rates were simply ramped
Asthma

13

up or down. The model is also unsuitable for examining the force-length relationship of smooth muscle since there is no spatial component.

Further developments were needed and, in 2000, Mijailovich et al. [89] incorporated spatial dependencies into the four state Hai-Murphy model. These spatial dependencies were similar to those of Huxley - they assume that the binding of myosin to actin can only take place up to a certain displacement from the equilibrium position of the myosin head to the binding site on the actin filament. Crossbridge detachment rates are relatively high outside this region while latchbridge detachment rates are significantly lower. The model was used to study the force-length relationship of the muscle and the extent to which each of the attached populations are responsible for it.

Wang et al. [129] went on to incorporate the regulatory mechanisms of the phosphorylation rate constants into the model of Mijailovich et al.. They assumed that calcium activates myosin light chain kinase (MLCK) leading to sharp rise in the rate of phosphorylation. Myosin light chain phosphatase (MLCP) activity governs the rate of dephosphorylation and is assumed to be transiently activated by calcium to cease contraction. MLCP is assumed to be inhibited by contractile agonists such as acetlycholine to enhance calcium sensitivity of ASM cells. This model will provide the basis for all simulations on activated ASM tissue throughout this thesis and will be discussed at length in Chapter 3.

The models of Wang et al., Hai-Murphy and Mijailovich et al. each account for the active side of ASM contraction but do not consider the passive events that take place alongside active contraction. These passive events are likely to have significant effects on the speed and duration of contraction and relaxation and are therefore important to incorporate into models that aim for a complete representation of ASM contraction.

In this regard, Donovan et al. [35] extended upon Huxley’s model to describe the behaviour of filaments which crosslink actin filaments within the cell, providing it with a structural cytoskeletal framework. The model differs from that of Huxley in that crosslinkers are assumed to bind at a continuum of binding sites along the actin filament and assumes both symmetric binding and unbinding of crosslinkers. Fundamentally, however, the models are quite similar: crosslinkers are assumed to exist in either bound or unbound states and the rate of binding and unbinding is governed by the strain imposed upon the crosslinker.

2.2 Asthma

As yet, there is no known function of ASM that is of benefit to mankind. While some speculate that it exists as some kind of evolutionary slip-up [2], others argue that it may
Asthma

have some more worthwhile functions such as controlling the ventilation/perfusion ratio [70], stiffening the airways [17, 12, 62] and breathing movements during embryonic development [120, 84].

In any case, it is certainly true that when ASM contracts excessively, there are serious implications in the form of asthma and airway hyper-responsiveness. The contributing factors towards asthma and airway hyper-responsiveness remain poorly understood, however, it is likely that the following dominate the response of the asthmatic airway to bronchoconstriction: 1.) fundamental changes in the properties of ASM governing crossbridge cycling, 2.) a change in the structural and mechanical properties of the non-contractile components of the airway wall 3.) changes in the interactions between the airway wall with its ASM lining and the surrounding lung parenchyma [113, 38, 13] and 4.) an increase in ASM mass [101, 97, 64, 75].

Firstly, it is possible that there are fundamental differences in the actin-myosin interactions between asthmatic and non-asthmatic airways although there is a lot of experimental controversy on this point [20, 119]. The excessive presence of the latch state phenomenon in asthmatic airways could be the most important of these differences [44, 32]. The latch state has been postulated to result in increased airway hyper-responsiveness through its potential to decrease the stretch experienced by the muscle during tidal breathing and deep inspirations [47].

It is thus crucial to further develop our understanding of this phenomenon. In the coming section, the experiment designed by Dillon et al. [31] to capture the latch state will be detailed along with a more in-depth description of how it arises and its implications.

As mentioned, several models have been formulated to account for latch state behaviour. In Chapter 5, we will use the model of Wang et al. and Donovan et al. to obtain a family of force-velocity curves which signify the latch state and show that crosslinkers as well as latchbridges contribute to force maintenance.

Several groups have developed experimental procedures to investigate the likely detrimental effects of the latch state on tidal breathing and deep inspirations. Analysis of the results of these experiments and model findings are therefore highly relevant to our examination of latch state behaviour and will also be discussed in the coming sections.

Secondly, the plasticity of the ASM tissue may also affect its response to tidal breathing. The transition of the tissue from a solid-like state at rest to a fluid-like state under imposed oscillations gives rise to a reduction in mean force and stiffness observed during such experiments. This decrease in mean force and stiffness may be vital for airway relaxation and avoiding airway stiffening associated with the latch state. In experiments on tissue strips
ex-vivo, the prevalence of the latch state gives rise to muscle stiffness while tidal oscillations and deep inspirations reduce muscle stiffness leading to relaxation. Abnormalities in the plasticity of the airway wall may also become apparent in asthma when it is believed that load fluctuations acting on crossbridges somehow become compromised [28]. This effect may lead to a reduction in the sensitivity to tidal breathing, a vital mediator of airway relaxation.

The plasticity of the tissue is also closely associated with its response to a single large stretch or a deep inspiration. Deep inspirations are well-known routes to airway relaxation as the extent of fluidization observed after a deep inspiration can be far greater than with tidal breathing alone. However, it is not clear why deep inspirations fail to reverse bronchoconstriction in asthmatic airways. It is likely to be a combination of abnormalities in the plasticity of the tissue and increased latch state behaviour or an increase in ASM mass.

Thirdly, one of our main objectives is to determine the extent to which the behaviour outlined above contributes to airway narrowing and relaxation in intact airways. In other words, how do changes in the nature of the ASM tissue affect the response of the airway that it lines?

Our model is capable of reproducing most of the properties of ASM tissue outlined above and described in this chapter. We will couple this model with a model of the airway to determine the extent to which the complexity of the ASM model is needed. The outcome of this study will shed light on the answer to one of the most important questions in its field: how do changes in the interdependence of the airway wall with its ASM lining affect the response of the airway to bronchoconstriction? In the coming review, these thoughts along with others will be further developed with analysis from mathematical models used to test the plausibility of each of the arguments regarding ASM properties and contributors to airway hyper-responsiveness.

### 2.3 Shortening Velocity and the Latch State

Dillon et al. [32] stimulated a strip of ASM tissue with agonist and found that a load applied shortly after the stimulation produced a greater shortening velocity than the same load applied several minutes later, see Figure 2.1. They concluded that the dephosphorylation of myosin can produce an attached, non-cycling, crossbridge (latchbridge) which is responsible for force maintenance in the tissue.
In a similar procedure, Kamm et al. [65] found that there is a temporal correlation between phosphorylation and maximum shortening velocity, indicating that myosin phosphorylation in smooth muscle functions in regulating the crossbridge cycling rates.

The decrease in phosphorylation that occurs alongside the decrease in crossbridge cycling rates during the force maintenance period observed by Dillon et al. and Kamm et al. has been termed the latch state. The attainment of the latch state has been postulated to be one of the reasons asthmatic airways fail to fluidize in response to deep inspirations and can develop a weakened sensitivity to tidal oscillations [39]. Failure to break crossbridges could result in latchbridge formation, stiffening the airways and decreasing the intracellular load against which the muscle shortens [44].

There is some controversy surrounding the issue of increased shortening velocity in asthmatic airways. Chin et al. [24] have reported no significant differences in their study on human trachealis muscle. However, the quality of the lungs used and the severity of asthma of their donors was unclear. At the same time, other studies have indicated that the properties of the trachealis many be different to that of small airways in asthma and it has been suggested that changes to ASM in asthma may only reside in the small airways.
In another study a greater rate of shortening was reported in the hyper-responsive Fisher rat [128] while a study performed on non-asthmatic human bronchial rings showed that shortening velocity increased when the sensitivity of the tissues to contractile agonists was increased [91]. A study performed on rat trachealis in vitro has demonstrated that an increase in shortening velocity could lead to the excessive airway narrowing characteristic of airway hyper-responsiveness [20]. This increase could alter the muscles response to mechanical stretch and result in increased airway constriction. For instance, ASM with a higher isotonic shortening velocity shortened more quickly and to a greater extent under conditions that mimic tidal breathing. The prevalence of the latch state in asthmatics could indicate an underlying frozen network of tightly bound bridges at low strains. This may serve to lower the load against which the muscle shortens, producing higher velocities as the rate of actin and myosin interactions increases, less perturbed.

While other mechanisms of force maintenance have been proposed (such as regulation of crossbridge cycling rates) the spatial extension of Hai-Murphy’s latch scheme is the most well accepted as it captures the direct relationship between myosin phosphorylation and shortening velocity at zero external load along with the reduction in crossbridge cycling rates during stress maintenance [47].

In Chapter 5 we also use our models to reproduce the phosphorylation versus velocity curves recorded by Kamm et al. [65]. We find that it is not possible to obtain the direct proportionality of phosphorylation and velocity observed experimentally when latchbridges are not included in the model.

### 2.3.1 Plasticity

Along with latch state behaviour, the ability of ASM to adapt to changes in length may also affect its shortening velocity and the extent to which the muscle shortens. This property of ASM has been demonstrated by Naghshin et al. [93], Bosse et al. [16], Wang et al. [131] and others. Length adaptation means that when a shortened or lengthened strip of ASM tissue is left to equilibrate for several hours, it is capable of generating active and passive force curves in response to stretch, similar to those generated at its original length. Length adaptation to shortened lengths may increase the shortening velocity at the adapted length, leading to greater shortening and narrowing of the airways, worsening the symptoms of an asthma attack. It may also affect relaxation rates of excessively shortened airways due to the higher resting tension.

Mechanisms of adaptation of ASM to shorter working lengths may also be attributed
to the remodelling of the contractile units within the cell. Donovan [33] has developed an adaptation of the Hai-Murphy model which explicitly incorporates the length distribution of thick sliding filaments to account for dynamic passive length adaptation. While the model cannot account for the behaviour seen at the shortest time scales or the passive length-tension curve, it can reproduce data from medium to long time scales by postulating that the degree of crossbridge binding is high in regions of actin filament overlap. The equilibrium force-length curves shift over a slow time scale with changes in length, as seen in the experiments of [131, 93, 16].

The empirical model of Lambert and Pare [73] supports the idea that the cell is able to adjust the number of contractile units to accommodate large changes in cell dimension by modulating the optimal overlap of actin filaments. However, their model does not address the molecular mechanisms by which the plastic adaptation of the cell is achieved.

The failure of activated muscle to re-shorten completely is related to the plasticity of the contractile responses [112]. While stretch-induced plastic remodelling cannot be the primary mechanism accounting for the observed force-length relationship of activated tissue (because of the rapidity, magnitude and nature of the response) [44] it is unreasonable not to include the influence of passive mechanical properties in a model of activated ASM dynamics [8]. One of the main arguments to support this is that the passive viscoelastic property of the connective tissue is likely to contribute to the internal mechanical load to oppose shortening [20].

Several experimental groups have examined the behaviour of the non-contractile structures that give rise to the plasticity of ASM cells. Furuike et al. [48] showed that the folding and unfolding of filamin A crosslinkers is reversible and may function to suppress the undulation of the actin filaments within the cytoskeletal network. The network may stabilize cell shape against a small shear force and increase its deformability against a large shear force. DiDonna et al. [29] used a filament network model crosslinked by unfolding crosslinkers to show that under sufficient strain, the network spontaneously self organises. This supports the experimental results of [30]: at moderate applied stress the system appears to adjust its mechanical properties so as to achieve a strain rate in which a significant fraction of its crosslinkers are poised at the brink of unfolding.

A mechanism of regulation of passive stiffness has been demonstrated by Raqeeb et al. [106]. In brief, it was shown that in the absence of myosin light chain kinase (MLCK), passive stiffness recovery following length oscillations is unaffected, but reduced in the absence of calcium. This indicates the existence of some other calcium initiated pathway which regulates passive stiffness and is independent of the regulation of MLCK.
Deng et al. [28] quantified cytoskeletal remodelling in response to applied mechanical stress and found that a progressive increase in actin remodelling and cell stiffening occurs alongside an increase in contractility and numbers of strong actin structures. They went on to infer that in asthma, mechanical stress might be increased due to wheezing etc.. The subsequent mechanically induced cytoskeletal remodelling may affect ASM function by altering force generation and normal regulation of cytoskeletal structure.

As mentioned, Donovan et al. [35] has put forward an extension of Huxley’s crossbridge model to passive biological soft tissue. The basis of the model is that proteins that crosslink actin filaments within the cell can attach and detach at both positive and negative strains in a symmetric fashion and at a continuum of binding sites. The model exhibits cytoskeletal fluidization and re-solidification in response to stretch as seen experimentally.

### 2.4 Force-Length Loops

It has been demonstrated by Mijailovich et al. [89] along with many others [8, 130, 117, 10, 24] that when length oscillations are applied to a strip of activated ASM, the force generated decreases transiently, see Figure 2.2 for the experimental results featured in Bates et al. [10]. Depending on the amplitude and frequency of the oscillations, the system gradually reaches a state characterized by fewer attached crossbridges, lower mean force and stiffness and increased adenosine triphosphate consumption [89]. We term this state the ‘dynamic steady state’.

Such experiments have been devised due to the belief that reduced sensitivity in the response of ASM to the magnitude of tidal oscillations could increase the likelihood of airway hyper-responsiveness [113]. This weakened sensitivity can also lead to a state closer to static equilibrium, characterized by slow bridge cycling and decreased rates of adenosine triphosphate utilization, i.e., the latch state [53].

The importance of considering the dynamic steady state is highlighted by the fact that even small tidal stretches can give huge decreases in stiffness compared with isometric steady state conditions [44].

Several experimental groups have imposed length oscillations of various frequencies and amplitudes to tissue strips in order to examine the dynamic force-length relationship of the muscle. Gunst et al. [50] have shown that banana shaped force-length curves are obtained whereby the peak isometric force is nearly independent of frequency of cycling. In bronchial tissue, dynamic force at peak cycle length remained higher than the isometric force at all frequencies of cycling.
Figure 2.2: Banana shaped force-length curves are obtained when tidal oscillations are imposed upon the tissue. The peak isometric force is nearly independent of frequency of cycling and the loops become progressively more bent as the amplitude of the oscillations increases. This image has been taken from [10]. Permission to use this image is not required.

Fredberg et al. [44] showed that the quantity of crossbridges decreases and the rate of cycling increases with increasing amplitude of stretch. The outcome is that the static equilibrium is never attained while oscillations are imposed. This led to the notion of ‘dynamically determined contractile states’ in which maximally contracted ASM can be maintained in a steady state of decreased stiffness as long as the tidal oscillations are sustained.

The reason for these dynamically determined contractile states in response to small tidal oscillations is proposed to be due to the perturbed binding of myosin to actin. This reduces the active forces generated by the muscle compared with an isometric contraction at the same mean muscle length.

As mentioned, several modelling groups have attempted to reproduce the experimentally
observed force length behaviour in mathematical studies. Using their modified Hai-Murphy model, Mijailovich et al. [89] examined how crossbridge distributions change in response to tidal oscillations. They showed that the reversal in size of populations of latchbridges and crossbridges between isometric steady state (when the level of latchbridges is higher) to dynamic steady state at high length perturbations is explained by the increased rates of crossbridge detachments at higher rates of crossbridge cycling. Their model predicts an almost linear relationship between adenosine triphosphate utilization and the loss modulus, consistent with experimental results on force development. However, for large strains the model fails to predict banana-shaped loops and the excessive increase of cycling rate predicted by the model does not agree with experiments. This is most likely due to discrepancies in the rate functions for displacements outside the crossbridge binding region.

Anafi and Wilson [3] formulated a model to describe the force length behaviour of ASM and incorporated it into a model for the dynamic behaviour of intact and constricted airways. Their model predicted the experimental data of Shen et al. [114] (sawtooth waves where peak length was held constant) and Fredberg et al. [44] (sinusoidal waves in which mean length was held constant) but was not defined outside the scope of periodic oscillations; it does not describe the basic molecular processes that underlie the force-length loops.

Bates et al. [2] formulated an empirical model of the activated ASM strip consisting of a passive elastic component in series with a force generator. The force-length loops predicted by the model do not become progressively more bent as amplitude increases. They reasoned that this may be due to the non-linearity in stiffness seen in all soft biological tissue and modified the passive component accordingly to get the observed loop shape. This model does not account for the fact that the experimental loops exhibit peak forces that vary little with oscillation amplitude.

Bates et al. went on to develop a model that consists of a non-linear viscoelastic component, representing the connective tissue in series with a force generator to represent the contractile machinery [10]. The model can account for the transient steady state oscillatory force-length behaviour of both passive and active ASM. However, it cannot predict the sustained decrement in isometric force seen when strips of activated ASM are subject to large fast stretches.

Brook [19] developed a model of the whole ASM cell that combines crossbridge cycling and actin-myosin turnover under imposed length perturbations and dynamic parallel to serial transitions of contractile units with force transmission pathways that occur through changes in length. The model indicates that the dynamic rearrangement of contractile
machinery is likely to underlie many of the features associated with tidal breathing. While it is an improvement on the modified Hai-Murphy model employed by Mijailovich et al., the model does not satisfy the elusive result that peak forces vary little with oscillation amplitude.

Given that the mathematical models outlined above do not completely account for the observed force-length relationship of ASM, perhaps a different modelling approach is needed, an approach that does not rely on any underlying molecular mechanisms governing the shape and properties of the force-length loops but instead ascribes them to system properties at a higher level of structural organization. The theory behind the approach is that the cytoskeletal scaffolding is in a continuous state of remodelling whereby it can be demolished under some circumstances and stabilized in others [40]. By observing that ASM cells fluidize and slowly re-solidify in response to stretch in the same way as soft glassy materials such as slurries, several groups have hypothesized that this approach could be recruited to explain the enigma surrounding the failure of deep inspirations to relax asthmatic airways as well as the mechanisms responsible for the observed force-length loops [41].

Observations regarding the plasticity of ASM have led us to think of passive and active mechanisms and their interactions in a fundamentally different way: a structurally stable system under low shear conditions that spontaneously transitions toward a fluid-like state once a certain shear threshold has been exceeded. It is in this sense that we have brought the worlds of ASM and complex fluids together with the goal of explaining the muscle response to tidal oscillations and deep inspirations.

2.5 Deep Inspirations and Soft Glassy Materials

One of the most potent mediators of airway narrowing in normal subjects is taking a deep inspiration. Figure 2.3 shows the experimental observations of Bates et al. [10] of the force generated by a strip of ASM tissue following the imposition of a single large stretch in between sinusoidal length oscillations. The drop in mean force following the deep inspiration is thought to relax the airways and prevent bronchospasm in response to some bronchoconstrictor (e.g., inhaling agonist). The impairment of this mechanism observed in asthma has been linked with a reduction in stretch of ASM. In 1868, Salter [110] wrote

*During a spontaneous asthma attack, the asthmatic loses the ability to dilate the airways with a deep inspiration, almost as if the airways had narrowed and then*
become frozen in the narrowed state.

Since then it has been found that when healthy volunteers inhale some of the most potent bronchoconstrictors, a deep inspiration is sufficient to avoid subsequent bronchospasm. In addition to this, if healthy, non-asthmatic and non-allergic subjects refrain from taking deep inspirations, within 15 minutes their airways become hyper-responsive to a degree that is indistinguishable from their asthmatic counterparts [68, 44]. From this we can conclude that it is abnormalities in the ASM as opposed to allergy-related factors which cause the impairment of the bronchodilatory effect of deep inspirations in asthmatics. Furthermore, this impairment is likely to be due to increased stiffening of the airways.

It has been observed that the behaviour of ASM is analogous to that of complex fluids. For instance, it has been demonstrated by Anne-Marie Lauzon’s group that when length oscillations are applied to a strip of inactivated ASM it fluidizes [35]. The extent of fluidization is proportional to the amplitude of the length oscillations. The crosslinker model we use can capture this result but, interestingly, it is also a key feature of models based on complex fluids.

Complex fluids, such as toothpaste, have a coexistence between two phases. They are suspensions of macromolecules which fluidize when stress is applied to them. Models of complex fluids describe state transitions that arise as the concentration of strong bonds within the system changes. They provide a relationship between the viscosity and elasticity of the system to imposed shear rate, a relationship which may be key to explaining the asthmatic response to deep inspiration. Since ASM modulates its mechanical properties and remodels its internal cytoskeletal structures in the same ways as soft glassy materials, the origin of the bronchodilatory effect of a deep inspiration and the reasons why asthmatic airways fail to fluidize in response to them, may lie in the glassy dynamics of the cell.

This approach has the potential to be highly valuable in improving our understanding of the causes of asthma and airway hyper-responsiveness. The glass hypothesis can indicate the rates at which plastic remodelling can progress and can help to establish a link between the macroscopic behaviour of the tissue and that of its structural constituents [94].

A few mathematical studies have already adopted this theory. The model of Murtada [92] suggests that the matrix in which the actin filaments are embedded has viscous properties. The model describes the contraction/relaxation of the contractile units as well as crossbridge interactions with the thin filaments but is not able to predict the profiles of shortening velocity obtained experimentally in isotonic protocols.

