UNIVERSITY OF CALGARY

Effects of Active Length Changes on Steady-State Force Production in Mammalian Skeletal Muscle

by

Rachel Ann Schachar

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

FACULTY OF KINESIOLOGY

CALGARY, ALBERTA

NOVEMBER, 2002

UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Effects of Active Length Changes on Steady-State Force Production in Mammalian Skeletal Muscle" submitted by Rachel Ann Schachar in partial fulfilment of the requirements for the degree of Master of Science.

Dr. Walter Herzog, Supervisor, Faculty of Kinesiology

Dr. James Wakeling, Faculty of Kinesiology

Dr. Douglas A. Syme, Department of Biological Science

December 19, 2002
Date

ABSTRACT

History-dependence is a well accepted property of skeletal muscle. Following active stretching, the steady-state muscle force is increased, and following active shortening, it is depressed compared to the force produced for the purely isometric contraction at the corresponding length. Despite much work in this area of skeletal muscle biomechanics many questions remained unanswered. Therefore, the purpose of this thesis was to systematically investigate the effect of history-dependence in whole skeletal muscle on the descending limb of the force-length relationship.

Steady-state forces following active stretching were greater than the isometric forces at the initial (and therefore the final) muscle length, and were smaller following active shortening than the isometric forces at the corresponding muscle length. Force enhancement and force depression increased with increasing amounts of stretching and shortening, respectively. Force enhancement increased with increasing final muscle length, whereas force depression increased with decreasing final muscle length. Novel to the literature was the observation that force enhancement exceeded the maximal isometric forces at the optimal muscle length for some stretch conditions, and was associated with a contribution from a passive component.

PREFACE

Chapters 3 and 4 of this thesis are stand alone papers and are based on the following manuscripts:

Schachar, R., Herzog, W., Leonard, T.R. (2002). Enhancement above the initial isometric force on the descending limb of the force-length relationship. *Journal of Biomechanics* **35**, 1299-1306.

Schachar, R., Herzog, W., Leonard, T.R. (2002). Effects of stretching and shortening on isometric forces on the descending limb of the force-length relationship. *Journal of Biomechanics* (Accepted November 2002).

Consequently, there is some repetition in the methods sections of these chapters.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks and appreciation to:

Dr. Walter Herzog for his continuous guidance, high expectations, feedback, support, and encouragement...as well as his advice about life.

Drs. Douglas Syme and James Wakeling for taking the time to serve on my supervisory / examination committee.

Tim Leonard and Hoa Nuygen for their outstanding technical support and their valuable time.

Treny Sasyniuk for her wonderful friendship, wisdom, and advice.

Jorge, Antra, Yukiko, Azim and Eun-Jeong, for being great officemates & friends, and for being there for me when I needed them.

The "Bat-Cave" Boys (J.P., Danny, Brian, & Spencer) for being great friends, making me laugh, and for forcing me take some time out (now and then) to have a bit of fun.

All the members of the Human Performance Laboratory for helping me out along the way and for making the HPL the wonderful place it is.

DEDICATION

To my wonderful family – Mom, Dad, Jaime & Leah, your love, patience, and encouragement were so important. I admire you all so much. Thank you for always being there.

And to my very best friend – Kirk Bothwell, thank you so much for being there for me always. Without your love, support, continued encouragement, and all your hugs, this would not have been possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

EPIGRAPH

All religions, arts and sciences are branches of the same tree. All these aspirations are directed toward ennobling man's life, lifting it from the sphere of mere physical existence and leading the individual towards freedom.

- Albert Einstein

CHAPTER 1: INTRODUCTION

Muscles are fascinating biological engines we use everyday; they are the motors of human locomotion. Skeletal muscle allows us to stand without the worry of falling over, run and jump with speed and power, and it gives our body its shape. However, despite years of research, dating back to the early description of muscles by Aristotle in 384 B.C, our understanding of how muscles work is limited.