The constitutive model formulated by Meyer [87] describes the pseudoplasticity of passive skeletal muscle. By assuming that muscle fibres have a complex, non-linear viscosity,
their model can account for the time and strain dependent responses to tensile loads.

A number of other groups have formulated mathematical models to represent the viscoelastic and glassy dynamics of the cell [69, 111, 83, 109]. These models have been used to investigate how glass transitions manifest within soft biological materials [111]. The ability of these models to reproduce rheological data is a strong indication that such cell systems exhibit behaviour akin to the solid-gel transitions observed in soft glassy materials [69]. However, the approach used does not consider any underlying biochemical or biomechanical pathways which may explain such behaviour.
Deep Inspirations and Soft Glassy Materials

Figure 2.3: In response to a deep inspiration there is a transient decrease in oscillatory force along with a sustained amplitude dependent decrease in force and stiffness. The model of Bates et al. [10] can account for the transient decrease in oscillatory force following a deep inspiration but cannot produce sustained amplitude dependent decrease in force and stiffness. In the experimental protocol, length oscillations were imposed upon a tissue strip for 120 seconds during which time a deep inspiration was imposed. The procedure was repeated for deep inspirations of varying amplitudes and it was found that larger deep inspirations cause progressively larger decrements in isometric force and increasingly delayed force recovery. Permission to use this image is not required.

The constitutive model formulated by [103, 132, 104] for complex fluids will be adopted for ASM in this body of work to test the extent to which the theory of soft glassy materials can be applied to ASM. It will be shown that, not only can the model account for the fluidization of passive muscle in response to stretch and produce the isotonic shortening velocity profiles in response to varying calcium and agonist levels, but it can also improve upon the force-length loops previously obtained with the modified Hai-Murphy model in that it
predicts a transient decay in force with ongoing tidal length oscillations and force length loops that are more similar in shape to those observed experimentally. The constitutive formulation can also predict the decrease in force observed following a deep inspiration.

2.6 Interactions Between the Parenchyma and ASM Tissue: Intact Airway Models

While experiments performed on tissue strips are essential for many reasons, they are not necessarily representative of conditions in vivo. Therefore, when considering dynamic airway contraction, it is necessary to consider the correct morphology i.e. the airway as opposed to the tissue strip. In mathematical models of the airways, both the contractile and passive forces generated by the ASM cells and the elastic parenchymal tethering forces should be included in order to obtain good agreement with experimental data [9]. Simulation studies with such models allow us to test the plausibility of different mechanisms of inducing airway hyper-responsiveness such as the effects of increased muscle mass. While ASM models are crucial to have on their own - ASM cells express essential mechanical features that are characteristic of the contractile response at the tissue level [42] - the impairment of physiological protection in asthma could be due to abnormalities in the mechanical coupling between the airways and parenchyma [45].

To highlight the significance of airway models, consider the problem of increased muscle mass [74]. The stiffening of ASM during activation can become exacerbated such that in response to a deep inspiration it stretches little [47]. Increased ASM mass reduces the stretch experienced by the muscle and results in airway hyper-responsiveness. In addition to this, it has been shown that increased heterogeneity in the airway response to stimulation leads to a greater overall increase in airway hyper-responsiveness [18]. Therefore, while tissue models are vital for improving our understanding of the underlying contractile processes that govern ASM behaviour, certain aspects of intact airway properties are impossible to predict with them.

Several mathematical models of the lung have been developed and applied to the understanding of airway hyper-responsiveness in asthmatic airways. The model of Lambert et al. [76] consists of an equation for the pressure gradient in the flow and airway pressure-area curves for 17 generations of the bronchial tree. Using the pressure-area measurements of Hyatt et al. [61] on the 1st 3 generations of human airways and Weibel’s data [133] at a single transmural pressure for the remainder of the tree, Lambert constructed a complete
description of airway behaviour from the trachea to the terminal bronchioles. In Chapter 4, we will use this description to model the airway response to pressure oscillations and varying concentrations of agonist in the upper and lower regions of the bronchial tree.

Other airway models have been used to examine the effects of airway wall thickening [18], the heterogeneity of airway narrowing [126], the dynamics of bronchoconstriction [9], increased ASM mass [78] and agonist concentrations on the airway response [72]. The models have shown that changes in the heterogeneity of airway narrowing can greatly affect overall airway resistance [126] and that the impaired effect of deep inspirations in asthma may increase airway resistance due to a heightened response to bronchoconstrictors [72, 112].

The model of Donovan et al. [34] is used to explore the interactions between deep inspirations and fluidization and finds that their combination is highly effective at reversing airway closure while neither is effective alone. The failure of the bronchodilatory effect of a deep inspiration in asthmatic airways may be due to an impairment of this interaction. The simplification of the ASM force to a prescribed external force allows complex stability analysis to be performed on the system. However, it has also been shown that ASM dynamics have the ability to modulate transitions between open and closed states. As such, the extent to which they should be incorporated has yet to be determined.

Several experiments on intact airways have shown that when the airway is injected with a contractile agonist, pressure oscillations of any reasonable sort are ineffective in reducing the net level of constriction. Tidal breathing, even with deep inspirations has almost no effect on mean airway area when the muscle is subject to varying levels of agonist. LaPrad et al. [77] and Lavoie et al. [77] have shown that simulated breathing reversed bronchoconstriction most effectively when the severity constriction was small and the depth of breathing large.

While these experimental results are essential for strengthening our faith in the mathematical model we study (e.g., the modified Hai-Murphy model of Wang et al. coupled with the airway can reproduce these results), they are only performed on large airways. In this thesis, we are most interested in the entire lung response to stimulation, particularly that of the smallest airways.
3

Mathematical Modelling of Airway Smooth Muscle

3.1 The Crossbridge Model

The model we will focus on is that of Wang et al. [129]. As mentioned, it includes Ca$^{2+}$ activation of MLCK, Ca$^{2+}$ activation of MLCP and agonist inactivation of MLCP. A schematic diagram of the crossbridge model is shown below: $k_1$ is the rate of myosin head phosphorylation by MLCK, $k_2$ is the rate of myosin head dephosphorylation by MLCP and $f_p(x)$, $g_p(x)$ and $g(x)$ are the attachment and detachment rates of crossbridges and latchbridges.

A detailed derivation of the rate functions is given in [129]. The rate of phosphorylation is given by

$$k_1 = \frac{k_{1a} c^A}{k_{1b} + c^A},$$

where $c$ represents the concentration of calcium within the cell. The following ordinary differential equation (ODE) is used to determine the rate of dephosphorylation, $P$: 
The Crossbridge Model

\[
\tau_p \frac{dP}{dt} = k_{on}(c)(1 - P) - k_{off}(a)P,
\]
\[
k_2 = k_2 P^2
\]
\[
k_{on}(c) = k_{on1} + \frac{c^2}{k_{on2} + c^2}, \] \[k_{off1} + \frac{k_{off2}a}{1+a}, \]

where \( a \) represents the concentration of agonist within the cell.

Crossbridge cycling is determined by the rates of myosin phosphorylation and the extent of strain imposed upon the crossbridges. The variable \( x \) represents the distance between the equilibrium position of the myosin head and the nearest binding site on the actin filament. The rates of attachment and detachment are as follows:

\[
f_p(x) = \begin{cases} 
\frac{f_{p1}x}{h}, & 0 \leq x \leq h \\
0, & \text{otherwise}.
\end{cases}
\]

\[
g_p(x) = \begin{cases} 
3(f_{p1} + g_{p1}), & 0 < x \\
\frac{g_{p1}x}{h}, & 0 \leq x \leq h \\
\frac{4g_{p1}x}{h}, & x > h.
\end{cases}
\]

\[
g(x) = \begin{cases} 
20g_1, & 0 < x \\
\frac{g_1x}{h}, & 0 \leq x \leq h \\
g_1, & x > h.
\end{cases}
\]

where \( 0 \leq x \leq h \) defines the region in which the myosin head can bind to an actin filament, here we have set \( h = 1 \). The rate of crossbridge detachment is low within the binding region (or the region where the crossbridge exerts a contractile force). When \( x \) is negative, the crossbridge opposes contraction and the rate of detachment is relatively large. When \( x \) is larger than \( h \), the rate of detachment increases with \( x \). The rate of attachment is assumed only to be non-zero within the binding region as crossbridges cannot bind to a binding site that is too far away. The rate at which a dephosphorylated crossbridge detaches is much smaller than the rate at which a phosphorylated crossbridge detaches.
The distribution of crossbridges is determined by:

\[
\frac{\partial M}{\partial t} - v_a(t) \frac{\partial M}{\partial x} = -k_1 M + k_2 M_p + g(x) AM,
\]

\[
\frac{\partial M_p}{\partial t} - v_a(t) \frac{\partial M_p}{\partial x} = k_1 M - (k_2 + f_p(x)) M_p + g_p(x) AM_p,
\]

\[
\frac{\partial AM_p}{\partial t} - v_a(t) \frac{\partial AM_p}{\partial x} = k_1 AM + f_p(x) M_p - (g_p(x) + k_2) AM_p,
\]

subject to the constraint \( M + M_p + AM_p + AM = 1 \), where \( v_a(t) \) is the velocity of the actin filament relative to the myosin filament, defined to be positive during shortening. As such, we define

\[
v_a(t) = -\gamma_a \frac{dL}{dt},
\]

where \( L \) is the length of the tissue and \( \gamma_a \) is a proportionality constant.

It is assumed that both crossbridges and latchbridges are capable of generating a force that depends on their displacement. The total force generated by smooth muscle cells depends the spring force according to Hooke’s Law and the displacement of the \( AM \) and \( AM_p \) distributions:

\[
F_a = \kappa \int_{-\infty}^{\infty} x(AM + AM_p) dx + \kappa(L(t) - L_{ref}),
\]

where \( \kappa \) is a proportionality constant and \( L(t) \) refers to the length of the cell. Differentiating Equation 3.2 with respect to time and rearranging yields:

\[
\frac{dL}{dt} = \frac{dF_a}{dt} - \kappa \int (f_p(x) M_p - g_p(x) AM_p - g(x) AM) dx}{\kappa + \kappa \gamma_B \int (AM_p + AM) dx}.
\]

The relative airway area (R.A.A.) is taken to be

\[
R.A.A. = \frac{(1 - \beta F(t))^2}{(1 - \beta F(0))^2},
\]

where \( \beta \) is a proportionality constant as in [129]. Although a rather crude first attempt at representing the airway, this formulation agrees well with the experimental data of airway radius profiles in response to varying calcium and agonist levels also presented in the publication. However, effects of transmural pressure or the location of the airway are not considered. As we shall see in Chapter 4, more realistic predictions of the airway response
are obtained when the tissue is coupled with the airway.

### 3.1.1 The Effects of Varying Calcium and Agonist Concentrations on Airway Contraction.

The model equations were transformed to a system of ODE’s via the method of characteristics using a large spatial domain to account for the decay boundary conditions, see Section 3.4 for a more detailed explanation. This ensures that the population distributions are equal at both boundaries of the domain and do not come close to the edge of the domain. These ODE’s were solved numerically using the fourth order Runge-Kutta method. A time-step of 0.001s and a space-step of 0.01 were used throughout. This method is tested for convergence in Appendix 1 along with a numerical scheme that employs a non-uniform mesh to reduce computation time for simulations which require a large spatial domain.

The simulations of Wang et al. [129] have been reproduced in this section to illustrate the model predictions of the crossbridge population response to varying calcium and agonist concentrations. The R.A.A and population densities, shown in Figure 3.2, change according to the phosphorylation rates shown in Figure 3.1.

![Figure 3.1: (a.) The calcium and agonist concentrations and (b.) The resulting phosphorylation and dephosphorylation rates.](image)

- As the level of calcium initially rises, the phosphorylation rate increases and the initial high level of dephosphorylated myosin decreases. The relative airway area decreases as the number of cycling crossbridges increases. As calcium acts to activate MLCP
Figure 3.2: The four state crossbridge model subjected to the calcium and agonist concentration depicted in Figure 3.1. As the force increases due to the increases of crossbridges and latchbridges, the relative airway area decreases according to Equation 3.4.

on a slower time-scale, the level of latchbridges gradually increases as crossbridges increasingly become dephosphorylated.

• The active force generated by the muscle decreases as these latchbridges slowly detach, giving rise to a transient increase in airway relaxation.

• This process repeats itself to a slightly lesser extent as calcium is increased for the second time. The level of dephosphorylation is high relative to its initial level due to the abundance of calcium now present within the cell. A smaller contraction is thus generated as fewer crossbridges become phosphorylated and commence cycling compared with the first increase in calcium.

• As the level of calcium is sustained, there is a balance between phosphorylation and dephosphorylation. Because of this, a high level of latchbridges is maintained. When calcium is decreased, this balance is demolished. Both rates decrease causing a drop in the level of latchbridges and force generated due to a decline in the availability of crossbridges.

• The large ramp in agonist produces a large decrease in airway area, followed by
relaxation once the agonist is removed. Agonist inhibits dephosphorylation leading to a huge increase in cycling crossbridges as myosin phosphorylation now dominates. As these crossbridges gradually become dephosphorylated, the latchbridge pool increases, reaching a peak when the inhibition of dephosphorylation is removed.

### 3.2 The Crosslinker Model

The crosslinker model of Donovan [35] is given by

\[
\frac{\partial n}{\partial t} - v_p(t) \frac{\partial n}{\partial y} = \alpha(y)(1 - N) - \beta(y, c(t)) n, \tag{3.5}
\]

where \(\alpha(y)\) and \(\beta(y, c(t))\) are the attachment and detachment functions respectively, \(y\) is the crosslinker strain, \(v_p(t)\) is the relative filament velocity, \(c(t)\) is the calcium concentration within the cell and \(N = \int_{-\infty}^{\infty} n(y, t) dy\) is the total fraction of bound crosslinkers.

This model, based upon the sliding filament model of Huxley, differs from the crossbridge model described previously in that it assumes symmetric binding and unbinding of crosslinkers and that the force they generate is highly nonlinear. In addition to this, crosslinker dynamics are not governed by MLCK or MLCP activity. We combine this model with the crossbridge model to determine the extent to which cytoskeletal remodelling accounts for the passive stiffness generated by the cell.

It is assumed that crosslinkers only exert a force when positively strained. The crosslinker generated force is thus given by:

\[
\phi(t) = \eta \int_{-\infty}^{\infty} \rho(y) n(y, t) dy,
\]

where \(\rho(y)\) is the relationship between crosslinker force and strain:

\[
\rho(y) = \begin{cases} 
0, & y < 0 \\
\rho_2 y^2 + \rho_3 y^3, & y \geq 0.
\end{cases}
\]

To account for the static elastic properties, we include a linear spring in parallel with the crosslinker generated force, so that the total force is

\[
F_p(t) = \phi(t) + F_0 + \psi(L(t) - L_0),
\]
where $F_0$ is the basal force, $\psi$ is the spring modulus.

The attachment function $\alpha(y)$ is taken to be

$$
\alpha(y) = \begin{cases} 
\alpha_1 & -1 < y < 1 \\
0, & \text{otherwise}
\end{cases}
$$

under the assumption that crosslinkers can only bind at relatively low strains. These strains have been normalized to be in the region of $(-1, 1)$. We take the relative filament velocity, $v_p$, to be positive during shortening as before and set $v_p(t) = -\gamma_p \frac{dL}{dt}$.

The force generated by these crosslinkers to resist the contraction or relaxation of the cell contribute to the stiffness of the cell and is referred to as passive stiffness. Evidence indicates that passive stiffness depends on the calcium concentration of the cell. Raqeeb et al. [106] demonstrates that the recovery in passive stiffness following length oscillations is reduced in the absence of calcium. Additionally, the viscosity of actin filament gels has been observed to increase with increasing calcium concentration, [21]. We have therefore modified the original crosslinker model to incorporate the dependency of passive stiffness upon calcium concentration.

Since data does not exist to fit the passive stiffness directly to the cell’s calcium concentration, we have fitted the crosslinker detachment rate, $\beta$, empirically to the data of Raqeeb et al. in which calcium is either on or off. Raqeeb et al. have shown that in the absence of calcium, the passive stiffness is reduced by around 50%. We have modified $\beta$ accordingly so that under low calcium concentrations the crosslinker generated force is approximately half of that generated under high calcium conditions.

The crosslinker detachment rate function now varies with calcium as shown in Figure 3.3 and is given by

$$
\beta(y) = \beta_1 + \beta_2 \frac{|Q(c)y|^m}{y_{\max}^m + |Q(c)y|^m},
$$

where $Q(c) = 1 - \text{Tanh}(c - 1)$. With increasing calcium, crosslinkers at increasing strains remain attached. This models the strengthening of crosslinkers under high calcium concentrations and will be discussed in greater detail in Section 5.2. All of the model parameters are given in [35].

The steady state solution,

$$
n_s(y) = \frac{\alpha(y)}{\beta(y, c(t))} (1 - N_s),
$$
Figure 3.3: The rate of crosslinker detachment according to the imposed strain. In this work, the detachment rate function, $\beta(y, c(t))$, is regulated by calcium.

is taken to be the initial distribution of crosslinkers.

The simulations of Donovan et al. [36] have been reproduced to illustrate crosslinker function. A strip of tissue is given a half sinusoidal stretch of 17% of reference length for 0.25s. Figure 3.4 shows the crosslinker distribution in response to this stretch: the displacement moves some bound crosslinkers into regions of unbinding as they become highly strained. Meanwhile, crosslinkers attach in positions of low strain. This process continues and eventually the steady state is reattained.

There exists some controversy surrounding the symmetry of the crosslinker force function. Donovan et al. have assumed that it is asymmetric and that crosslinkers are only capable of generating force at positive strains. This is because a strip of inactivated tissue will go slack if compressed. However, the symmetry could depend on the activation level of the tissue: if only stretched crosslinkers can generate force once the muscle is fully activated, the airway would continue to contract (albeit on a very long time-scale) after crossbridge cycling has ceased due to the small positive force exerted by the stretched crosslinkers. This is very unlikely to happen in vivo. If compressed crosslinkers could also generate force, the total passive force would eventually decrease to zero. Airway narrowing would cease on the same time-frame as the crossbridge cycle.
Coupling of the Crossbridge and Crosslinker Models (the Parallel Model)

Within airway smooth muscle, we assume that there is a basal force, crosslinker and crossbridge generated forces and elastic recoil forces, represented here using Hooke’s law. Thus, the contributions for a muscle strip are:

- **Basal Force**: $F_b$
- **Crosslinker Force** (passive): $F_P(t)$
- **Crossbridge (active) and Latchbridge (passive/active)** Force: $F_a(t)$

The **Linear Spring** force is given by:

$$F_s(t) = (\psi + \kappa)(L(t) - L_0)$$

where $L(t)$ is the current strip length, $L_0$ is the reference length and $(\psi + \kappa)$ is the elastic coefficient of the muscle strip.
We assume that these forces act in parallel within the tissue. The total force, $F(t)$, generated by the muscle is now described by

$$F(t) = F_b + F_p(t) + F_a(t) + F_s(t).$$

In length controlled experiments such as those of Bates et al. [10], $dL/dt$ is known and the crossbridge and crosslinker models are solved in parallel to find the total force.

When the force is controlled i.e. in the experiments of Wang et al. [129] and Dillon et al. [31], the modelling approach becomes a little more complex as the velocity must be found. As mentioned, we assume that the crosslinker velocity, $v_p(t)$ and the crossbridge velocity, $v_a(t)$, are proportional to the length change of the tissue strip according to

$$v_p(t) = -\gamma_p \frac{dL}{dt},$$
$$v_a(t) = -\gamma_a \frac{dL}{dt},$$

where $\gamma_p$ and $\gamma_a$ are proportionality constants. In these force controlled procedures, the following balance equation must hold

$$F_e(t) = F(t),$$

where $F_e$ is some known external force imposed on the tissue strip.

We differentiate 3.6 with respect to time and substitute for $F_a(t)$, $F_p(t)$ and $F_s(t)$ to yield

$$\frac{dL}{dt} = \frac{dF_e/dt - \kappa \int (f_p(x)M_p - g_p(x)AM_p - g(x)AM) \, dx - \eta \int \rho (\alpha(1 - N) - \beta n) \, dy}{\kappa + \psi + \kappa \gamma_B \int (AM_p + AM) \, dx + \eta \gamma_p \int \rho' n dy}.$$  

(3.7)

### 3.4 Numerical Method

We have used the method of characteristics [27] to solve both the crossbridge and crosslinker systems of equations. This method reduces our PDE’s to systems of ODE’s which are then solved using the Runge Kutta fourth order method [22, 4, 121].

To demonstrate this method, we will use the crosslinker model as an example. The crossbridge model is dealt with in a similar fashion.
The distribution of crosslinkers is given by

\[
\frac{\partial n}{\partial t} + \gamma \frac{dL}{dt} \frac{\partial n}{\partial x} = \alpha(x)(1 - \int_{-\infty}^{\infty} ndx) - \beta(x)n.
\]

Letting \( \frac{dx}{dt} = \gamma \frac{dL}{dt} \) and solving for \( x \), we find

\[
x(t) = x_0 + \gamma (L(t) - L_0),
\]

where \( L_0 \) is the initial length of the muscle strip.

By the chain rule,

\[
\frac{d}{dt} n(x(t), t) = \frac{\partial n}{\partial t} + \frac{dx}{dt} \frac{\partial n}{\partial x} = \frac{\partial n}{\partial t} + \gamma \frac{dL}{dt} \frac{\partial n}{\partial x}.
\]

The PDE has thus been transformed into the following system of ODE’s

\[
\frac{d}{dt} n(x(t), t) = \alpha(x(t))(1 - \int_{-\infty}^{\infty} n(x(t), t)dx) - \beta(x(t))n(x(t), t),
\]

\[
= f(x(t), n(x(t))).
\]

This system is now discretized in time using the fourth order Runge Kutta method:

\[
n_1^j = f(x_0^i + \gamma (L[j\Delta t] - L_0), n^i),
\]

\[
n_2^j = f(x_0^i + \gamma (L[(j + 0.5)\Delta t] - L_0), n^i + 0.5n_1^i),
\]

\[
n_3^j = f(x_0^i + \gamma (L[(j + 0.5)\Delta t] - L_0), n^i + 0.5n_2^i),
\]

\[
n_4^j = f(x_0^i + \gamma (L[(j + 1)\Delta t] - L_0), n^i + n_3^i),
\]

\[
n_{j+1}^i = n_j^i + \frac{1}{6}(n_1^i + 2n_2^i + 2n_3^i + n_4^i),
\]

where \( \Delta t \) and \( \Delta x \) are the time and space steps respectively and \( n_j^i \) approximates \( n(j\Delta t, i\Delta x) \), the distribution of crosslinkers at time \( j\Delta t \) and strain \( i\Delta x \). We have taken \( \Delta t = 0.001s \) and \( \Delta x = 0.01 \) in all our model simulations.
4

Coupling the Airway Wall with the Airway Smooth Muscle Tissue

4.1 Introduction

It is important to have models of the ASM tissue as they are crucial in advancing our understanding of the underlying mechanisms behind ASM contraction. They can predict the mechanisms responsible for the ability of ASM to adapt to changes in length or maintain force, for example. They can also be used to support hypotheses from experimentalists about the sub-cellular structures and pathways that control the muscle properties.

However, it is also vital to consider the complete lung morphology. This approach allows us to predict the airway response to changes in the heterogeneity of the lung response or changes in the mass of the muscle that surrounds the airways. It also enables us to determine conditions under which small airways will close and what types of pressure oscillation protocols may be imposed to prevent closure.

As a first step toward a full representation of the lung, we consider a single airway. The main goal of this chapter will then be to focus on the response of a small airway to imposed pressure oscillations. However, since experimental data only exists for large airways, we will first use our parallel model, coupled with the airway, to ensure that the results it predicts for such protocols on a large airway agrees reasonably well with experimental data.

In determining small airway responses, we are not interested in the transient towards a steady state, only in which of the two stable states the airway eventually tends to: the open branch or the closed branch. This allows for the possibility of using a hugely simplified ‘constant force’ model to investigate the impact of the complex ASM properties, represented...
by the parallel model, on the airway response. The predictions of the constant force model will again be compared with data from experiments performed on large airways as well as results obtained using the force implied by the parallel model.

In terms of complexity, one small step up from the constant force model is the ‘dynamically determined contractile force’ model. Here the ASM generated force is given by the strain experienced by the muscle tissue. Several experimental groups have shown that when tidal length oscillations are imposed (to mimic tidal breathing) the resulting mean force decreases with the strength of the oscillations [89]. It is thought that airway stiffening can be avoided in this way as even small oscillations (quiet tidal breathing) has the potential to be a powerful mediator of bronchoconstriction. By mapping pressure oscillations to length oscillations, we can approximate the force as the mean force generated by the tissue subject to the given length oscillation protocol.

Using the simple and complex models coupled with the airway, we will examine the stable open and closed states of the small airway in response to pressure oscillations. This will enable us to determine how realistic it is to couple the constant force or dynamically determined force model with the airway as opposed to the parallel model. If this approach proves successful, computation time will be significantly reduced. As mentioned, the long term goal is to formulate a method of representing the complete morphology of the lung. This involves modelling thousands of airways, using the parallel model to represent the ASM tissue encircling each of them. But coupling the parallel model with the airway means solving more than 800,000 ODE’s at each time step using the Runge-Kutta fourth order method (RK4), a process that is tedious for one airway and almost intolerable for thousands. This makes clear our motivation to take a simplified approach to modelling the ASM force using the methods outlined above and described in this chapter.

4.2 The Airway Model

The 27 airway orders, as described by Horsfield [58], are composed of more than 30,000 distal terminal bronchioles branching from the trachea, see Figure 4.1b. We use the pressure-radius relationship of Lambert et al. to describe the influence of pressure changes on each of these airway orders [76], see Figure 4.1a. This relationship is defined as follows:

\[
R^2(P_{tm}) = \begin{cases} 
  R_i^2(1 - P_{tm}/P_1)^{-n_1} & \text{for } P_{tm} < 0 \\
  r_{imax}^2 - (r_{imax}^2 - R_i^2)(1 - P_{tm}/P_2)^{-n_2} & \text{for } P_{tm} \geq 0,
\end{cases}
\]

(4.1)
The transmural pressure $P_{tm}$ acting along the airway wall describes the airway lumen radius $R(P_{tm})$ for a given airway order. The maximal radius $r_{\text{max}}$, the radius at $P_{tm} = 0$, $R_i$ and the parameters $P_1$, $P_2$, $n_1$ and $n_2$ depend on the airway order.
Figure 4.1: (a.) The pressure-radius relationship as described by Lambert et al. [76]. Smaller airways such as the terminal bronchioles, are extremely compliant and there is a sharp transition to closure as the pressure decreases beyond 1 cmH₂O. Larger airways, such as the bronchus, are far less compliant and do not close as pressure drops. (b.) The branching pattern of the airways, as illustrated by [81] (permission to use this image is not required).
The transmural pressure $P_{tm}$ is given by

$$ P_{tm} = P_0 + P_i - BR_{ref} \frac{F}{r} + \tau(r), $$

(4.2)

where $P_0$ is the base transmural pressure, $P_i$ is the imposed pressure, $R_{ref}$ is the radius given by Equation 4.1 at $P_{tm} = P_0$ cmH$_2$O and $F$ represents the active and passive ASM generated forces. $B$ is a dimensionless parameter but here represents the degree to which the ASM generated force affects the airway response. Recoil forces opposing contraction, termed ‘tethering forces’, are given by the Lai-Fook [71] model and are described by

$$ \tau(r) = 1.5\mu \left( \frac{R_{ref} - r}{R_{ref}} + 1.5 \left( \frac{R_{ref} - r}{R_{ref}} \right)^2 \right), $$

where $\mu$ is the parenchymal shear modulus. An increase in tethering force is brought about by the expansion of the lung parenchyma as the radius of the airway, $r$, decreases.

The ODE used to model the dynamic variations in the transmural pressure upon the airway radius is

$$ \frac{dr}{dt} = \rho(R(P_0 + P_i - BR_{ref} \frac{F}{r} + \tau(r)) - r). $$

(4.3)

This approach assumes that the rate of adaptation of the airway depends on how fast and to what extent it is stretched from its fixed point, $R(P_{tm})$ and is derived from Newton’s Law of Cooling. The Laplace Law, $F/r$ was used as the simplest way of representing the relationship between changes in airway radius and ASM generated force. In all upcoming simulations, we used parameters given in the supplementary material of [100] for a given airway order and took the ASM generated force, $F$, to be either constant, dynamically determined or predicted by the parallel model.

### 4.2.1 Determining the Fixed Points of the System

Studying the movement of the trajectory around the fixed points of Equation 4.3 is one of our main approaches to determining why a small airway closes (or remains open) in response to certain stimuli. For small, compliant airways, three regions of stability emerge: an ‘open’ branch of stable fixed points, an unstable branch en route to closure and a closed branch of stable fixed points. Deep inspirations and tidal oscillations in the physiological range cannot force the path of the trajectory to reverse its direction: once the tissue is activated and closure ensues, it is impossible to obtain reopening unless the muscle is deactivated. This becomes clearer by examining equation 4.2; as the airway closes, $r$ becomes increasing
small, causing $P_{tm}$ to move further along the negative x-axis. An imposed deep inspiration, even out of the physiological range, may carry $P_{tm}$ back up the Lambert curve on the inhalation but would carry it back down again on the exhalation.

We find these fixed points by solving $\frac{dr}{dt} = 0$, starting at $F = 0$. The first point on the open stable branch of fixed points is found using fzero, a numerical root finding routine based on Newton’s method, in Matlab and $r = R_{ref} \text{mm}$ as an initial guess. $F$ is then incremented, using the value of $r$ at the previous fixed point as an initial guess. The closed stable branch of fixed points is found in a similar fashion.

The unstable branch of fixed points is found by starting at the mean value of $F$ on the open stable branch and using $r = 0.1 \text{mm}$ as an initial guess. The rest of the points on the unstable branch are found by taking increments and decrements of $F$ as before. The curve of stable and unstable equilibria can be written as force as a function of radius, termed $F_{eq}(r)$.

We define the airway to be in an open state if the solution remains on the open branch of fixed points and closed if it remains on the closed branch of stable fixed points.

Figure 4.2 shows the response in airway radius when the parallel model is relaxed, then immediately maximally activated over the course of 1 second. Maximal activation is imposed here via a ramp in calcium and agonist concentrations from 0.01 to 1. Initially the airway is open and the force is small. As the force starts to increase with activation, the airway starts to narrow and the trajectory travels down the branch of open fixed points (in blue). Around the point at which the stable and unstable branches meet, the muscle is now fully activated and the force starts to fall as the radius continues to decrease. Once the solution is carried down the unstable branch of fixed points and the radius of the airway is approximately constant, the force begins to rise again, now in the closed stable state.

4.2.2 Constant Force

In the simplest case, the transmural pressure was found at each time-step by taking $F$ to be a piecewise constant which was ramped up or down to reflect varying agonist concentrations. The airway radius was then found using the Euler method to solve Equation 4.3.

4.2.3 A Dynamically Determined Force

As demonstrated by Mijailovich et al. [89] and others, when tidal length oscillations are imposed upon the ASM tissue in its maximally activated state, the mean force generated
by the tissue decreases and this reduction is dependent upon the amplitude and frequency of the imposed oscillations.

In terms of complexity, one step up from the constant force model would be to incorporate this force dependency upon imposed strain. Since we are looking at the airway response to pressure oscillations, we need to find a map between pressure and length. Using this map, we can find the force for a given pressure oscillation protocol if we know the corresponding amplitude of length oscillations.
To determine these contractile states, sinusoidal length oscillations were applied to maximally activated tissue (the parallel model uncoupled from the airway) and the mean steady state force was determined for various amplitudes and frequencies. Empirical curves were
The Airway Model

then fitted to the data and we found that

$$F(a, f) = 0.75 + 0.25 \times (0.3566 \times \text{Exp}[-\nu(f) \times a] + 0.669 \times \text{Exp}[-5.2 \times a]),$$  \hspace{1cm} (4.4)

where

$$\nu(f) = -3.078 + 26.5 \times (1 + \text{Tanh}[1 \times (f - 1.75)]) - 2.5, $$ \hspace{1cm} (4.5)

and $f$ and $a$ represent the frequency (in Hz) and amplitude (as a fraction of change from reference length) of the length oscillations. These dynamically determined contractile states are illustrated in Figure 4.3. For a given pressure oscillation protocol, $P_i(t)$, we linearised the Lambert pressure-radius relationship (Equation 4.1) about $P_{tm} = 10\ \text{cmH}_2\text{O}$, giving the line

$$d(P_i) = R(P_0) + R'(P_0)P_i.$$ 

This line is illustrated in Figure 4.4 along with the Lambert pressure-radius relationship. Linearisation about the point $P_{tm} = 10\ \text{cmH}_2\text{O}$ is necessary due to the steepness of the curve at transmural pressures below 10 cmH$_2$O; the physiological range of tidal breathing is around 4% stretches in length of the ASM tissue [89]. If such a linearisation had not been employed, a tidal breath corresponding to a 5cmH$_2$O (for instance) would lead to an almost 20% change in airway radius.

As $P_i$ changes with time, the corresponding change in length of the tissue is found using the map

$$a = \frac{|d(P_0 + P_i) - d(P_0)|}{d(P_0)}.$$ 

This value represents the amplitude of the length oscillations in Equation 4.4. We took the frequency of the length oscillations, $f$, to be the same as the frequency of the imposed pressure oscillations. Hence, the dynamically determined $F = F(a, f)$ can be found for any pressure oscillation protocol.

This maximal force was multiplied by the same piecewise constant value employed in the constant force model to reflect varying muscle activation levels, given that force generated has an approximately linear dependence on muscle activation.
Figure 4.3: The dynamically determined contractile states of ASM.

Figure 4.4: The linearisation of the Lambert curve about $P_{tm} = 10$ cmH$_2$O was used to find the changes in tissue length corresponding to changes in applied pressure. The linearisation is shown here for an order 2 airway.
The Airway Model

4.2.4 Force Predicted by the Parallel Model

In this case, the parallel model is coupled with the airway. Using Equation 3.1, the velocity of the muscle is taken to be

\[ v(t) = -\gamma \frac{dL}{dt} = -\frac{2\pi \gamma}{R_{ref}} \frac{dr}{dt} \]

and the PDE’s describing active and passive ASM properties, described in Chapter 3, are solved alongside Equation 4.3, taking

\[ F(t) = \kappa \int_{-\infty}^{\infty} x(A M_p + A M) dx + \psi \int_{-\infty}^{\infty} \rho(y)n(y, t) dy \]

as the force in Equation 4.2. The mechanism by which the contractile agonist regulates MLCP activity is a built-in feature of the active ASM model meaning force does not need to be multiplied by some piecewise constant fraction as in the previous two cases. We have taken the crosslinker generated force function, \( \rho(y) \), to be a symmetric function of strain in this chapter. This is because stretched crosslinkers will continue to generate a small but significant force following muscle activation and eventually cause the airway to close, when, if crosslinkers are not include in the model, the airway would have remained open. Since it is unlikely that crosslinkers alone are capable of closing an airway, we examined the model to see what was causing this to happen and found it was due to the asymmetry of the force function.

When the airway is stimulated and begins to contract, crosslinkers detach and reattach in positions of low strain as in Figure 3.4. If \( \rho \) is symmetric, the force generated by crosslinkers would eventually tend to zero. An asymmetric \( \rho \) causes a small, positive, force to be generated by stretched crosslinkers while no compressed crosslinkers are capable of generating a negative force to counteract it. In this way, the force continues to increase an the airway closes as the transmural pressure decreases.

\( \rho \) was taken to be asymmetric in unactivated tissue because such tissue will go slack if compressed. We could argue that the nature of \( \rho \) changes with muscle activation as an increase in calcium leads to greater rates of actin polymerization. Perhaps it’s most likely that the true function is neither symmetric nor asymmetric, however enough data does not exist to construct such a function. It should be mentioned however that this feature is simply an artefact of the model: in-vivo the extra-cellular matrix (not considered by the model) would serve as a passive restraint against shortening forces.
4.3 Experiments on Large Airways

As mentioned, little data exists for large intact human airways and absolutely none for small airways. As a method of validating our modelling approach, we look to the experimental results of Lavoie et al. [79]. We hypothesize that if our models can reasonably reproduce the key features of this data and agree well with each-other, then our idea of replacing the parallel model with a simplified model is strengthened.

Lavoie et al. sets out to show that the relaxing effects of taking a deep breath are determined by the extent of stretch imposed upon the airway. It is one of very few studies to examine the effects of tidal breathing and deep inspirations on intact human donor airways.

4.3.1 Airway Response to Varying Degrees of Activation (Protocol A.)

Subject to a given pressure oscillation regime, the response of the airway was recorded for varying acetycholine concentrations applied at the beginning of the experiment. Ten minutes following the application of acetycholine, pressure oscillations that caused a 20% luminal area expansion were imposed for another 10 minutes before the airway was left to relax. The experimental results are shown in the top panel of Figure 4.5.

It was found that, as acetycholine increased, the magnitude of the tidal area fluctuations, induced by the pressure oscillations, decreased, indicating increased airway stiffness.
Experiments on Large Airways

Figure 4.5: The experimental results of Lavoie et al.. In protocol A, the airway response to varying degrees of activation is measured (top), while in protocol B, the airway response to increasing amplitudes of pressure oscillations is found (bottom panel). Reprinted with permission of the American Thoracic Society. Copyright ©2015 American Thoracic Society. The American Journal of Respiratory and Critical Care Medicine is an official journal of the American Thoracic Society [79].

Model Predictions

There is very little difference in the predictions of both the constant force and dynamically determined force models to Protocol A. The case of a dynamically determined force is shown in Figure 4.6a. As mentioned in Section 3.1, we are unconcerned with the transients to steady state following oscillations. As such, we are satisfied that both models can qualitatively reproduce the key feature of this experimental result: in both cases, the extent of airway stiffening increases with the level of activation.

As with the highly simplified models, the parallel model predicts that as the dose of acetylcholine increases, the magnitude of airway strain decreases, see Figure 4.6b.
4.3.2 Airway Response to Increasing Amplitudes of Pressure Oscillations (Protocol B.)

Six 10 minute periods of simulated breathing are performed with the airway subjected to a constant dose of acetylcholine, applied at the start of the experiment. In each 10 minute period, pressure oscillations of increasing amplitudes are imposed. The experimental results are shown in the bottom panel of Figure 4.5.

The increases in tidal pressure oscillation amplitude led to corresponding increases in tidal area fluctuations. This result indicates that deep breathing can reverse bronchoconstriction more effectively than quiet tidal breathing.

Constant Force and Dynamically Determined Force Models

Both the constant force and dynamically determined force models show that the tidal area fluctuations experienced by the airway increase with the magnitude of the pressure oscillations imposed upon them. Results are shown in Figure 4.7 for an order 16 airway. Reopening of the airway can be obtained in both cases but to a slightly lesser extent with the constant force model. This is simply because the force does not decrease with increasing oscillation amplitudes as it does in the dynamically determined case. Decreasing ASM force means increasing transmural pressure leading to an increase in airway radius as the trajectory moves up the Lambert pressure-radius curve in Figure 4.1a.
Figure 4.6: Lavoie protocol A for an order 22 airway: both models are capable of predicting that airway strain decreases as the degree of activation increases. In both figures, activation increases from blue to green to red.
Figure 4.7: Lavoie Protocol B for an order 16 airway: both models predict an increase in airway strain with increasing tidal area fluctuations. The increase in the moving average (red line) indicates a degree of airway reopening that is larger in the case of a dynamically determined force.

**Parallel Model**

Again the parallel model predicts that airway strain increases with the amplitude of imposed oscillations. The parallel model also predicts a gradual reopening of the airway as the strain increases, in qualitative agreement with experimental observations and the simplified force models.
Higher Airway Order Result

The simulations in the previous section were obtained using the pressure-radius relationship from an order 16 airway. If the experiment is repeated using an order 22 airway, the results are markedly different for the dynamically determined force and constant force models. Figure 4.9 shows that both models do not predict any airway reopening while Figure 4.10 shows that the parallel model does predict a reasonable degree of reopening under the same conditions. Because of the stiffer pressure-radius relationship, in the constant force model the radius varies little with pressure oscillations and the mean pressure changes little from its pre-oscillation value.

In the parallel case, as before, as the degree of strain imposed upon the tissue is increased, the ASM force decreases causing the airway to gradually reopen. Had the linearisation been taken about a steeper part of the pressure-radius curve, the dynamically determined force model would compare well with the parallel model. This result serves to show that this dynamically determined force approach may break down for larger, stiffer, airways and indicates the importance of passive stiffness.
Figure 4.9: Lavoie Protocol B for an order 22 airway: both models predict an increase in airway strain with increasing tidal area fluctuations. However, the constant moving average (red line) in both models indicates no airway reopening in contrast with the experimental results and the parallel model.
Figure 4.10: Lavoie Protocol B for an order 22 airway: as with the order 16 airway, the parallel model again predicts an increase in strain with increasing area fluctuations as well as a gradual reopening of the airway.

We have validated our approach by demonstrating that the constant force and dynamically determined force models both agree reasonably well with experimental data and (crucially) with the parallel model. All models predict that similar variations in airway area are obtained when increasing concentrations of agonist are applied to the airway. To a degree, the models also agree that increasing amplitudes of imposed pressure oscillations cause the airway to re-lengthen gradually.

We repeated the experiment of Lavoie on an order 22 airway and found that a constant moving average is predicted with the simplified models in contrast with the increasing moving average predicted by the parallel model and seen experimentally. This indicates that the linearisation we choose for the dynamically determined contractile state model may break down for larger airways. However, since our main concern is whether airways will close or remain open in response to certain chemical and mechanical stimuli, we can conclude that, for large airways that never close, the complicated model can be replaced with a highly simplified one.