Possibly the most basic property of skeletal muscle is its ability to produce force. Muscle is structurally divided into fascicles (or fibre bundles), fibres (single cells), myofibrils, sarcomeres, and myofilaments (Figure 1-1). Muscle fibres are composed of a repeat of sarcomeres in series, with the sarcomere being the basic contractile unit of a muscle. Skeletal muscle contraction occurs, within the sarcomere, through an interaction of the thick (myosin) and thin (actin) myofilaments. The most widely accepted theory of muscle contraction is the cross-bridge theory proposed by Huxley in 1957. This theory describes how the thick myofilaments attach to neighbouring thin myofilaments forming a "cross-bridge". The cross-bridge is believed to rotate, pulling the thin filament past the thick filament and towards the centre of the sarcomere, resulting in functional shortening and thus force production. It has been well known for over a century that the force a muscle can exert depends on its length (Blix, 1894) and experimental evidence for this, and in support of the cross-bridge theory for muscle contraction, lies in the force-length relationship for skeletal muscle (Gordon et al., 1966).

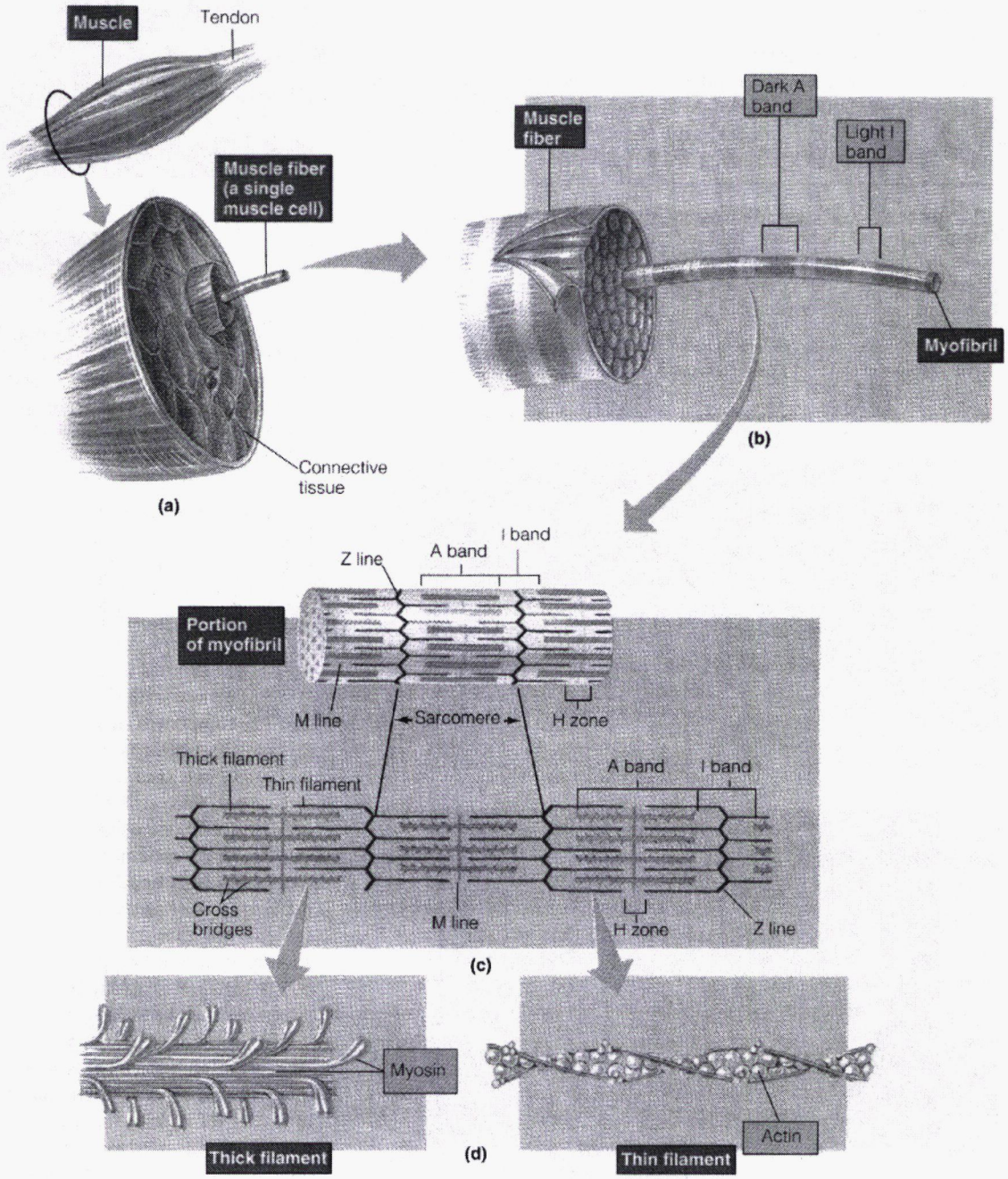

Figure 1-1. Schematic illustration of the levels of organization in skeletal muscle. The whole muscle is structurally divided into fascicles (bundles of fibres) (a). These fascicles are composed of muscle fibres, which are made up of myofibrils (b). Each myofibril is a collection of sarcomeres in series composed of myofilaments (c) (actin and myosin (d)). (Sherwood, 1997)

The skeletal muscle force-length relationship is presently accepted as one of the most important features supporting the sliding filament theory of muscle contraction, and is of fundamental importance in the study of muscle physiology and muscle mechanics. However, some questions regarding the force-length relationship still remain to be investigated. To understand how we control the complex movements of human locomotion, knowledge about skeletal muscle force production is important.

It has been assumed previously that, for a given level of activation, the steady-state force a muscle can exert is a function only of its length and the speed of contraction. However, there are several indications that preceding movements influence the force a muscle can produce. For example, the maximal isometric force a muscle can produce is reduced if, prior to the development of the steady-state isometric force, the muscle is allowed to actively shorten, and is enhanced if, prior to the development of the steady-state isometric force, the muscle is actively stretched. These properties are collectively referred to as history-dependent effects.