We will now begin our investigation into each of our model predictions to the small airway response to varying pressure oscillation protocols following an initial application of acetylcholine. The outcome of this study will indicate the importance of the complexity of the ASM generated force: is it necessary to include ASM phenomena such as force
maintenance and the way in which passive stiffness is regulated to obtain an accurate prediction of the small airway response? In other words, to what extent do these properties play a part in determining whether an airway will remain open, or close?

4.4 The Small Airway Response

An order 2 airway is subject to sinusoidal pressure oscillations of varying frequency and amplitude around a mean of $P_0 = 10 \text{ cmH}_2\text{O}$ following activation. Depending on the oscillation protocol employed and the nature of the ASM generated force, the airway will either reach an open or closed stable steady state, see Figure 4.2.

4.4.1 The Constant Force Model

Taking $F = \hat{F}$ to represent maximal activation, the force is ramped instantaneously up from 0 to $\hat{F}$ while oscillations commence. It is found that airways subjected to high frequency, high amplitude oscillations will remain open while all other will close. This result was expected from both a physiological and mathematical viewpoint. When ASM tissue strips are subject to imposed tidal load fluctuations, the force and stiffness generated by the tissue decreases. This dynamic state is characterized by fewer bound populations as they become more and more perturbed with the strength of the imposed oscillations. This decrease in force and stiffness relaxes the airways and is a powerful mechanism of preventing bronchoconstriction. Mathematically, when the force, $F$, in Equation 4.2 decreases, the transmural pressure increases causing the trajectory to move up the Lambert pressure-radius curve.

It should be stressed that the open and closed airway regions depend greatly on the parameter $B$ in Equation 4.2: for values of $B$ outside a small interval around the value choosen here, airways will either close (for large $B$) or will remain on the open stable branch of fixed points (for smaller $B$) for all pressure oscillation protocols.

We offer two explanations for how certain pressure oscillations cause the airway to close. The first relates to the relationship between the parameter $\rho$ in Equation 4.3 and the frequency of the oscillations. The second relates to a curve of saddle-node bifurcations that occur between the open and closed regions of Figure 4.11.
In the first case, consider Figure 4.12 which shows the trajectory of 1 Hz and 2 Hz oscillations of amplitude 10 cmH$_2$O. As the force is increased, the radius decreases and starts to oscillate around the end of the stable branch of fixed points. When the frequency is high, the airway remains open. $R(Ptm)$ decreases with downward oscillations, but with high frequency relative to $\rho$, $r(t)$ decreases slowly toward $R(Ptm)$ and has not decreased enough to close the airway before the subsequent upward oscillation. The upward oscillation causes a large increase in $R(Ptm)$ (relative to the increase with lower amplitude oscillations) and this increase serves to decrease $F/r(t)$. This leads to an increase in $Ptm$ and the cycle starts again. In other words, the trajectory will be pulled up to the open stable state before it has time to be pulled further toward the closed stable state. Closure in the 1 Hz case is caused by the trajectory falling too far into the unstable region on the downward pressure oscillation before being pulled up on the subsequent upward pressure oscillation.

Another way of visualising this is that for large $\rho$, the boundary between open and closed states is shifted to the right, Figure 4.13. This is because $\rho$ is directly proportional to the distance the trajectory is from equilibrium: if the frequency is small relative to $\rho$, the trajectory will move further into the unstable region leading to a fall to the closed stable state.

As mentioned, this can also be explained by studying the saddle-node bifurcations of the system i.e. the point at which the open stable periodic solution collides with the unstable periodic solution and ceases to exist. For each pressure oscillation protocol, the value of
the force, $F$, at which the saddle-node bifurcation occurs is found. In the simulations above, $F$ was ramped up to $\hat{F}$. If the saddle-node bifurcation for a given frequency and amplitude occurs at values of $F$ below $\hat{F}$, the airway will close since the closed solution is the only stable one. Alternatively, the airway will stay open for values of $F$ above $\hat{F}$. Oscillation amplitudes and frequencies that have a saddle-node bifurcation very close to $F = \hat{F}$ determine the boundary between the open and closed states. In the following, we have taken $\hat{F} = 1$.

For a given pressure oscillation protocol, the value of $F$ at which the saddle-node bifurcation occurs was found as follows.

For each amplitude and frequency, the stable branch of periodic solutions is found. An example of a typical trajectory is shown in Figure 4.14. Starting at $t = 0$, $r = R_{\text{ref}}$ and $F = 0$, the mean of the periodic solution the system tends to is found. The process is repeated, increasing $F$ each time to find the stable branch and taking each point on the branch to be the mean of the periodic solution.
(a) Closure with low frequency (1 Hz) oscillations relative to $\rho$.

(b) 2 Hz oscillations: The airway remains on the open stable branch if the frequency is fast enough relative to $\rho$.

Figure 4.12: Constant force model subjected to oscillations of amplitude 10 cmH$_2$O and frequencies (a) 1 Hz and (b) 2 Hz
Figure 4.13: Constant Force Model: decreasing $\rho$ shifts the boundary between open (red) and closed (blue) states to the left.
Figure 4.14: Constant Force Mode: starting at $r = R_{ref}$ and marching forward in time to find the unstable periodic solution for a given $F$ and pressure oscillation protocol.

Next the unstable branch of periodic solutions is found. To achieve this, we let time run backwards so that the trajectory is repelled from the stable solution and approaches the unstable solution. For a given frequency and amplitude, starting at $r = 0.01$, $F = 0.1$ and marching back in time (i.e., back to the unstable periodic solution), the mean of the solution the system tends to is found. The process is repeated as before, increasing $F$ each time, giving the unstable branch of periodic solutions. A typical trajectory is shown in Figure 4.15.

The stable and unstable periodic fixed points are shown in Figure 4.16 for various pressure oscillation protocols. The figure shows that the value of $F$ at which the saddle-node bifurcation takes place depends on the frequency and amplitude of the pressure oscillations. For a given force, we have taken the mean of the periodic solution associated with it to be one point on the force-radius curve. In this way, the time component of the periodic solutions has been neglected. Had it not been, vertical oscillations would appear around the force-radius curves in Figure 4.16.

For each pressure oscillation protocol, an interpolating function is made from the branch of stable periodic solutions and again from the unstable branch. Using `fzero` in Matlab, the value of $F$ at which the two curves intersect is found, giving the saddle-node bifurcation for that particular protocol. The curve of saddle node bifurcations that occur close to $F = \hat{F} = 1$ is shown in black in Figure 4.11.
Figure 4.15: Constant Force Model: tracking back in time to find the unstable periodic solution for a given $F$ and pressure oscillation protocol.
Figure 4.16: The airway will close if the saddle node bifurcation occurs before $F = \hat{F} = 1$ (mN). $A$ and $f$ represent the amplitude (in cmH20) and frequency (in Hz) of the pressure oscillations respectively. The pink, green and red curves represent stable branches, while the light blue, dark blue and black represent unstable branches. Figure 4.16b is a zoomed image of Figure 4.16a and is included for the sake of clarity.
4.4.2 The Dynamically Determined Force Model

As previously described, the amplitude of the pressure oscillations is mapped to the pressure-radius curve by linearising the order 2 curve about $P_{tm} = 10 \text{ cmH}_2\text{O}$. Radius is then mapped to length and used as input in Equation 4.4. As such the force varies at every time step depending upon the displacement of $P_i(t)$ from its initial value. A more complex pattern now emerges (Figure 4.17) which can again be explained by the relationship between $\rho$ and the frequency of the pressure oscillations.

Figure 4.17: DDCS Force Model: When fluidization is taken into account, a greater region of open (red) states is obtained compared with the constant force model.

Figure 4.19 show how the boundaries between open and closed states is shifted up or down depending on the magnitude of $\rho$. Typical trajectories are shown in Figure 4.18 for oscillations of frequency 0.2 Hz and 2 Hz and amplitude 6 cmH$_2$O. The fall to closure in the 0.2 Hz case is caused by the relatively long transient time - the radius narrows to a greater degree due to the long downward pressure oscillation. This pushes it further into the unstable region and eventually down to the closed state. If higher frequency oscillations are imposed, the airway will remain open: the trajectory does not have time to fall too far below the open stable branch before it is picked up again by the upward oscillation.
Figure 4.18: Dynamically determined force model: The airway remains on the open stable branch if the frequency is fast enough relative to $\rho$. The open stable branch of fixed points is shown here in green and the unstable in blue. Figure 4.18b is included as a zoomed image of Figure 4.18a for clarity.
Figure 4.19: DDCS Force Model: decreasing $\rho$ shifts the boundary between open (red) and closed (blue) states to the left as in the case of constant force.
4.4.3 The Parallel Model

The open and closed states predicted by the parallel model vary significantly from those of the constant force and dynamically determined force models, see Figure 4.20. Generally, for a given frequency, high and low amplitudes cause reopening and closure respectively. More specifically however, the parallel model predicts closure for high frequency oscillations and reopening for low frequency oscillations of the same amplitude. This result was not expected, it was thought that a figure more similar to Figure 4.11 would be obtained. The result brings the question of whether the parallel model is best at representing ASM generated force when the airway is coupled with the tissue or is it actually representative of what occurs in vivo. Since the latter is counter-intuitive and there is no experimental data to verify its prediction, an investigation into what is causing this result is clearly needed.

The obvious answer is numerical error, however, convergence testing indicates that this is not the case (see Appendix 1).

Our next avenue of investigation was the study of the force and radius trajectories predicted by the model in both cases. To understand why the airway closes in the 0.4 Hz case, it helps to compare the force-radius curves predicted by the parallel model with the curve of equilibria, \( F_{eq}(r) \). Figure 4.21a shows the model predictions for the force-radius curve. Each dot represents the trajectory at times when \( P_i(t) = 0 \) cmH\(_2\)O. The blue/green curve gives the unstable/stable equilibria. Figure 4.21b is a zoomed version of this. The further the trajectory is to the right of the equilibria, the more it will be attracted to the closed stable state. However, it is difficult to see from Figure 4.21 how far the mean of each trajectory is from \( F_{eq}(r) \).

In Figure 4.22 we have taken the mean of the 0.2 Hz and 0.4 Hz trajectories at \( P_i(t) = 0 \) cmH\(_2\)O subtracted from the corresponding equilibrium solution for a given value of \( r \), i.e. the mean of \( F(r) - F_{eq}(r) \), where \( F(r) \) is the force predicted by the parallel model at \( r \) and \( F_{eq}(r) \) is the fixed point at \( r \). \( R(P_{tm}) - r \) determines how fast the solution moves away from the fixed points while \( F(r) - F_{eq}(r) \) represents the (horizontal) distance the solution is from the fixed point at \( r \). Closure ensues in the 0.4 Hz case because the average rate with which the trajectory moves away from the fixed points is higher along with the distance the trajectory is to the right of the curve of fixed points. In other words, the trajectory moves further into the unstable region in the 0.4 Hz case and is increasingly attracted to the closed stable solution.
Figure 4.20: The open (red) and closed (blue) states predicted by the parallel model.

We found that the 0.4 Hz oscillations cause the trajectory to move slightly faster and further away from the open stable branch than their 0.2 Hz counterparts, see Figure 4.22. In other words, 0.2 Hz oscillations hug the curve of fixed points slightly more. This difference, subtle as it is, eventually causes the trajectories to branch, with the case of higher frequency oscillations falling to closure.
Figure 4.21: (a) The curve of equilibria (stable in black, unstable in red) along with the force-radius curves predicted by the parallel model for 0.2 Hz and 0.4 Hz oscillations of amplitude 3 cmH$_2$O. Points show the trajectory at every $t$ where $P_i(t) = 0$ cmH$_2$O. (b) A zoom of Figure 4.21(a) is shown for clarity. In this case, the unstable branch and closed stable branch are outside the plot region.
Figure 4.22: \( F(r) - F_{eq}(r) \) and \( R(P_{tm}) - r \) at every \( t \) where \( P_i(t) = 0 \text{ cmH}_2\text{O} \). \( F_{eq}(r) \) is the fixed point at \( r \). \( R(P_{tm}) - r \) determines how fast the solution moves away from the fixed points while \( F(r) - F_{eq}(r) \) represents the (horizontal) distance the solution is from the fixed point at \( r \). The average of \( F(r) - F_{eq}(r) \) and the average of \( R(P_{tm}) - r \) are shown in black (0.4 Hz) and pink (0.2 Hz). When 0.4 Hz oscillations are imposed, the average rate with which the trajectory moves away from the fixed points is higher along with the distance the trajectory is to the right of the curve of fixed points.
4.5 Discussion

We have investigated several ways of modelling the ASM generated force in determining the airway response to imposed stimuli: a constant force, a dynamically determined force and force generated by the crossbridge cycle, development of latchbridges and cytoskeletal remodelling. Given that the latter is highly complex, difficult to analyse and comes with long computation times, one of our main concerns was whether the model could justifiably be replaced with a simplified version.

We first looked at the experiments of Lavoie et al. [79] on large intact human donor airways. All three models fitted the data reasonably well, predicting that tidal area fluctuations increased with decreasing doses of acetlycholine and that airway strain increases with the amplitude of imposed pressure oscillations. The models also predict a gradual reopening of the airway with increasing area fluctuations.

Satisfied with the results for large airways, we moved on to address our main goal for this chapter: can the simplified models predict similar open and closed airway regions for small airways to the parallel model, once activated and subject to pressure oscillations? The answer is yes, for the most part. All the model predictions highly depend upon the parameter $B$, for $B$ smaller than that chosen, all airways will remain open and close for larger values of $B$. However, for a small interval of values of $B$, significantly different patterns of open and closed regions emerge depending on the model used to describe the ASM generated force.

The constant force model predicts the airway will remain open only for high frequency, high amplitude oscillations. This can be explained by a curve of saddle-node bifurcations that occur at $\hat{F}$, the value the force is ramped up to. If the saddle-node bifurcation for a particular pressure oscillation protocol occurs above $\hat{F}$, the airway will remain open and close for values below $\hat{F}$.

The dynamically determined force model predicts a greater region of open states compared with the constant force model due to the lower values of force employed for the given oscillation regime. The boundary between open and closed states can be explained by the dependency of the parameter $\rho$ on the frequency of the imposed oscillations: if $\rho$ is large enough relative to the frequency, the trajectory will fall too far into the unstable region on the downward oscillation before being pulled up on the subsequent upward oscillation.

The predictions of the parallel model are markedly different to the simplified models: for a certain amplitude, the model predicts closure for high frequency oscillations and reopening for low frequency oscillations. As mentioned, this result is unexpected and led
us to question the viability of using the parallel model to represent the forces generated by ASM tissue. While the subtle differences in the movements of the trajectories around the stable branch of equilibria answers the question from a mathematical viewpoint, several questions remain regarding the physiological meaning of the result. It is difficult to envision how such patterns could emerge in vivo given the well established result that ASM fluidizes with imposed shear and the extent of fluidization is proportional to the magnitude of this shear.

To understand why this was the case from a mathematical perspective, we looked at the mean displacement of the force from the equilibrium force at a fixed radius and fixed $P_i$. We found that force was further from equilibrium in the high frequency case which eventually led to closure as the trajectory is continually pulled further into the unstable region.

In conclusion, the complex ASM properties captured with the parallel model is the fundamental difference separating the results predicted by the parallel model with the two highly simplified models studied here. While this is true only for a small range of parameters, it has demonstrated that behaviour characteristic to ASM at the tissue level has profound effects at the airway level. In Chapter 5, such properties will be examined in greater detail with particular emphasis on the passive processes that take place within the cell. We are particularly concerned with these passive mechanisms due to the hypothesis that abnormalities in their behaviour may lead to an increased resistance to applied loads, thus stiffening the airways to prevent relaxation.
Properties of Airway Smooth Muscle

Latchbridges are slowly cycling crossbridges meaning they are part of the active side of ASM contraction. However, their low detachment rates also mean that they can act to reduce the load against which the muscle shortens, thereby increasing the cell's resistance to imposed load fluctuations. In this sense, latchbridges, along with cytoskeletal remodelling, contribute to the passive processes governing the passive stiffness generated by the cell. The goal of this chapter is to learn more about such passive mechanisms as it has long been believed that abnormalities in their behaviour can have serious implications in the pathogenesis of asthma and airway hyper-responsiveness. We want to know which filaments contribute to passive ASM processes, how the activity of these filaments regulated and what consequences could such passive events have in relation to airway diseases.

5.1 Latch State Behaviour

Dillon et al. [31], demonstrated the latch state phenomenon by showing that ‘if an isometrically contracting muscle is released to an isotonic load at various times during the force maintenance period, it will contract with an initial velocity that decreases as a function of the amount of time spent in isometric contraction’. This reduction in velocity is correlated with a decrease in myosin phosphorylation and a decrease in energy consumption. Dillon et al. demonstrates latch state behaviour as follows:

1. A smooth muscle tissue strip is activated with potassium chloride in the presence of calcium.

2. A force drop is applied to the first strip one minute later. It is applied to the second strip ten minutes later. In both cases, the velocity of the muscle strip is recorded.
3. The process is repeated for force drops of varying fractions of the initial force and force velocity curves are plotted.

![Figure 5.1: Experimental data from Dillon et al. [31]. Shortening velocity is measured when a given load (on the x-axis) is applied to the muscle 1 and 10 minutes following stimulation with a contractile agonist. Permission to use has been granted with licence number 3643530793774.](image)

A force drop applied ten minutes after activation produced a much lower velocity than a drop of the same magnitude imposed one minute after activation. It is suggested that the dephosphorylation of an attached crossbridge arrests crossbridge cycling. ‘Such a cross-bridge is termed a latchbridge, capable of maintaining force but acting as an internal load on the remaining crossbridges to produce the decrease in shortening velocity’ [31]. The force-velocity curves obtained by Dillon et al. are given in Figure 5.1.

In this section we investigate this hypothesis with our four state, Hai-Murphy based model. We will see that an increase in latchbridges following muscle stimulation is consistent with a decrease in shortening velocity and myosin phosphorylation.

However, it is a widely held belief that latchbridges are not the only source of passive stiffness in smooth muscle tissue [51, 140, 21]. It has been postulated that the ‘freezing’ of cytoskeletal filaments following tissue stimulation contributes to the passive force generated
by smooth muscle. We want to investigate the extent to which this behaviour contributes toward the latch state.

In order to get a clear vision of this, we replace latchbridges with crosslinkers in the modified Hai-Murphy model of Wang et al. [129]. In other words, latchbridges are removed from the model and the remaining three state model is solved in parallel with the crosslinker model of Donovan et al. [35]. The procedure of Dillon et al. is followed with the modification that force drops are applied just after force has reached a plateau (around 10 seconds after activation) and long after force has reached a plateau (180 seconds after activation). This modification was needed as the time constants in our model were built around mouse data as opposed to swine data, as utilised by Dillon et al..

We will see that crosslinkers can account for the separation of the force velocity curves associated with latch state behaviour. However the three state crosslinker model cannot account for all features of the latch state.

5.2 Capturing Latch State Behaviour

5.2.1 The Crossbridge Hypothesis

Equation 3.3 describes the shortening velocity of the tissue in response to an applied external force, $F_e(t)$, when latchbridges are assumed to be solely responsible for the passive force generated by the muscle.

The model predictions of the dynamic response to the procedure of Dillon et al. are now detailed. In Figure 5.2a force increases rapidly following activation as the crossbridge and latchbridge populations increase. An approximately steady force is maintained after this point while crossbridges are dephosphorylated and move into the latchbridge pool. It is this transition that leads to separation of force velocity curves obtained at various times following activation. Soon after a near steady force has been attained, the crossbridge population is high while the latchbridge population is low. A force drop applied at this point will result in a higher shortening velocity being obtained than at any subsequent point in time. Since crossbridges and latchbridges have high and low detachment rates respectively, there is little to resist the applied load when the level of latchbridges is low as crossbridges are easily broken. The opposite is true several minutes following stimulation when the level of latchbridges is high: their low detachment rates mean they are capable of generating relatively strong forces to resist the contraction, leading to lower shortening velocities compared with the high crossbridge state seen shortly after stimulation.
**Force-Velocity Relationship in the Absence of Latchbridges**

To highlight the effect of the latchbridges, we compare this to a three state model, devoid of passive mechanics. A schematic for this model is as follows:

\[
\begin{align*}
M & \xrightarrow{k_1} M_p \\
& \xleftarrow{k_2} M_p \\
g_p(x) & \xrightarrow{f(x)} AM_p
\end{align*}
\]

which translates to the following system of PDE’s to describe the corresponding population distributions

\[
\begin{align*}
\frac{\partial M}{\partial t} - v(t) \frac{\partial M}{\partial x} &= -k_1 M + k_2 M_p, \\
\frac{\partial M_p}{\partial t} - v(t) \frac{\partial M_p}{\partial x} &= k_1 M - (k_2 + f_p(x)) M_p + g_p(x) AM_p, \\
\frac{\partial AM_p}{\partial t} - v(t) \frac{\partial AM_p}{\partial x} &= f_p(x) M_p - g_p(x) AM_p,
\end{align*}
\]

subject to the constraint \( M + M_p + AM_p = 1 \). In the absence of crosslinkers and latchbridges, Equation 3.7 becomes

\[
\frac{dL}{dt} = \frac{dF_e/dt - \kappa \int (f_p(x) M_p - g_p(x) AM_p) dx}{\kappa + \kappa \gamma_B \int AM_p dx}.
\]

The force-velocity curve obtained soon after activation is almost identical to the one obtained long afterwards indicating a fast force recovery after stimulation, see Figure 5.3. Due to the rapid cycling nature of phosphorylated crossbridges, their steady state population is attained quickly. If a force drop is imposed soon after activation, a near steady state level of crossbridges will be available to resist the drop. If the force drop is imposed much later, a similar level of crossbridges will be available to provide resistance. The velocities are almost equal in each case leading to a negligible separation between the force velocity curves, indicating no latch state behaviour.
Figure 5.2: The four state model: a) The force generated by the muscle rapidly increases upon full activation, b) the force-velocity relationship obtained when force drops of varying magnitudes are applied 5 seconds after and 2 minutes after activation with calcium and agonist, c) upon activation, the crossbridge population rises steeply before falling transiently as cycling ceases and crossbridges are dephosphorylated, increasing the latchbridge pool.
Figure 5.3: The three state model: Force drops of varying magnitudes are applied soon after (green) and several minutes after (blue) (once a steady state has been attained) activation with calcium and agonist. a) A sharp rise in crossbridge generated force is observed upon activation, b) Little separation of the force-velocity curves is obtained in the absence of latchbridges, indicating no latch state behaviour, c) A steady state level of crossbridges is attained relatively quickly in the absence of latchbridges.
5.2.2 The Crosslinker Hypothesis: An Alternative Approach to Capturing Latch State Behaviour

The density of latchbridges increases hyperbolically with time following muscle activation. The same is true following stretch as the initial distribution of crossbridge populations are torn apart before rising transiently back to steady state. The force-velocity relationship predicted by the 3 state and 4 state models is hence unchanged if muscle activation is replaced with a stretch in length.

To model the effects of density of crosslinkers on latch state behaviour, a stretch is imposed on the 3 state + crosslinker model. We have found that, if crosslinkers are added to the three state model, i.e., if latchbridges are replaced with crosslinkers, separation of the force-velocity curves is obtained. The imposition of stretch here is crucial, it is the only way we have of disturbing the distribution of bound crosslinkers at steady state. This could be changed with a calcium or agonist dependent crosslinker attachment function, for example. The equation found to describe the velocity of the muscle in response to an applied external load when crosslinkers are assumed solely responsible for the tissue’s passive stiffness is given by

$$\frac{dL}{dt} = \frac{dF_e/dt - \kappa \int (f_p(x)M_p - g_p(x)AM_p)xdx - \psi \int \rho(\alpha(1 - N) - \beta n)dy}{\kappa + \psi + \kappa\gamma_B \int AM_pdx + \psi\gamma_L \int \rho n dy}.$$

The force-velocity curves for the three state + crosslinker model are shown in Figure 5.4. Following stretch/activation, the distribution of crosslinkers is skewed toward positive strains. A force drop applied at this point will push the distribution further into the region of positive strain in which the rate of detachment is high. These crosslinkers provide little resistance to the imposed load as they detach rapidly, analogous to crossbridges.

A force drop applied when the population has returned to steady state will produce a lower shortening velocity. This is because, at steady state, there is an assumed symmetric distribution of crosslinkers centered around zero strains. When this distribution is shifted to the right with an imposed load, the crosslinker detachment rates in this region are relatively low. These crosslinkers are capable of generating relatively strong forces to resist the applied load due to their low detachment rates. In this way force can be maintained while there is a large degree of separation of the force-velocity curves, indicating a high degree of latch state behaviour. Ways in which crosslinkers may contribute to the latch state are discussed in an upcoming section.
Capturing Latch State Behaviour

5.2.3 Different Length Change Scheme Leads to Very Different Model Results: A Feature of the Crosslinker Model

Depending on whether a stretch or contraction is imposed as the tissue is activated, the three state + crosslinker model predictions vary dramatically while the four state model predictions remain the same. When the tissue is contracted by 17% during activation, the force velocity curves obtained with the crosslinker hypothesis are not well separated, see Figure 5.5. This is in stark contrast to the well separated curves obtained when a stretch is imposed during activation, Figure 5.4. To understand this, it is necessary to look at the movement of the crosslinker distribution during stretch and contraction, Figure 5.6.
As described in the previous section, during a stretch, crosslinkers are pushed into regions of high positive strain where their rate of detachment increases with their level of strain. This leads to high shortening velocities being obtained when force drops are applied to crosslinker distributions that are initially skewed towards positive strains as they detach rapidly, providing little resistance.

During a contraction, low strained crosslinkers are pushed into regions of high negative strain. A force drop then imposed on such a crosslinker distribution will shift the population close to zero strains in which the rate of detachment is low. Hence, a relatively low shortening velocity is obtained as these crosslinkers are capable of exerting a relatively strong resistive force. Whether this result can be reproduced experimentally and is representative of in-vivo conditions is unknown and likely to remain so. Since the tissue is activated, in vivo, latchbridge activity would mask the higher resistive crosslinker generated force in the latter scenario.

It is worth noting that this feature of the crosslinker model is independent of the nature of the force function: similar results are obtained when $\rho$ is symmetric, see Figure 5.7. As mentioned in Chapter 3 and 4, there is some controversy surrounding the force response of
crosslinkers to compression. In Chapter 4, $\rho$ needed to be symmetric to prevent a continual increase in force generation once active force had reached a steady state. However, the fact that inactivated tissue goes slack when compressed gave rise to the original definition of $\rho$. We hypothesize that crosslinker generated force is likely to change with muscle activation and provide this result simply as an interesting feature of the model.

Figure 5.6: Depending upon whether the tissue is stretched (a) or contracted (b) during activation, the model predictions for the force velocity curves are very different. The crosslinker distribution is skewed to the right following a stretch, leaving a large population of fast-detaching crosslinkers available to resist an imposed force drop. The opposite is true when the distribution is skewed to the left following a contraction.
Figure 5.7: A force drop is applied to one tissue strip 10s after the stretch/activation (blue). It is applied to another long after the stretch/activation (green). The nature of the crosslinker force function is independent of the degree of separation of the force-velocity curves obtained following stretch or contraction. Instead it is the distribution of crosslinkers causing this effect, see Figure 5.6.

5.2.4 Theories on the Link Between Active and Passive Smooth Muscle Dynamics

The active side of smooth muscle contraction has been extensively studied however much less is understood about the underlying passive dynamics, particularly crosslinkers. Several interesting theories and observations have been proposed regarding crosslinker dynamics, some of which will be presented in this subsection.

We have seen that crosslinkers can contribute to the latch state but mechanisms which regulate their behaviour to enable them to do so remain unclear. For instance, there is a possibility that once the cell has contracted, newly bound crosslinkers can lower the
crossbridge strain thus contributing to the latch state. Dense bodies within the cell provide anchoring points for crosslinkers and actin filaments. The accumulation of these filaments at dense bodies increases the strength of the cytoskeleton for mechanoprotection and increases the rigidity of the cytoskeletal lattice hence lowering the intracellular load [52]. In this way, crosslinkers in vivo may contribute to the decrease in shortening velocity following activation as the resistive force generated by the cell, caused by their reconfiguration, increases.

The rheology of this actin network changes with applied shear stress from a linearly deforming gel at low stress to a non-newtonian fluid at high stress [137]. These changes are brought about by mechanical and chemical events that lead to the remodelling of the actin filament lattice. Figure 5.8 shows a schematic which suggests a pathway of events leading to the formation of structure following activation or mechanical stimuli.

Figure 5.8: An increase in crosslinker strain stimulates a chemical pathway leading to an increase in intracellular Ca\(^{2+}\) [59, 23]. Contractile activation increases actin polymerization leading to strengthened crosslinkers, [86]. Focal adhesion kinase (FAK) has been linked to actin cytoskeleton polymerization, cytoskeletal remodelling and enhancing the adhesion strengthening rate [123, 49, 88].

An outline of the possible roles of crosslinker proteins as mediators of the mechanical adaptation of smooth muscle tissues to environmental stimuli is given below.

- An increase in crosslinker strain stimulates a chemical pathway leading to an increase in intracellular Ca\(^{2+}\) [135, 80].
- Contractile activation increases actin polymerization leading to strengthened crosslinkers [86].
- Agonist stimulation of focal adhesion kinase (FAK) and paxillin phosphorylation leads to active tension development and myosin light chain phosphorylation, [124]. FAK
has also been linked to actin cytoskeleton polymerization and cytoskeletal remodelling and its activity may depend upon the length of the cell [123, 49]

- During the early stages of adhesion, FAK up-regulates integrin activation to enhance integrin binding and enhance the adhesion strengthening rate, [88, 63].
- FAK expression has been associated with a transient inhibition of Rho activity, suggesting a down-regulation of cytoskeletal tension, [88].
- Contractile stimuli cause α-actinin crosslinkers to be recruited to adhesion junctions. This strengthens the connections between membrane adhesion junctions and actin filaments. A strong and rigid framework has been created, well suited to the transmission of force generated by crossbridge cycling, [52].

Given the above information, we suggest that the regulation of cytoskeletal remodelling, induced by the reconfiguration of crosslinkers, may take place as follows. Upon stimulation with agonist, FAK is activated and myosin phosphorylation takes place, initiating the crossbridge cycle. The activation of FAK leads to actin remodelling and crosslinker polymerization. These events may increase crosslinker strain, leading to an increase in intracellular calcium [135, 80, 59, 23], enhancing myosin phosphorylation which feedbacks to an increase in crosslinker binding. FAK strengthens crosslinkers, increasing their resistance to imposed strain, and its transient inhibition of Rho decreases cytoskeletal tension. This may result in a reduction in intracellular calcium thus halting the crossbridge cycle. In this way, the regulation of cytoskeletal remodelling by FAK may help to sustain isometric force.

5.2.5 Phosphorylation vs Velocity Curves

As mentioned previously, Hai and Murphy [53] used their model to obtain the force velocity curves of Dillon et al. [31]. They did this by evaluating the velocity at steady state using different phosphorylation rates to reflect a decrease in phosphorylation following tissue activation. The force velocity curves, reproduced from [53], along with their imposed phosphorylation rate curve are shown in Figure 5.9.
Figure 5.9: (a) The time dependent (normalised) phosphorylation rate used by Hai and Murphy [53] and (b) the force-velocity curve produced by the model. The curve obtained shortly after isometric stimulation is given in green while the curve obtained long after is given in blue. The phosphorylation rates used to obtain these force velocity curves change according to the phosphorylation profile depicted in (a).

Figure 5.10 shows the crossbridge model prediction for the level of phosphorylation in the presence, Figure 5.10a, and absence, Figure 5.10c, of latchbridges following tissue activation. We take phosphorylation to be a finite integral over the phosphorylated population:

$$\int_{-1}^{1} M_p dx + \int_{-\infty}^{\infty} AM_p dx.$$
This is due to the distribution of the phosphorylated myosin population which is non-zero everywhere in the domain and decreases in the binding region where the level of attached crossbridges increases from zero to become relatively large. If we were to take the integral over the entire domain, any changes in phosphorylation would be tiny due to the constant high level of phosphorylated myosin away from the binding region. We therefore restrict ourselves to the displacements at which the distributions change most.

After a steep initial rise in phosphorylation following tissue activation with agonist and calcium (Figure 5.10), our latchbridge model shows the decrease that follows in accordance with experimental results. The decrease is due to a portion of latchbridges that formed during stimulation becoming phosphorylated. Once phosphorylated, myosin detaches and becomes dephosphorylated once more until a steady state distribution has been attained. Phosphorylation may be regarded as being directly proportional to the rate of crossbridge cycling. Therefore, the decrease in phosphorylation predicted by the 4 state model is consistent with its prediction of a decrease in shortening velocity following activation.

Figure 5.11b shows the phosphorylation against shortening velocity curves obtained by Kamm et al. [1]. Following activation and the rise to force maintenance, force is dropped to 1% of its steady state level. The shortening velocity and phosphorylation are recorded and the procedure is repeated at several times. It is found that there is a direct proportionality between phosphorylation and shortening velocity.

Hai and Murphy use this proportionality to infer values for the phosphorylation at each of the time points under consideration. The velocity at these points is then found and curves similar to those in Figure 5.11(a) are obtained.

An advantage of our model over that of [53] is that the rate of phosphorylation, $k_1$, is not required to be a decreasing function of time in order to achieve a decrease in phosphorylation. Regulatory mechanisms such as the influence of calcium on MLCK activity and phosphorylation is a built in feature of the model of Wang et al.. Figure 5.11a shows the relationship between phosphorylation and shortening velocity predicted by the latchbridge model when subjected to the protocol of Kamm et al..
Figure 5.10: In the absence of latchbridges, the steady state distribution is reattained rapidly as phosphorylated myosin detaches and returns to its dephosphorylated state. Since this is the only route back to steady state, it is impossible to obtain any decrease in phosphorylation following tissue activation with the 3 state + crosslinker model, in contradiction with the experimental results of [65] (permission is not required to use this image). This indicates that latchbridges are required for a complete representation of ASM.
Figure 5.11: In accordance with experimental results, our four state latchbridge model shows that shortening velocity decreases with phosphorylation. The data in Figure 5.11b is taken from [1]. Permission is not required to use Figure 5.11b.

5.2.6 Conclusions

We have seen that the latchbridge hypothesis can account for all features of latch state behaviour:

- Separation of force velocity curves obtained during the force maintenance period,
- Decrease in phosphorylation following a plateau in force,
- A direct relationship between phosphorylation and shortening velocity.

In spite of the fact that the crosslinker hypothesis can only account for the first of these properties, our investigation into the matter led to a deeper understanding of the dynamical features of the crosslinker model and some of the pathways which lead to the regulation of passive stiffness. The reconfiguration of crosslinkers to low-strain distributions following stretch/activation can contribute to reducing the intracellular load, thereby increasing the cells ability to resist applied loads. This can be seen as a high degree of separation in the force-velocity curves predicted by the 3 state + crosslinker model. In this sense, we conclude that cytoskeletal modelling can contribute to latch state behaviour but cannot account for all properties associated with it as the 4 state model can. In addition to this, we will find in an upcoming section that crosslinkers are essential for a complete representation of smooth
muscle mechanics due to the fact that their behaviour is regulated by a pathway separate to that of the crossbridge cycle.

For now, we continue to investigate mechanisms of passive stiffness generation within ASM cells. We have seen that latch state behaviour is directly related to passive events regulated by calcium initiated pathways. Prevalence of this behaviour can stiffen airways and exacerbate symptoms of airway hyper-responsiveness.

Along with the latch state, the adaptation of processes involved in passive stiffness generation to shorter working cell lengths can have detrimental effects relating to sustained airway narrowing. In the following section, we investigate mechanisms underlying the ability of passive stiffness to adapt in such a way.
5.3 Length Adaptation

The force generated by ASM determines the extent to which the muscle will shorten. Active and passive length-tension relationships can be shifted along the length axis by adapting the muscle to different lengths [93, 16, 131]. If this length adaptation occurs in vivo, the muscle would be able to generate more active force at a shorter length and could reduce the relaxation induced by tidal breathing because of the higher resting passive stiffness. Such adaptation could contribute to airway hyper-responsiveness through excessive airway narrowing.

Length adaptation to shorter working lengths occurs over the course of 8 days in the experiments on Naghshin et al. [93]. These experiments are set up to mimic in vivo conditions more closely than previous experiments that demonstrate length adaptation: tissue strips were incubated in a carbon dioxide controlled incubator during the adaptation process and stimulation was not applied to speed up the process. In contrast with this, in vitro muscle preparations at 4° stimulated regularly with electrical field stimulation (EFS) take around 30 minutes to adapt. The experiments at body temperature show that length adaptation could occur in-vivo. If this is the case, adaptation-induced shortening may be brought about through airway wall remodelling due to chronic asthma.

The shift in the length-tension curves is likely to be due to the restructuring of the cytoskeleton: the remodelling of the crosslinkers along with adaptation of the contractile proteins to changes in the degree of actin filament overlap. It is thought that the latter gives rise to the active length-tension curve but questions remain concerning the involvement of passive ASM structures in the length adaptation process: Could the freezing of a newly formed network of cytoskeletal proteins following the restructuring of the contractile machinery account for the passive length-tension curve?

We use a variation of the traditional crossbridge model of Hai and Murphy, developed by Donovan [33], to capture the active side of ASM mechanics. Another crossbridge population is introduced to the model of [129] to represent crossbridges that are unable to become phosphorylated and start cycling. This addition ensures that crossbridge binding sites are preferentially available within the actin filament overlap region and dependent upon the distribution of myosin filaments. This model can account for the shift in active length-tension curves following muscle adaptation at a given length but not their passive counterparts. We hypothesize that, by incorporating the passive crosslinker model into the system, the increase in passive stiffness with increasing length can be observed along with the shift in passive length-tension curves.
The model, given in [33] is as follows:

\[
\frac{\partial M}{\partial t} - v_a(t) \frac{\partial M}{\partial x} = -(k_1 + f_e(x))M + k_2 M_p + g_e(x) P + g(x) AM,
\]

\[
\frac{\partial M_p}{\partial t} - v_a(t) \frac{\partial M_p}{\partial x} = k_1 M - (k_2 + f_p(x) + f_e(x)) M_p + g_p(x) AM_p,
\]

\[
\frac{\partial AM_p}{\partial t} - v_a(t) \frac{\partial AM_p}{\partial x} = f_p(x) M_p - (k_3 + g_p(x)) AM_p + k_4 AM,
\]

\[
\frac{\partial P}{\partial t} - v_a(t) \frac{\partial P}{\partial x} = f_e(x) M_p + f_e(x) M - g_e(x) P,
\]

subject to the constraint \( M + M_p + AM_p + AM + P = 1 \), where \( v_a(t) = -\gamma_B dL/dt \) is the velocity of the actin filament relative to the myosin filament, defined to be positive during shortening.

The total force is again given by:

\[
F(t) = \kappa \int_{-\infty}^{\infty} x(AM_p + AM) dx + \kappa (L(t) - L_0) + \phi \int_{-\infty}^{\infty} \rho(y)n(y,t) dy.
\]

The experimental procedure of Naghshin et al. [93] for finding the length-tension curves is as follows:

- An inactivated muscle strip, set at its in-situ length \( L_{\text{insitu}} \), is shortened quickly to 84% \( L_{\text{ref}} \).
- It is then stretched every 5 minutes in 8% \( L_{\text{ref}} \) increments to 116% \( L_{\text{ref}} \).
• Passive force is recorded during each stretch and active force is measured 60s later as the peak force reached during a 10s imposition of electrical field stimulation.

• Another tissue strip is passively shortened and the procedure is repeated a few days later once it has equilibrated.

5.3.1 Length-Tension Curves

The inverted quadratic shape of the active length-tension relationship, shown in Figure 5.12, demonstrates that the muscle generates maximum force at its initial adapted length. When the adapted muscle is quickly shortened, the overlap of actin filaments within the cell moves away from its optimal value. This reduces the number of available binding sites and in turn reduces the active force generated at this length as less crossbridges have been formed. As the muscle is stretched back to its original length, the overlap of the filaments increases and a maximum number of crossbridges can bind, hence maximum force is generated.

![Active length-tension curves predicted by (a.) the experimental data from Naghshin et al. and (b.) the parallel model of Donovan. The active force generated by the muscle reaches a peak at the adapted muscle length.](image)

Meanwhile, when the initial distribution of crosslinkers is pushed towards the region of negative strain, little passive force is generated due to the inability of compressed crosslinkers to generate force. As the distribution of crosslinkers is pushed further towards the region of positive strain, the passive force generated increases considerably. This is due to the nature of the force function: the force produced by positively strained crosslinkers increases rapidly according to the magnitude of the strain imposed upon them. The distributions of crosslinkers following each stretch are shown in Figure 5.13 along with the crosslinker force...
function, $\rho(y)$, to explain the rise in passive stiffness. Following stretch, some crosslinkers will detach and move back to their steady state positions. The steep rise in passive stiffness due to this movement is shown in Figure 5.14.

The force generated by the crosslinkers will continue to increase until the distribution is pushed outside of the region of low detachment. At this point, a large force drop will occur. However, the stretches imposed upon the tissue are not large enough for such a force drop in this experiment.

5.3.2 Passive Stiffness Recovery

Naghshin et al. also investigated the passive force recovery following a large stretch in muscle length in inactivated tissue. The profile of the passive stiffness following a stretch of 30% initial muscle length is recorded over the course of 80 minutes: every 5 minutes, passive stiffness is measured as the peak force reached during a fast 5% stretch and release. The data is fitted to a three component exponential function: $a \exp(-bt) + c$, where we have found $b=-.0744/s$, $a=10.04$ and $c=2.836$ ($a$ and $c$ are constants).