History-dependent effects have been observed for a long time, dating back to the early 1950's (Abbott and Aubert, 1952). However, most studies have presented data that are only illustrative, or that come from experiments performed on isolated muscle fibres. As a result, it is difficult to assess the importance of history-dependent effects for whole muscle. It is necessary to investigate and to gain knowledge of these effects in intact skeletal muscle. Therefore, the aim of the present thesis was to systematically study the impact of history-dependent effects, specifically active muscle stretching and shortening, in whole mammalian muscle. For this purpose, experiments were performed on in situ cat semitendinosus and soleus muscle.

1.1 New and Notable

There are three new and notable results that emerged from this thesis and should be emphasized. This study was the first to:

1) Show that whole muscle, in an in-situ muscle preparation, behaves in a stable manner following active stretching on the descending limb of the force-length relationship. Steady-state forces following active stretching were found to be significantly greater than the purely isometric forces at the initial muscle length on the descending limb of the force-length relationship (Chapter 3).

2) Show force enhancement following active muscle stretching that exceeded the maximal isometric forces at optimal muscle length. Force enhancement above the isometric plateau was found for 6 out of 9 stretch conditions on the descending limb of the force-length relationship (Chapter 4).

3) Reveal that part of the force enhancement produced following active muscle stretching is caused by a passive structure. It was concluded that there is an active and a passive component of force enhancement. The active component allows for force enhancement to reach values corresponding to the isometric plateau and the passive component gives an extra force, allowing force enhancement to exceed the isometric plateau (Chapter 4).

1.2 Overview of Thesis

The thesis continues with a review of the literature in chapter 2, aimed at providing a presentation of the relevant issues related to skeletal muscle force production, stability, force enhancement following stretching, and force depression following muscle shortening. Chapters 3 and 4, based on two stand-alone manuscripts, explain the experiments carried out to illustrate the changes in skeletal muscle force production following different stretching and shortening conditions. Chapter 5 includes a summary of the results and a general conclusion of the thesis.