Figure 5.15 shows the crosslinker model predictions to this protocol of Naghshin et al.. The experimental data and model predictions vary substantially in terms of the time course to recovery with the model predicting a much faster return to a baseline force. This may be for a number of reasons: 1.) Naghshin et al. measure the passive stiffness every 5 minutes, making it difficult to see how fast force decreases in the initial period following stretch, 2.) a stretch of 30% is likely to irreversibly damage mouse ASM tissue so the model was not fitted to such data 3.) the passive stiffness recovery rates in mouse and rabbit tissue are likely to be different.

Another experiment designed to study the rate of passive stiffness recovery following stretch was performed by Sharon Bullimore’s group. The (unpublished) experimental data obtained in response to a stretch of 10% reference length in mouse ASM tissue is shown in Figure 5.16 along with the crosslinker model predictions. After the stretch, there is an immediate drop in force as highly strained crosslinkers detach rapidly. The transient force recovery that ensues is due to the remaining attached crosslinkers tending towards positions of low strain. The stress relaxation predicted by the model is best fitted with a multi-exponential like that of Naghshin et al. rather than a power law found by several experimental studies [6, 127, 11]. Although we have found that both a power law and a multi-exponential fit the model predictions and data well over the time course of the experiment, Figure 5.17 demonstrates that the model predictions of the force response to
Figure 5.13: The shift in the crosslinker distribution with each stretch gives rise to the steep rise in passive stiffness observed by Naghshin et al.

the same stretch and hold over a longer time period are far better represented with a multi-exponential rather than a power law fit. While it is true that some experimental data exhibits power law behaviour over several decades, eventually the passive force generated must flatten out, it cannot continue to decrease indefinitely.

Figure 5.14: Passive length-tension curves predicted by (a) the crosslinker model and (b) the experimental data from Naghshin et al.. The passive force curve continues to increase beyond the adapted length.
Figure 5.15: Passive stiffness recovery with time: (a) model results and (b) experimental data from Naghshin et al. The tissue is stretched by 30% of its original length and the passive force recovery is recorded over a period of time. Passive stiffness recovers slowly back to steady state following an initial large decrease.

Figure 5.16: Passive stiffness recovery following a 10% stretch: experimental data of Sharon Bullimore (unpublished) and model predictions. Both the data and model predictions can be represented with a power law or a multi-exponential fit.
Figure 5.17: Passive stiffness recovery following a 10% stretch: over longer timescales, a multi-exponential best represents the model predictions.

5.4 The Regulatory Mechanisms of Passive Stiffness

We have seen that our two hypotheses can account for the separation of the force-velocity curves observed by Dillon et al., indicating that crosslinkers as well as latchbridges are likely to contribute to latch state behaviour. In this section, we test our hypotheses against the experimental results of Raqeeb et al. [106]. In these experiments, the redevelopment of active force and passive stiffness is measured after softening induced by length oscillations. This is done under a variety of experimental conditions to examine the dependence of the forces upon different chemical stimuli including calcium and MLCK.

The experimental set-up is as follows: electric field stimulation (EFS) is applied for 9 seconds and the force is measured. Raqeeb et al. define the active force to be the maximum total force reached during EFS. To quantify the passive stiffness, the tissue is stretched and held for 5s, 60s after the EFS has been removed. The maximum total force minus the basal force during this stretch-and-hold period is defined to be the passive stiffness. Active and passive force measurements are recorded in this way and they are taken to be the steady state values which are used to normalize all subsequent measurements.

Fluidization is then induced by applying length oscillations for 60 seconds at a frequency
of 2 Hz and amplitude of 5% of the reference length. Immediately after this, the passive force is measured, followed one minute later by the active force.

EFS is imposed at 5 minute intervals for 30 minutes after the length oscillations and the active and passive forces are measured in each interval as described above. This process is illustrated in Figure 5.18.

The experimental results of Raqeeb et al. are given in Figures 5.19 and 5.20. They have found that the recovery of passive stiffness is reduced in the absence of calcium but is unaffected by the absence of MLCK. Active force is abolished in both cases. The study into how passive stiffness is regulated can be considered an extension of our previous study into latch state behaviour. We now want to learn more about the degree to which passive stiffness due to crosslinkers or latchbridges is regulated under the various experimental set-ups considered by Raqeeb et al..

We have previously found that latchbridges are essential for a complete representation of smooth muscle mechanics: without them it is not possible to obtain a decrease in phosphorylation following muscle stimulation. However, we also found that crosslinkers can contribute to the latch state under certain circumstances. In this section we will show that crosslinkers too are essential for a complete airway smooth muscle model.
Figure 5.18: The experimental procedure performed in Raqeeb et al. [106]. Active force is taken to be the peak force reached during EFS. A stretch of 5% Lref is imposed 1 minute after EFS. The peak force reached during the stretch is taken to be the passive force. Permission is not required to use this image.
The Regulatory Mechanisms of Passive Stiffness

Figure 5.19: Experimental data reproduced from [106]: In the absence of calcium, the active force is abolished while the passive stiffness is reduced. If latchbridges were the only source of passive stiffness, the passive force would also be abolished.
The Regulatory Mechanisms of Passive Stiffness

Figure 5.20: Experimental data reproduced from [106]: The recovery in passive stiffness is dependent upon some calcium initiated pathway apart from the one involving MLCK. In addition to this, passive stiffness can recover independently of active force. The experiment is performed in a control environment as well an MLCK free environment. In the absence of MLCK the active force is abolished while the passive stiffness is unaffected.
5.4.1 The Latchbridge Hypothesis

If we assume that passive stiffness is solely due to the presence of slowly cycling, dephosphorylated crossbridges (latchbridges), the results of Raqeeb et al. cannot be satisfied by the four state mathematical model as it stands. During electrical field stimulation (EFS), cycling crossbridges move into the latchbridge pool. Therefore, the maximum active force reached will be proportional to the passive force measurement.

Raqeeb et al. demonstrate that the inhibition of MLCK does not affect the recovery of passive force. In the absence of MLCK, phosphorylation of myosin cannot take place. Thus, crossbridge cycling and the subsequent development of latchbridges is not possible: zero active force due to the inhibition of MLCK results in zero passive force. Therefore, if we assume that dephosphorylated myosin cannot bind to actin, the assumption that latchbridges alone are the source of passive stiffness cannot account for these experimental results.

If dephosphorylated myosin could attach to actin, then, in the absence of MLCK or calcium, we would essentially have the crosslinker model but with much slower cycling rates. In theory, it is possible that this could explain how MLCK does not affect passive stiffness but quite a lot of fine-tuning would be involved to find an attachment rate function: the level of latchbriges present immediately before the measurement of passive stiffness in the presence of MLCK should be similar to that present beforehand in the absence of MLCK. Other rates in the model would have to be modified too because currently the rate of passive stiffness recovery following EFS takes longer than 1 minute. Experimental data does not exist to construct a quantitative model of this kind.

In the following sections, we assume that crosslinkers are the source of ASM passive stiffness. In this way, the passive force measurements are completely independent of crossbridge cycling. There is evidence that calcium regulated Rho sends mechanical signals to crosslinking proteins to in-turn regulate their dynamics [123]. A more detailed description of this process is given in Section 5.2. As mentioned in Chapter 3, we choose to modify $\beta$, the crosslinker detachment function, such that crosslinkers have the ability to stay bound at higher strains in the presence of calcium. Specifically, we make $\beta$ calcium dependent to model the assumption that crosslinkers become stiffer with increasing calcium. The way in which this is modelled is depicted in Figure 5.21.
5.4.2 The Crosslinker Hypothesis

Here we assume that the movements of crosslinkers give rise to ASM passive stiffness and that they detach according to the level of calcium present within the cell. The solutions of the three state + crosslinker model in each of the three experimental set-ups is given in Figures 5.22 and 5.23. There is no dependence of MLCK upon crosslinking activity and the crosslinkers operate independently of the crossbridges. Hence, active force is abolished while passive force is unaffected. In the absence of calcium, MLCK cannot be activated so the active force is again abolished. With the calcium dependent crosslinker detachment rate function the recovery in passive stiffness is reduced in the absence of calcium. Thus, all the results of Raqeeb et al. are satisfied with this three state model coupled with the calcium dependent crosslinker model.

Figure 5.21: The crosslinker detachment function, $\beta$ is now calcium dependent. It has been shown that passive stiffness recovery following length oscillations decreases in the absence of calcium. If we assume that crosslinkers become stiffer with increasing calcium concentrations, the results of Raqeeb et al. can be obtained.
(a) Model Prediction: Active Force with Ca\(^{2+}\) inhibition.

(b) Model Prediction: Passive Stiffness with Ca\(^{2+}\) inhibition.

Figure 5.22: The active force is abolished while the passive force recovery is reduced in the absence of calcium in accordance with experimental results.
Figure 5.23: Active force is abolished in the absence of MLCK as phosphorylation cannot take place. Passive stiffness recovery is unaffected by the absence of MLCK but is reduced in the absence of calcium. This is due to the calcium dependent crosslinker detachment rate function we have employed.
5.5 Conclusions

Understanding mechanisms behind passive ASM events is crucial to enhancing our knowledge regarding the pathogenesis of airway hyper-responsiveness and asthma. Passive events play major roles in sustaining contraction (latch) and have the ability to adapt to large changes in length which may lead to sustained contraction of excessively narrowed airways. The focus of this chapter was to determine which filaments are likely to be involved in bringing about these detrimental states and how their behaviour is regulated.

We have found that latchbridges are needed to capture all the features of latch state behaviour but have also demonstrated that crosslinkers may contribute to properties associated with the latch phenomenon. Furthermore, crosslinkers are required to reproduce the shape of the passive length tension curve of Naghshin et al..

To learn more about how these passive events are regulated, we studied the experimental observations of Raqeeb et al.. It was found that passive stiffness is unaffected by the absence of MLCK but is reduced in the absence of calcium. This indicates that passive and active mechanisms are regulated via separate, calcium initiated pathways. From this, we concluded that the latchbridge hypothesis could not account for the recovery in passive stiffness demonstrated by Raqeeb et al. and conducted a deeper examination of crosslinker mechanics and their sensitivity to calcium.

The rigidity of smooth muscle actin filament gels containing alpha-actinin has been shown to increase with calcium concentrations [14]. We incorporated this finding into the crosslinker model by employing a calcium dependent crosslinker detachment rate. Thus, as calcium increases, crosslinkers are able to remain attached at higher strains. We have found that, with this calcium dependent crosslinker detachment rate function, the regulation of crosslinker generated passive stiffness is calcium dependent and MLCK independent, in accordance with the experimental results of Raqeeb et al.. Together with the result that crosslinkers can account for latch state behaviour, we conclude that crosslinkers are likely to contribute substantially to the passive stiffness generated by airway smooth muscle cells.

This hypothesized calcium dependency is empirical and fitted, rather than directly based on data. In the future we hope to take a more quantitative approach by conducting a thorough analysis of experiments performed on passive biological tissues. This may begin with a mathematical representation of the pathway of mechanical and chemical events that lead to cytoskeletal remodelling illustrated in Figure 5.8.

Using the parallel model, we have been able to account for several fundamental properties of ASM such as the latch state, the direct relationship between shortening velocity and
phosphorylation and the shift in the passive length-tension curve following muscle adaptation. We have learned about the sub-cellular filaments underlying these properties and have determined the pathways in which they are likely to be regulated.

However, the parallel model is unable to capture several key experimental observations regarding the force-length relationship of ASM. This leads us to question whether this approach may be too simplistic and instead we take a constitutive approach to combining the active and passive force generating mechanisms.

The past few years has seen the growth in momentum of the hypothesis that the rheological connection between ASM and soft glassy materials may explain the enigma surrounding deep inspirations. Specifically, the phase transitions exhibited by materials such as toothpaste is similar to the fluidization - re-solidification process of ASM in response to imposed shear. We hypothesize that employing a constitutive formulation used to capture the behaviour of such materials may shed light on why the bronchodilatory effect of deep inspirations fails in asthmatics.
A new way of combining active and passive sides of airway smooth muscle contraction

6.1 Introduction to complex fluids

Up to this point, we have combined the crosslinker and crossbridge models in parallel to obtain a representation of fully activated ASM contraction. However, a parallel or series approach to combining the models may be too simplistic; we want to know if an alternative approach to combining these models may give similar (or better) reproductions of experimental data. Specifically, we want to learn more about how passive and active events within ASM cells interact. Another advantage of using this different modelling approach is that it can teach us more about the system we are modelling. It forces us to think of the system in a different way which can only be a positive thing.

In this chapter, we focus on the analogy between ASM and complex fluids, or soft glassy materials. These are materials that fluidize or exhibit strong shear thinning once a certain yield stress has been exceeded. Their viscosity decreases substantially and they flow. Both ASM and complex fluids, such as toothpaste, are in solid-like, elastic states, under low shear but transition towards fluid-like states under shear. It is thought that ASM does this through the restructuring of its contractile apparatus [90] as well as the remodelling of its actin cytoskeleton [35].

Factors which may affect the asthmatic airway response to stretch include the heterogeneity of airway narrowing [55], increased airway closure [67] along with abnormalities in
the restructuring of the cells contractile and cytoskeletal apparatus. In the latter case, a move to a ‘frozen’ configuration of crosslinkers and latchbridges may lead to sustained airway narrowing, characteristic of severe asthma attacks [98, 96]. Hence, studying the glassy dynamics of the cell may shed light on mechanisms underlying several anomalies, some of which have been reported as far back as 1868 [110].

For instance, it has been found that when non-asthmatics inhale bronchoconstrictors, taking a deep inspiration (DI) will help them to overcome subsequent bronchospasm. This DI will relax the airway, most likely due to the fluidization that is taking place. If they do not take a DI, bronchospasm will ensue. Asthmatic airways do not have the ability to fluidize or relax under the same circumstances.

Another finding is that when non-asthmatics refrain from taking a DI, within 15 minutes they will experience airway hyper-responsiveness to a degree that is indistinguishable from that of asthmatics. This rules out that allergies or abnormal tissue are the cause of the failure of this bronchodilatory effect. Deepening our investigation into cytoskeletal remodelling and crossbridge cycling may indicate the degree to which they could be responsible for this behaviour. Studying the glassy dynamics of cell should also provide us with a new conceptual framework from which novel pathways and phase transitions may be explained or discovered.

In this chapter, we look to the theory of complex fluids as a way of modelling smooth muscle fluidization in response to shear as well as the interactions between passive and active smooth muscle components. We will explore a constitutive model formulation used to capture the behaviour of complex fluids, and apply it to ASM experimental protocols. We want to know if we can use the transition between solid and fluid phases exhibited by complex fluids theory as a way of combining our active and passive models. The complex fluids model of Weber et al. used in this study is given in [132]. In the coming sections, we examine the constitutive model predictions of the inactivated tissue response to imposed length perturbations and find that it can adequately account for the proportionality between fluidization and the extent of imposed shear. We move on to explore the length-tension relationship of activated tissue and find that the force-length loops predicted by the constitutive formulation display a transient reduction in force with time as well as loop shapes that are similar to those observed experimentally compared with those predicted by the parallel model. This new approach also accounts for the reduction in mean force following a deep inspiration.

To truly determine whether this approach could replace the parallel configuration, we study the model’s predictions of the dynamic response to varying chemical stimuli.
Several physical models have been formulated to describe muscle mechanics. Zahalak [139] use a spring in series with a force generator to describe skeletal muscle contraction. Meyer et al. [87] use a spring in parallel with a series arrangement of a dashpot and spring to capture skeletal muscle contraction. Syong and Seow [122] model smooth muscle with two springs in series, each representing the actions of crossbridges and crosslinkers, with a force generator in between them. Bates et al. [10] also models smooth muscle with a more complex system of two Maxwell bodies in parallel, one in series with a force generator.

The model we will study here is simply a spring in series with a dashpot and is typical of that found in the complex fluids literature. We choose this for its simplicity and its ability to represent the viscoelasticity of ASM tissue. However, the crucial difference between our model and previous physical models is that the spring constant and dashpot resistance depend upon the level of tightly bound crossbridges, latchbridges and crosslinkers, as defined by the active and passive models described in Chapter 3.

### 6.2 Complex Fluids

Complex fluids are fluids that are biphasic. This means that they are composed of solid-like and fluid-like elements. At rest, they consist of a suspension of particles that are clustered together to form a highly viscous compound, like honey. When shear is imposed upon them, they fluidize, like applying force to toothpaste makes it runnier.

In the same way as complex fluids, ASM fluidizes when shear is imposed upon it and it fluidizes to an extent that is proportional to the level of shear applied. To make it clear why the theory of complex fluids is applicable to smooth muscle, consider the same set-up in a strip of inactivated tissue. At low levels of applied stress, crosslinkers are stretched or compressed to lengths which generate relatively little resistive force. As the applied stress increases, the crosslinkers are pushed further away from their initial steady state distribution. As they become increasingly strained, the resistive force they generate increases rapidly.

In order for the steady state distribution to be reattained, these crosslinkers break and gradually reattach at low strains. During this period, a fluid-like state is observed as the initial level of bonds has decreased substantially, thereby reducing the viscosity of the muscle.
6.2.1 Experimental Observations of Complex Fluid Behaviour

To demonstrate how the constitutive model works to describe the behaviour of complex fluids, we look at the experiments modelled in [132]. A linearly increasing or decreasing ramp in stress is imposed upon the complex fluid over a time of 300 seconds, Figure 6.1(a). Plotting the stress versus the absolute value of the velocity in 6.1(b), we can see how the fluid moves between its two states: when the stress passes a critical level, the rate of deformation increases exponentially and the material exhibits a fluid-like state.

When the applied stress is decreased, the rate of deformation stabilizes to a level higher than that observed at the initial point. This shows the material is closer to a fluid-like state following applied stress and indicates the presence of structures within the material that affect its rheology on a slower time-scale.

It is worth noting that in order to obtain the separation of the shear-stress curves at low stress, the up ramp and down ramp were fitted separately. The shear elastic modulus, $G$, was modified so that it was approximately an order of magnitude larger in the up-ramp case.

![Figure 6.1](image-url)  
(a) Stress is prescribed according to the linear ramp shown in Figure 6.1a. The shear rate exhibited by the fluid is dependent upon the branch of prescribed stress under consideration. Image taken from [132]. Permission to use this image has been granted with licence number 3643530392163.
6.3 Complex Fluid Model Formulation

As mentioned, the complex fluids model studied in this chapter is derived from a spring and a dashpot in series. The elastic modulus of the material is given by $G$ and the concentration of the solid phase of the material is given by $\Phi$. With a spring constant given by $G/\Phi$ and a damper with viscosity $\eta$, we have that

$$\sigma = \eta \dot{x}_1 = \frac{G}{\Phi} x_2,$$

where $x_1$ is the displacement of the damper and $x_2$ is the displacement of the spring. We differentiate with respect to time to obtain:

$$\dot{\sigma} = \frac{G}{\Phi} \dot{x}_2 - \frac{G}{\Phi^2} \dot{\Phi} x_2$$

(6.1)

$$= \frac{G}{\Phi} (\dot{\gamma} - \frac{\sigma}{\eta}) - \frac{1}{\Phi} \dot{\Phi} \sigma$$

(6.2)

$$\frac{\Phi}{G} \dot{\sigma} = -\frac{\sigma}{\eta} + \dot{\gamma} + \dot{\Phi} \frac{\sigma}{G}$$

where

$$\eta = 1 + \frac{\sigma y G}{\epsilon + |\dot{\gamma}|}$$

and

$$\Phi = c_1 \int_{-1}^{1} n dx + c_2 \int_{0}^{1} (AM + AM_p) dx$$

We take the shear elastic modulus, $G$, to be constant throughout and set $G = 1$. We tailor the concentration of the solid phase, $\Phi$, and spring constant to ASM behaviour. $\Phi$ is taken to be a linear combination of the level of tightly bound crosslinkers, crossbridges and latchbridges, $n(x,t)$, $AM_p(x,t)$ and $AM(x,t)$ respectively at strain $x$ and time $t$. The limits in these integrals refer to the regions in which crosslinkers and crossbridges can bind. $\dot{\Phi}(t)$ represents differentiation of $\Phi(t)$ with respect to time.
In physical terms, this means that with a large number of elastic elements or low strained bonds, the spring constant, \( G/\Phi \), is small giving an elastic material. When the absolute value of the velocity, \( |\dot{\gamma}| \), increases, the viscosity, \( \eta \), decreases. The number of elastic elements, \( \Phi \), decreases and the spring becomes redundant. In other words, with high magnitudes of velocity, the material fluidizes.

The parameter values are as follows: \( c_1 = 0.2, c_2 = 1, \epsilon = 10^{-5} \) (all dimensionless) and \( \sigma_y = 45(\text{mN}) \).

Interestingly, the derivative of \( \Phi \) with respect to time is left out of the complex fluids model given in [132]. Figure 6.3(a) shows the stress strain curve obtained in response to the linear ramp in stress depicted in Figure 6.1(a), when the derivative of \( \Phi \) is included in the constitutive equation. Clearly the omission of \( \dot{\Phi}(t) \) does not make a huge difference to the results of the paper, however, omitting this derivative has drastic consequences to the results our ASM tissue strip experiments (see Figure 6.5).

This complex fluids approach could be a stepping stone to simplifying the model (by approximating \( \Phi \)) but this was not the original aim. We used the complex fluids model given in [132] which is typical of that found in the complex fluids literature. The spring constant is dependent upon the level of tightly bound populations and the damper resistance was a typical representation of the viscosity. Since the model we employ is composed of two fundamental elements, a spring and a dashpot, we reason that it is in essence a constitutive formulation inspired by similarities with complex fluids.
Passive ASM

Eventually, we want to see if we can use the theory of complex fluids to model the forces exerted by fully activated ASM. In order to find out if we can do this, we first investigate the outcome in the simplest case - can a complex fluids representation model the forces exerted by inactivated/passive ASM?

Passive ASM processes, such as cytoskeletal remodelling induced by the reconfiguration of crosslinkers, are regulated via different chemical and mechanical stimuli to active processes such as crossbridge cycling. However, such remodelling may act to decrease the intracellular load against which the muscle shortens. This may lead to sustained contraction or even excessive contraction as crossbridge cycling is less perturbed.

This reconfiguration of crosslinkers is captured with the model of Donovan et al. [35] for passive ASM tissue. The parameters for the constitutive model were found using the Markov Chain Monte Carlo method, fitted against the data obtained from an experiment of Sharon Bullimore described in [35]. The procedure is as follows:

1. A strip of inactivated ASM is stretched by 17% and left to relax for 10 seconds.
2. The length of tissue is oscillated at varying fractions (5%, 10% and 20%) of the pre-stretch amplitude for 60 seconds.
3. The tissue is left to re-equilibrate.

It is found that the extent of fluidization after the oscillations is proportional to the amplitude of the oscillations.

When we put the complex fluids model formulation derived above through this experimental regime, the force profiles predicted are similar to those obtained experimentally as well as those predicted by the crosslinker model of Donovan et al.. The original force profiles obtained with the crosslinker model are shown in Figure 6.4 along with the experimental data and the complex fluids model prediction for the passive force.

When the tissue is stretched, the concentration of the solid phase (the level of low strained bonds) decreases. The spring constant, $G/\Phi$, increases and the spring becomes stiffer. Meanwhile, as the velocity increases, the viscosity decreases. The tissue fluidizes giving rise to the large drop in force following the initial stretch.
Figure 6.4: The experimental data of Sharon Bullimore along with the crosslinker model both agree, qualitatively, that inactivated tissue fluidizes to an extent proportional to the level of stress applied to it. The constitutive model formulation can reproduce this property.
6.4.1 The Importance of the Derivative of $\Phi$ in the Constitutive Formulation

As mentioned, the constitutive formulation given in [132] does not take into account the rate of change of the concentration of the solid phase. We briefly look at the predictions of the model of Sharon Bulimore’s experiment without (Figure 6.5(a)) and with (Figure 6.5(b)) the term $\Phi(t)$ in the constitutive equation. Although both models predict that fluidization is proportional to the level of shear applied, when $\Phi(t)$ is omitted there is absolutely no fluidization following the initial stretch.

![Graph](image1.png)  ![Graph](image2.png)

(a) Constitutive Formulation without $\Phi$ derivative.  (b) Constitutive Formulation with $\Phi$ derivative.

Figure 6.5: The original constitutive equation does not take into consideration the derivative of $\Phi(t)$ with respect to time. In this experiment, there is a huge difference in the absence of $\Phi'(t)$, with absolutely no fluidization following the initial stretch.

6.5 Approximation for $\Phi$ in Isometric Stretch and Hold Procedures

Our aim in this section is to simplify the full constitutive formulation considerably by finding an approximation for the concentration of the solid phase, $\Phi$, in order to avoid solving for the crosslinker distribution at each time step. This will be particularly useful in speeding up parameter searches as the constitutive ODE and the approximation for $\Phi$ will be the only equations to solve. The procedure we have developed to find this approximation works well for stretch and hold procedures that require a large spatial domain. Computation
time is reduced substantially as the number of ODE’s to solve has been reduced to just 1. However, the approximation is unsuitable for experiments in which the tissue length is constantly perturbed. In this section, the isometric stretch and hold procedure of Sharon Bullimore is outlined. This is unpublished data of an inactivated tissue strip subject to fast stretches of 5%, 10% and 20% reference length. Following each stretch, the tissue is left to re-equilibrate for 1000 seconds. The experimental data is shown in Figure 6.6(a). We will compare our approximation for Φ with this data and the predictions of the crosslinker model.

We break up the spatial domain of the crosslinker distribution into two regions: one in which crosslinker attachment is possible (−1 ≤ x ≤ 1) and one in which it is not (−D ≤ x < −1 and 1 < x ≤ D), where −D ≤ x ≤ D defines the domain. In order to find Φ = \int_{-1}^{1} ndx, we must find the integral over the crosslinker distribution in each of these regions. Since the velocity in stretch and hold procedures is zero, we can integrate the crosslinker model of Donovan et al. (Equation 3.5) over −1 ≤ x ≤ 1 to obtain an ODE for Φ.

\[
\frac{d \Phi}{dt} = \left(1 - \int_{-D}^{-1} ndx - \int_{1}^{D} ndx - \int_{1}^{D} \alpha(x) dx - \int_{-1}^{1} \beta(x) ndx\right) \int_{-1}^{1} 2 \alpha_1 dx - \int_{-1}^{1} \beta(x) ndx, \tag{6.3}
\]

where \(\alpha(x)\) and \(\beta(x)\) are the attachment and detachment functions respectively and \(x\) is the crosslinker strain. Since the attachment function \(\alpha(x)\) is taken to be

\[
\alpha(x) = \begin{cases} 
\alpha_1, & -1 < x < 1 \\
0, & \text{otherwise}
\end{cases}
\]

Equation 6.3 becomes

\[
\frac{d \Phi}{dt} = \left(1 - \int_{-D}^{-1} ndx - \Phi - \int_{1}^{D} ndx\right) \int_{-1}^{1} 2 \alpha_1 dx - \int_{-1}^{1} \beta(x) ndx. \tag{6.4}
\]

Outside the region of attachment, the crosslinker distribution is given by:

\[
\frac{\partial n}{\partial t} = -\beta(y)n.
\]

We can solve this equation using integrating factors to find that

\[
n(x,t) = n(x,0)\text{Exp}(\beta(x)t) \text{ for } -D \leq x < -1 \text{ and } 1 < x \leq D.
\]
Substituting these expressions back into 6.4 and using the approximation

\[ \int_{-1}^{1} \beta(x)n(x,t)dx = \hat{\beta} \int_{-1}^{1} n(x,t)dx = \hat{\beta} \Phi, \]

where \( \hat{\beta} = \int_{-1}^{1} \beta dx \). We find that \( \Phi \) is the solution of

\[
\frac{d\Phi}{dt} = (1 - \int_{-D}^{-1} n(x,0) \exp(-\beta(x)t)dx - \Phi - \int_{1}^{D} n(x,0) \exp(-\beta(x)t)dx) \int_{-1}^{1} 2\alpha_1 dx - \hat{\beta} \Phi. \tag{6.5}
\]

Figure 6.6b shows the crosslinker model predictions of the passive muscle force response to instantaneous stretches of 5\%, (blue), 10\% (green) and 20\% (red) reference length, here taken to be unity. The initial crosslinker distributions are shifted along the strain axis by \( \gamma \Delta L \), where \( \Delta L \) is the degree of stretch (0.05, 0.1 or 0.2). Since the force is recorded immediately after stretch, there are no predictions of the force prior to stretch.

Figure 6.6c shows the constitutive model predictions of the passive force response under the same conditions as above. Here the approximation of \( \Phi \) has been used. While the time course of the force recovery agrees reasonably well with the data, using the approximation means the model cannot predict an increase in basal force with increasing stretch amplitude. Nonetheless, the approximation cuts down computation time significantly, making it easier to perform parameter searches with the Markov Chain Monte Carlo method [102, Chapter 15]. Its simplicity also makes it suitable for analysis of the bifurcations of the system to be carried out which may give rise to a deeper understanding of the phase transitions of the system.
Figure 6.6: A strip of tissue is stretched by varying percentages and left to equilibrate for 1000 seconds. The experimental data of Sharon Bullimore is shown in Figure 6.6a. The constitutive and crosslinker model predictions of passive force decline are shown in (b) and (c) respectively for the fist 100 seconds. An approximation for \( \Phi \) has been used in the constitutive model, reducing the number of ODEs to be solved at each time step from 1001 to 1.
6.6 Activated ASM

Now we want to see if the complex fluids representation can model the forces exerted by fully activated ASM. In this section, we obtain the constitutive model predictions for ASM force response to tidal length oscillations and deep inspirations.

6.6.1 The Isometric Case

Force-Length Loops

ASM is likely to be involved in the pathogenesis of airway hyper-responsiveness, contradictory to the initial view that airway inflammation had sole responsibility. With this in mind, why does the bronchodilatory effect fail in asthmatics? One possible answer stems from the reduction in stiffness generated by ASM in response to tidal oscillations. This may be a mechanism of avoiding airway responsiveness in vivo resulting from the reorganization of the cytoskeletal and contractile filaments to decrease contractility and stiffness.

However, since cytoskeletal remodelling takes place over a much longer duration of time than those considered in such experiments, it is unlikely that their behaviour causes much of a reduction in cell stiffness. Another possibility is that the crossbridge cycling rate is reduced as the length oscillations progress thus resulting in the decrease in stiffness and contractility.

It has been shown that even very small tidal oscillations can reduce airway stiffness and it has been suggested that this may come about through the regulation of crossbridge cycling. However, no mechanism has been identified to explain why this can fail in asthmatics. It may be that advential thickening observed in asthmatics acts to diminish the tidal forces to which the muscle is subjected. On the other hand, a reduction in load against which the muscle shortens, brought about by a prevalence of latch state behaviour, may be responsible.

It is perhaps most likely that many events contribute to the cells response to tidal length oscillations. This serves to highlight the benefits of a constitutive formulation: examining the response at a higher level of structural organisation rather than investigating the behaviour of micro-filaments and signalling pathways at the sub-cellular level provides us with an explanation of how passive and active events within the cell interact.

We now employ a soft glassy materials approach to modeling ASM behavior in response to tidal oscillations. This will allow us to study the stiffness (spring constant) and viscosity of the tissue over the duration of length oscillations and may give strength to the arguments discussed above.
The experimental procedure of Bates et al. [10] is as follows:

1. A strip of smooth muscle tissue is activated and allowed to equilibrate for 1 minute.

2. The length of the tissue strip is oscillated with an amplitude of 2%Lref and a frequency of 2 Hz.

3. Force is recorded throughout the experiment and force against length curves are obtained.

The results are shown in Figure 6.7 with (a) the crossbridge and crosslinker models in parallel and (b) the complex fluids formulation. When the parallel model is subjected to this protocol, it produces force-length loops that do not have the characteristic banana shaped reduction in stiffness over time. However, curves similar to the classical force-length loops can be obtained with the constitutive formulation under the same conditions. The damper is the cause of the hysteresis in the complex fluids model, without the damper there is none. This is a feature of complex fluids models which have similar properties to ASM. In the case of ASM, the damper could represent bonds within the tissue becoming more compliant as length oscillations progress or it may be a way of incorporating the effects of extracellular constituents like collagen into the tissue model.

The banana shaped loops come from the the spring constant $1/\Phi$ which has similar non-linearity. There is a little hysteresis in $\Phi$ which increases if the dephosphorylation rate is calcium dependent but it is not much. The force generated by the muscle decreases with ongoing imposed shear leading to the transient reduction in force observed experimentally and with the constitutive formulation. This not only indicates that a soft glassy materials approach may provide a more realistic representation of ASM mechanics but it can also pin-point the underlying mechanisms behind the reduction in stiffness and contractility.

In Figure 6.8, the time profile of the mean of the spring constant over each force-length loop is plotted. The reduction in spring constant indicates the muscle is becoming less stiff which in turn indicates a reduction in the rate of crossbridge cycling, consistent with the predictions of Fredberg [44].

However, the constitutive formulation is not able to predict the experimental finding that peak isometric force changes little with amplitude of oscillations. This is the case with the force-length loops produced by the parallel model but not the constitutive model in which the peak force changes substantially with the amplitude of the imposed oscillations, Figure 6.9. As suggested by Bates et al., this may be due to the non-linearity of the stiffness
of ASM tissue. Thus, modifying the shear elastic modulus, $G$, accordingly may serve to rectify this. However, such modifications are now beyond the scope of this thesis.
Figure 6.7: The length of an activated tissue strip is oscillated with an amplitude of 2%Lref and a frequency of 2 Hz. The experimental data (b) is shown along with the parallel (a) and constitutive model (c) predictions. Permission is not required to reproduce Figure 6.7b.
Figure 6.8: The mean of the spring constant \( \frac{G}{\Phi} \) over the course of each force-length loop decreases with time, indicating a reduction in stiffness due to a reduction in crossbridge cycling.

Figure 6.9: The force-length procedure of Bates for 1%, 2% and 4% of \( L_{ref} \) tidal oscillations of frequencies 2 Hz in fully activated tissue. Permission is not required to use this image.
Deep Inspirations

We have also tested the constitutive model against the deep inspiration procedure described in Bates et al. [10]. In the experimental procedure, oscillations of amplitude 2% of reference length and frequency 2 Hz are imposed upon the tissue for 60 seconds. A deep inspiration (DI) of amplitude either 5%, 15% or 25% reference length is then applied. The duration of the DI is 1 second after which oscillations were again imposed for a further 60 seconds. The experimental results are shown in Figure 6.10.

It was found that the extent of fluidization observed after the DI was proportional to the strength of the DI. For larger DI’s, the mean of the force oscillations following the DI did not recover to the pre-DI level for the remainder of the oscillations.

The model of Bates can demonstrate the former property but not the latter. They suggest that this is due to the absence of some mechanism in the model that is capable of causing a temporary reduction in the ability of the ASM to generate force.

On the other hand, the constitutive model does show a decrease in mean force following large amplitude DI’s, see Figure 6.11. This is due to the nature of the plasticity of the spring: following a large DI, the level of low strained bonds within the cell does not recover to its pre-DI level within 60 seconds. Since these bonds contribute to the force generating capacity of the cell, this finding may reveal the absent mechanism in the model of Bates et al..
Figure 6.10: Experimental Data of Bates et al. [10]. In response to a deep inspiration there is a transient decrease in oscillatory force along with a sustained amplitude dependent decrease in force and stiffness. This reduction in force is dependent upon the magnitude of the deep inspiration. Permission to use this image is not required.
Figure 6.11: The procedure of Bates et al. [10] used to investigate the effects of deep inspirations.

6.7 The Isotonic Case

The next step is to see if the complex fluids formulation can reproduce the results of a completely different experimental set-up. We now turn our attention to an experiment
described in Wang et al. [129] and discussed in Chapter 3 in which force is measured in response to changes in calcium and agonist concentrations. The tissue length is not controlled as it has been in previous experiments.

The crucial difference between ASM and complex fluids is that complex fluids do not react to changes in concentrations of agonist or calcium. As such, the outcome of this section will provide us with a true indication of whether we can apply the theory of complex fluids to airway smooth muscle phenomena. The concentration of the solid phase and the shear elastic modulus now depend on the level of calcium and agonist due to the dependence of crossbridge populations upon these substances.

Below the method of calculating the shortening velocity is described. In previous sections, \( \Phi \) has been defined as the integral over the attached populations from \(-1 \leq x \leq 1\). To avoid numerical errors in \( \Phi \) caused by the sharp ‘edge’, here we define

\[
\Phi = c_1 \int_{-\infty}^{\infty} q_1(y)ndy + c_2 \int_{-\infty}^{\infty} q_2(x)AMdx + c_2 \int_{-\infty}^{\infty} q_2(x)AM_pdx
\]

where

\[
q_1(x) = \text{Tanh}[20(x + 1.02)] - \text{Tanh}[20(x - 1.02)] \\
q_2(x) = \text{Tanh}[20(x + 0.02)] - \text{Tanh}[20(x - 1.02)]
\]

We know that the derivative of the force, \( \dot{\sigma} \), is proportional to the shortening velocity of the tissue, \( \dot{\gamma} \). As in Wang et al. we assume that, given a linear spring to represent the ASM generated force, the rate of change of force, \( \dot{\sigma} \), is directly proportional to the velocity, \( \dot{\gamma} \) according to \( \dot{\sigma} = -P\dot{\gamma} \). Therefore, we solve

\[
P\dot{\gamma} = \dot{\sigma} = \frac{G}{\Phi} (\dot{\gamma} - \frac{\sigma}{\eta} - \frac{\dot{\Phi}}{\Phi})
\]

for \( \dot{\gamma} \) given \( \Phi, G, P \) and \( \sigma \).

Because of the absolute value in the constitutive equation, we have two quadratic equations to solve for \( \gamma \) depending upon its sign. Each of these gives two possible solutions:

If \( \dot{\gamma} > 0 \), solve

\[
\dot{\gamma} = \frac{-B_p - \sqrt{B_p^2 - 4AC}}{2A},
\]

If \( \dot{\gamma} < 0 \), solve

\[
\dot{\gamma} = \frac{-B_n + \sqrt{B_n^2 + 4AC}}{-2A},
\]

where

\[
A = P - G/\Phi, \\
B_p = (P - G/\Phi)(\epsilon + \sigma_y G) + G\sigma/\Phi + \sigma\Phi'/\Phi,
\]
\[ B_n = (P - G/\Phi)(\epsilon + \sigma_y G) - G\sigma/\Phi - \sigma\dot{\Phi}/\Phi, \]

\[ C = G\sigma\epsilon/\Phi + \sigma\dot{\Phi}/\Phi(\epsilon + \sigma_y G). \]

We have found that choosing the smaller root will always give suitable values for \( \dot{\gamma} \), regardless of its sign. For values of \( \dot{\gamma} \) very close to zero, we solve both equations and take the solution closest to the value of \( \dot{\gamma} \) at the previous timestep.

Figure 6.12: The calcium and agonist concentrations used as inputs for the models.
Figure 6.13: The constitutive and parallel models prediction for the velocity, $\gamma$, in response to the calcium and agonist concentrations given in Figure 6.12.
Figure 6.14: The constitutive and parallel models prediction for the force exerted by the activated tissue strip.

Figure 6.12 shows the variation of calcium and agonist concentrations as described by Wang et al.. In response to these concentrations, the constitutive formulation qualitatively predicted the time course of both the force and velocity response of the muscle, see Figures 6.13 and 6.14. However, tight numerics were required: the space step need to be reduced by a factor of 10 to $\Delta x = 0.001$. Consequently, the time step need to be reduced to satisfy the
Courant-Friedrichs-Lewy condition [26]. We found that this was caused by the \( \Phi'(t) \) term in Equation 6.6 and that employing Tanh functions as described helped to resolve much of the numerical issues.

### 6.8 Conclusions

The goal of this chapter was essentially to determine whether our previous approach to modelling active and passive ASM interactions could be improved upon with a more complex, constitutive formulation. As such, we employed the complex fluids model of [132] as a way of combining active and passive mechanisms and set about the first of our two tasks: can the constitutive formulation reproduce experimental results, already well represented by the parallel model?

The fluidization observed in passive smooth muscle tissue in response to shear can be described by the crosslinker model. This result was unsurprising as complex fluids share similar rheological properties with passive soft biological tissue. The true test of the constitutive model was whether it could reproduce the time course of force generation in activated tissue in response to varying chemical stimuli. Even though the numerics required tightening, the model qualitatively agreed with the parallel model.

Our next task was to determine whether this new approach could improve upon existing results. We demonstrated that the constitutive model formulation can improve upon the parallel model predictions in response to the tidal length oscillation protocol of Bates et al. [10] in the sense that the force length loops are more similar in shape to those obtained experimentally and there is a transient reduction in force with time. However, the model does not predict the similarity in peak isometric force over a broad range of oscillation amplitudes. Nonetheless, the constitutive formulation predicts that it is the decrease in stiffness caused by a reduction in crossbridge cycling rate that gives rise to the transient decrease in force observed experimentally, as hypothesized by Fredberg et al. [44].

We went on to explore the anomaly of deep inspirations (DI’s). The constitutive formulation again improves upon the predictions of the parallel model and shows that a reduction in force following a DI is consistent with the temporary absence of some sub-cellular force generating mechanism. The decrease in the concentration of the solid phase, \( \Phi \), predicted by the constitutive formulation causes this decrease in force and explains how DI’s usually function to relax airways.

While it may not be perfect, this constitutive formulation has improved upon some existing model results and gave strength to theories from certain experimentalists. With future
simplifications of $\Phi$, (e.g., using the distribution moment approach of Zahalak [138]) this model could prove valuable in assessing the phase transitions brought about through mechanical stimuli such as taking a deep breath. Furthermore, it has provided us with a novel way of viewing the ASM cell that is structurally based and did not require modifications to the existing mechanisms of contractile regulation.
Discussion

One of the main goals of this project was to determine whether we can replace the parallel model with a simplified model. The idea behind this was to make a mathematical representation of the bronchial tree less computationally expensive. Formulating a model of the lung response constitutes modelling thousands of airways, each lined with a layer of ASM tissue. The properties of ASM tissue, many of which can be accounted for by the parallel model, govern the force generated by the tissue. One of our primary concerns was to determine the extent to which this complex behaviour at the tissue level affects the airway response and hence the need to represent it in a mathematical model of the airway.

There are numerous points regarding wall-tissue interactions that give us reason to believe a simplified representation of the tissue generated force would suffice: 1) ASM in vivo may behave significantly differently to that in tissue strip experiments. Evidence to suggest that length adaptation occurs in vivo is difficult to prove (though there are some indications [136]), or at least not to the same extent as observed in tissue strips [93]. 2) In modelling the airway, we are not interested in the transients to a steady state, only in whether the trajectory settles on the open stable branch of equilibria or falls to the closed stable branch.

Given that the mean force generated by ASM tissue is dynamically determined by the shear imposed upon it, we constructed curves to represent the amplitude and frequency dependent force response to tidal length perturbations. We compared this models predictions of the small airway response to varying pressure oscillations with the predictions obtained using a constant force and force determined by the development of latchbridges, crossbridge cycling and cytoskeletal remodelling (the parallel model).

In most cases, the parallel model agrees with the simplified models but this is very much
dependent upon the parameter $B$, used to represent the impact of the ASM force on the transmural pressure of the airway. For small values of $B$, the models agree that all airways remain open and will close for large $B$. However there exits a small interval of values of $B$ in which the model predictions vary significantly. The parallel model predicts reopening of the airway for some low frequency oscillations and closure for high frequency oscillations of the same amplitude. This result was unexpected to the point that it was the opposite of what was expected. It has been shown that ASM fluidizes to an extent that is proportional to the magnitude of shear imposed upon it. However, coupling the tissue with the airway has mitigated this effect in a way that may not be representative of what occurs in vivo. Having ruled out numerical errors, we found that this was due to the mean force on the high frequency trajectory being further to the right of the open stable branch of equilibria than its low frequency counterpart.

These unexpected results are unlikely to stem from errors in the parallel model due to its ability to capture much of the behaviour associated with ASM in tissue strip experiments. It may be a consequence of how the airway was coupled with the ASM model: the Laplace law in Equation 4.2 may not be an accurate reflection of the complex interactions between the ASM generated force and the airway radius.

However, there is no experimental evidence to determine whether this counter-intuitive result is actually wrong. If it is right, then the force reduction with increasing applied shear seen in tissue strips does not apply to the airway. Investigations into why this may be the case is a task best suited to a physiologist.

The study has shown that it appears acceptable to use a simplified model to account for ASM generated forces for preliminary investigations into the lung response to factors such as increased muscle mass. At the same time, however, the properties of ASM caused by crossbridge interactions and cytoskeletal remodelling on the micro-scale can have great impact on the airway response. Whatever the reason for the counter-intuitive result, the parallel model is the ‘gold standard’ in accounting for ASM properties from tissue strips. We want to further our understanding of events that take place within cells, particularly events associated with passive mechanisms, which may affect the airway response.

ASM tissue encircles the airways throughout the lung, from the trachea down to the terminal bronchioles. Along with airway inflammation, abnormalities in the interactions between the airway wall with its lining ASM is thought to be one of the main culprits at the root of diseases such as asthma and airway hyper-responsiveness.

In normal airways, the precise role of ASM in assisting lung function in a helpful manner remains unclear. However, what is abundantly clear is the detrimental effects ASM can
have when not functioning normally. Prolonged or enhanced stiffness of the airways leads to breathing difficulties and may stem from passive processes taking place within the cell.

In normal airways, stretch induced by tidal breathing or taking a deep breath are the most powerful mechanisms for the inhibition of bronchoconstriction. They relax the ASM and lower the mean force it can generate. The state of the muscle during tidal oscillations is associated with a decrease in latch state behaviour or other force generating mechanisms. In this sense, the force generated by the muscle is dynamically determined: the mean force generated by the muscle is directly related to the amplitude and frequency of the imposed oscillations.

In contrast with this, stiffening of the airways brought about by the prevalence of latch state behaviour or cytoskeletal remodelling could lower the internal load against which the muscle shortens. The degree to which stretch affects the tissue is thus lowered as the intracellular resistance has increased. Behaviour associated with the latch phenomenon enables the muscle to maintain force for prolonged periods with little energy expenditure. The weakened sensitivity to imposed strain, brought about by the cell’s progression into a ‘frozen’ like state with the prevalence of latch state behaviour, can have severe implications; fluidization in response to tidal breathing and deep inspirations is a fundamental part of airway relaxation. This makes clear the need for a detailed vision of the mechanism underlying these events.

We have found that such behaviour is likely to come about through a combination of processes. Latchbridge formation, a biproduct of crossbridge cycling, increases the cell’s resistance to imposed mechanical stimuli via their slow detachment rates. Since the early eighties, it has been widely accepted that the emergence of latchbridges following muscle activation enables the force maintenance associated with the latch phenomenon. Their formation is also associated with a fall in shortening velocity and rates of phosphorylation meaning they can account for all the features of the latch phenomenon. Through their low detachment rates, these latchbridges may lower the intracellular load. Actin-myosin interactions will be less perturbed by imposed load fluctuations which may cause faster rates of cycling and excessive airway narrowing to ensue.

In addition to latchbridge formation, we have found that cytoskeletal remodelling through crosslinker reconfiguration may also act to lower the load against which the muscle shortens, mitigating the degree of airway relaxation. Focal adhesion kinase (FAK) enhances the adhesion strengthening rate and actin polymerization. Its activity is regulated by mechanical stimuli such as crossbridge cycling or imposed length perturbations and is enhanced by the presence of calcium. FAK may help to reduce crosslinker strain thus lowering their
detachment rate and increasing their ability to resist imposed loads [123, 49, 88, 124, 135, 80].

While such cytoskeletal remodelling cannot explain the decrease in phosphorylation observed following activation, it can account for the passive length-tension curve observed by Naghshin et al. [93] as well as the shift of this curve at adapted lengths. This shift translates to a higher resting tension at shorter lengths, making it more difficult for excessively shortened airways to relax. In addition to this, we found that latchbridges alone cannot give rise to the passive stiffness generated by the muscle. This is because passive stiffness is unaffected by the inhibition of MLCK and reduced in the absence of calcium. If latchbridges were the sole mechanism of passive stiffness generation, it would be abolished in each case.

Learning more about how cytoskeletal remodelling is regulated should be a priority of future work concerning passive ASM processes. While it seems likely that it is regulated by some calcium initiated pathway involving FAK, much is yet to be understood. We know that remodelling can take place in the absence of crossbridge cycling so this calcium pathway must be separate from that which controls active dynamics. However, other factors in this pathway or aspects of the remodelling they govern remain unclear. Our first attempt at representing this pathway by employing a calcium dependent crosslinker detachment rate function worked but this approach was empirical and fitted rather than directly based on data. It would be of interest to study experiments performed on actin filament gels to infer a more realistic relationship between the rigidity of the cytoskeletal network and the concentrations of various stimulants.

The absence of the relaxing effects of taking a deep inspiration (DI) observed in asthmatics has been linked with a weakened sensitivity to stretch which may be brought about through a reduction in the intracellular load caused by a newly formed network of crosslinkers and latchbridges. Our need to understand more about how these processes interact with events related to active crossbridge cycling is clear, however, our current approach of combining active and passive dynamics in parallel makes it difficult to establish a link between the macroscopic behaviour of the tissue and that of its sub-cellular filaments. Rather than relying on specific molecular processes to explain the regulation of crossbridge cycling rates, we instead embraced the hypothesis that ASM modulates its internal structures in a fashion analogous to soft glassy materials.

We have learned a lot from the parallel model, particularly in relation to passive subcellular events and regulatory mechanisms, but the model cannot account for 1) banana shaped force-length loops, 2) the transient reduction in force with the onset of tidal oscil-
lations or 3) the reduction in mean force following a deep inspiration. We hypothesize that this may be due to the way in which active and passive sides of contraction are combined and we question whether a parallel configuration may be too simplistic.

We choose to take a constitutive approach, common to that seen in complex fluids literature, for a number of reasons: 1) There is little evidence to suggest some mechanism exists to regulate crossbridge cycling rates during tidal breathing, 2) ASM and soft glassy materials fluidize and re-solidify in much the same way, 3) Abnormalities in these phase transitions may be at the heart of why deep inspirations fail to dilate asthmatic airways, and 4) The way in which ASM behaves similar to glass is thought to be at the heart of the bronchodilatory effect of taking a deep inspiration.

This formulation captures the reduction in mean force following a deep inspiration, the transient reduction in force during tidal length oscillations and produces force-length loops that are more similar in shape to those observed experimentally. We found that it is the decrease in spring constant with continuous oscillations that gives rise to the banana shaped fall in the force-length loops, indicating a decrease in stiffness caused by reduced crossbridge cycling. Although peak isometric force varies substantially with oscillation amplitude and the force length loops cross unexpectedly in parts, preliminarily results are encouraging.

Following Bates et al. [10], the incorporation of non-linearity into the stiffness of the spring may improve the shape of the loops and a simpler representation of the concentration of the solid phase, Φ, should make analytic endeavors less complex.

Motivation for further analysis of the model can be summarised as follows: the bronchodilatory effects of DI’s may stem from the phase transitions captured using this approach. It has also been suggested that the glass hypothesis can indicate the rate at which plastic remodelling and biochemical mechanisms may take place. This may also shed light on our previous question regarding the mechanisms underlying cytoskeletal remodelling.

A less complicated representation of Φ may also help to fulfill one of the most important goals of this project. It is one way of representing, in a computationally efficient manner, the effects of ASM generated force on the airway response.

7.1 Future Work

A further study into the phase transitions predicted by the constitutive formulation may shed light on how abnormalities may arise following deep inspirations i.e why do asthmatic airways fail to fluidize in response to them? This may come about through a simplification of Φ and an analysis of the bifurcations of the system. The investigation may provide
insight into the rates at which cytoskeletal remodelling takes place and may assist in the study of how such remodelling is regulated.

Clearly ASM tissue influences the response of the airway it encircles. However, the PDE models used to describe the properties of ASM are highly complex, require stiff numerics and are difficult to analyse. Though a constant or dynamically determined approach to modelling the ASM force works well in many circumstances, an intermediate model in terms of complexity would be valuable. This may be obtained using the distribution moment approach of Zahalak [139].

An investigation into alternative methods of coupling ASM tissue with its surrounding airway wall is also needed as the current airway response predicted by the parallel model seems difficult to justify in a physiological sense.

The long term goal is to model the bronchial tree. Using the description of Lambert et al. [76], ASM tissue will be coupled with the airways to determine the effects of obesity, changes in the heterogeneity of airway narrowing etc. on lung function.
Appendices
Appendix A

List of Parameters

The parallel model parameters are as follows:
The order 2 airway model parameters are as follows:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{1a}$</td>
<td>0.5962s$^{-1}$</td>
</tr>
<tr>
<td>$k_{1b}$</td>
<td>1.35µM</td>
</tr>
<tr>
<td>$k_{on1}$</td>
<td>0.000125</td>
</tr>
<tr>
<td>$k_{on2}$</td>
<td>0.8988µM</td>
</tr>
<tr>
<td>$k_{off1}$</td>
<td>0.4629</td>
</tr>
<tr>
<td>$k_{off2}$</td>
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</tr>
<tr>
<td>$\tau_p$</td>
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</tr>
<tr>
<td>$\bar{k}_2$</td>
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</tr>
<tr>
<td>$g_1$</td>
<td>0.1211s$^{-1}$</td>
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<tr>
<td>$f_{p1}$</td>
<td>0.88s$^{-1}$</td>
</tr>
<tr>
<td>$g_{p1}$</td>
<td>0.22s$^{-1}$</td>
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<tr>
<td>$\beta$</td>
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<tr>
<td>$\gamma_a$</td>
<td>44.87</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>3mN$^2$</td>
</tr>
<tr>
<td>$\alpha_1$</td>
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<tr>
<td>$y_{max}$</td>
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<tr>
<td>$\beta_1$</td>
<td>0.01(1/s)</td>
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<tr>
<td>$\beta_2$</td>
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<tr>
<td>$m$</td>
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</tr>
<tr>
<td>$\rho_2$</td>
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</tr>
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<td>$\rho_3$</td>
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<td>$F_0$</td>
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<tr>
<td>$k_1$</td>
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<tr>
<td>$\gamma_p$</td>
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<tr>
<td>$\psi$</td>
<td>0.3(1/m$^2$)</td>
</tr>
<tr>
<td>$\delta$</td>
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</tr>
<tr>
<td>$f_{E1}$</td>
<td>4.32 * 10$^{-5}$(1/s)</td>
</tr>
<tr>
<td>$f_{E2}$</td>
<td>1.5 * 10$^{-5}$(1/s)</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>4</td>
</tr>
<tr>
<td>$g_{E1}$</td>
<td>3 * 10$^{-5}$(1/s)</td>
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</table>
The order 16 airway model parameters are as follows:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>$B$</td>
<td>$10^*R_{ref}$</td>
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<tr>
<td>$R_i$</td>
<td>0.073mm</td>
</tr>
<tr>
<td>$r_{\text{imax}}$</td>
<td>0.337mm</td>
</tr>
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<td>$P_1$</td>
<td>19.475cmH$_2$O</td>
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<tr>
<td>$n_1$</td>
<td>1</td>
</tr>
<tr>
<td>$n_2$</td>
<td>7.185</td>
</tr>
<tr>
<td>$\mu$</td>
<td>15cmH$_2$O</td>
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Appendix B

Convergence Tests

We have used the fourth order Runge Kutta method (RK4), Heun’s method and Euler’s method to solve the system of ODE’s obtained when the parallel model PDE’s have been reduced with the method of characteristics. We have tested the numerical convergence of the parallel model for two scenarios: a strictly isometric procedure and a stretch and hold procedure. In each case the error is taken to be

\[ E = \sum_{i=1}^{N} |d_{i}^{\Delta t} - d_{i}^{C}|, \]

where \( d_{i}^{\Delta t} \) is the final distribution of crossbridges, latchbridges or crosslinkers when a time-step of \( \Delta t \) has been used throughout calculations. The control solution is given by \( d_{i}^{C} \) in which a time-step of \( 10^{-8} \) has been used. A space-step of \( \Delta x = 0.01 \) has been used throughout giving \( N = 2D/\Delta x + 1 \) where \( x \in \{-D,D\} \) defines the initial spatial domain.

In the first of these set-ups, the length of the tissue is held constant while the muscle is activated over 25 seconds. Since the length of the tissue does not change, the method of characteristics is not used and the slopes of the error lines closely approximate the order of the numerical method used. The error graphs are shown in Figure B.1 for the crossbridge model.

In the second scenario, the length of the tissue is stretched by 20% of its reference length over the course of 1 second. The error graphs obtained are shown in Figures B.2 and B.3. We have found the slopes of the error lines can fall significantly below the order of the numerical method used, particularly for the RK4 method, when the method of characteristics is called upon. This result is to be expected. For instance, [37] have shown that PDE’s solved with the Eulerian-Lagrangian method suffer from order reduction due
to the modified method of characteristics being employed. The slope of the error for each method is given in the following tables:

**Strictly Isometric**

<table>
<thead>
<tr>
<th>Method</th>
<th>Distribution</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euler</td>
<td>$AM_p$</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>$AM$</td>
<td>0.97</td>
</tr>
<tr>
<td>Heun</td>
<td>$AM_p$</td>
<td>1.98</td>
</tr>
<tr>
<td></td>
<td>$AM$</td>
<td>1.99</td>
</tr>
<tr>
<td>RK4</td>
<td>$AM_p$</td>
<td>4.44</td>
</tr>
<tr>
<td></td>
<td>$AM$</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Table B.1: Error in each numerical method for a strictly isometric procedure (method of characteristics is not used).

**Stretch and Hold**

<table>
<thead>
<tr>
<th>Method</th>
<th>Distribution</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euler</td>
<td>$AM_p$</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>$AM$</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>$CL$</td>
<td>1.01</td>
</tr>
<tr>
<td>Heun</td>
<td>$AM_p$</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>$AM$</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>$CL$</td>
<td>1.75</td>
</tr>
<tr>
<td>RK4</td>
<td>$AM_p$</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>$AM$</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>$CL$</td>
<td>1.62</td>
</tr>
</tbody>
</table>

Table B.2: Error in each numerical method for a stretch and hold procedure.
B.0.1 A Strictly Isometric Case

Figure B.1: Error with the RK4 method and the Euler method for the crossbridge model when the muscle is activated and the length held constant. We have taken the true solution to be that obtained with a time-step of $\Delta t = 10^{-7}$. Since the method of characteristics is not used here, the slopes obtained with each numerical method is roughly the same as the order of the numerical method employed. These slopes are given in Table B.1.
B.0.2 A Stretch and Hold Procedure

Figure B.2: Error with the RK4 method and Heun’s method for the crossbridge model when the muscle is stretched after which its length is held constant. The slopes obtained with each numerical method are given in Table B.2. We have taken the true solution to be that obtained with a time-step of $\Delta t = 10^{-8}$. 
Figure B.3: Error with each numerical method for the crosslinker model when the muscle is stretched after which its length is held constant. The slopes obtained with each numerical method are given in Table B.2. We have taken the true solution to be that obtained with a time-step of $\Delta t = 10^{-8}$. 

(a) Euler Method

(b) Heun’s Method

(c) RK4 Method
B.0.3 The Non-Uniform Mesh Method

In some simulations, particularly those concerned with the airway, a huge spatial domain is required. As mentioned, we assume decay boundary conditions and this is enforced by using a big enough spatial domain with the method of characteristics to ensure that the populations do not fall off the domain and the population distribution at each boundary is equal.

The spatial domain usually required is $-\gamma(L_{\text{min}} - L_0) - 10 \leq x \leq \gamma(L_{\text{min}} - L_0) + 10$, where $L_0$ is the initial length of the tissue, $L_{\text{min}}$ is the shortest length reached during simulation and $\gamma$ is a proportionality constant between the displacement of the population distributions and the displacement of the tissue.

In simulations where the airway closes, the domain needed is $-2\pi\gamma - 10 \leq x \leq 2\pi\gamma + 10$. We have taken $\gamma = 12.87$ for these simulations meaning that with a domain spacing of $\Delta x = 0.01$, more than 16,000 ODE’s need to be solved at each time step.

However, looking at the population distributions throughout simulation, it is immediately obvious that significant change in the distributions only occurs in the region $-15 \leq x \leq 15$. Outside this region, populations are almost constant. This led to the idea that a coarse mesh could be used outside the region $-15 \leq x \leq 15$ and a refined mesh used within it. Specifically, the initial mesh was taken to be

$$x = \begin{cases} -2\pi\gamma : H : -15 \\ -15 + h : h : 15 \\ 15 + H : H : 2\pi\gamma. \end{cases}$$

The code was set up so that if the upper boundary of the refined mesh moved a certain distance away from 15, using the cubic spine interpolation feature of Matlab, the populations would be interpolated onto the mesh

$$x = \begin{cases} x_1 : H : -15 \\ -15 + h : h : 15 \\ 15 + H : H : x_N, \end{cases}$$

where $x_1$ and $x_N$ are the start and end points of the mesh given by the method of characteristics. This step ensures that the refined population stays within $-15 \leq x \leq 15$ and that interpolation does not need to be carried out at every time step.

To demonstrate the efficiency of this method, the crossbridge model was coupled with the airway model. The tissue was activated causing the airway to close.
• **Uniform Mesh**: A mesh with a uniform spacing of $\Delta x = 0.01$ was used and resulted in over 16,000 ODE’s. These were solved using the RK4 method. Computation time was 53 minutes.

• **Non-Uniform Mesh**: A coarse mesh with spacing $\Delta x_1 = 0.1$ and a refined mesh with spacing $\Delta x_2 = 0.01$ was used resulting in 4317 ODE’s which were also solved using the RK4 method. Computation time was 9 minutes.

Both cases are illustrated in Figure B.5

We conclude that, for our purposes, it is best to use a lower-order, lower-cost numerical method. Since we do not obtain fourth order convergence, the fourth order Runge-Kutta method is more expensive than needed, whether it is standard or adaptive. However, it would be interesting to see the efficiency and accuracy of an adaptive, lower-order method.

![Figure B.4: Model predictions of airway closure with a uniform and non-uniform mesh. The non-uniform mesh computation ran around 7.5 times faster.](image-url)
Figure B.5: Error resulting from interpolation using the non-uniform mesh method.
Bibliography


[81] *Lung Animation. commons.wikipedia.org*,