CHAPTER 2: REVIEW OF RELEVANT LITERATURE

The literature review presented here will provide background and rationale for the work done in the present study. Section 2.1 describes the basis of skeletal muscle force production, including the primary mechanism of muscle contraction - the cross-bridge theory. Section 2.2 describes one of the principal mechanical properties of skeletal muscle - the force-length relationship. Together these two sections give insight into why history-dependent properties of skeletal muscle have been a controversial and well-studied aspect of skeletal muscle biomechanics. Section 2.3 gives an overview of previous relevant and selected work in the area of force enhancement, and section 2.4 describes some of the proposed mechanisms for this phenomenon. Sections 2.5 and 2.6 cover the history-dependent phenomenon of force depression following active muscle shortening and the proposed mechanisms of force depression, respectively.

2.1 Skeletal Muscle Force Production

There currently exist different theories of skeletal muscle force production. At present, the primary mechanism of muscle contraction accepted in the field of muscle mechanics is the cross-bridge theory (Huxley, 1957; Huxley and Simmons, 1971). However, this theory does not explain all observed properties of skeletal muscle force production. Before the 1950's, the accepted mechanism for skeletal muscle contraction was the "continuous-filament theory", which was based on the idea that the contractile material, in the form of a continuous filament, shortened by a folding or coiling action (Huxley, 1974). The cross-bridge theory was aimed at expanding a theory of muscle contraction, the "sliding filament theory" that was proposed after the "continuous-filament theory"

(A.F. Huxley and Niedergerke, 1954; H.E. Huxley and Hanson, 1954). The cross-bridge theory can be explained conceptually as follows: side projections (cross-bridges), or the heads of the thick (myosin) filament, momentarily attach themselves to specialized sites on the thin (actin) filaments. The cross-bridges then propel the thin filaments past the thick filament to a new position, thereby producing force and contraction (Figure 2-1).

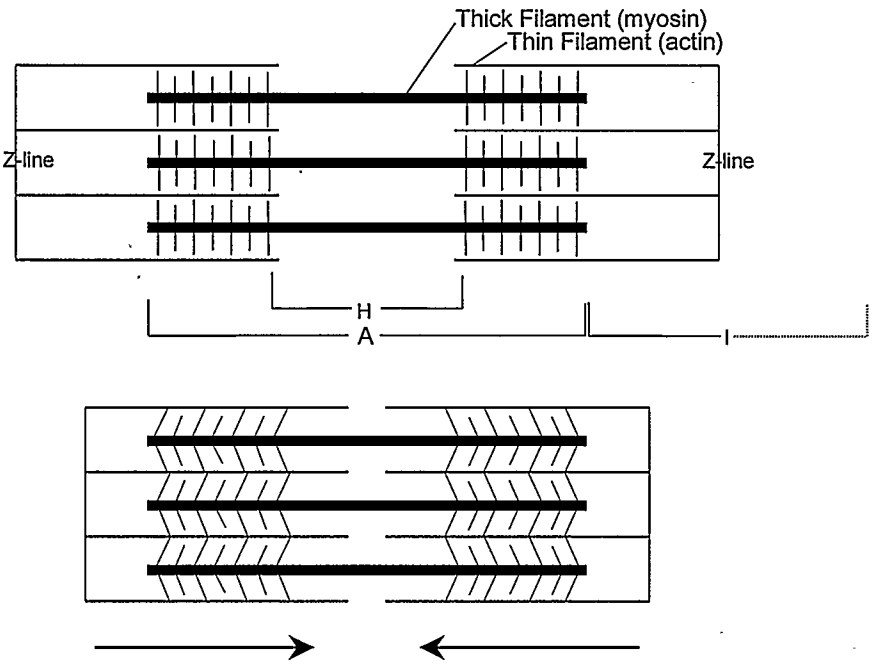

Figure 2-1. An illustration of the overlap of actin and myosin filaments in a relaxed sarcomere (A). Contraction is produced by the actin filaments sliding over the myosin filament, causing the Z-lines to move toward each other and the narrowing of the H-region.

One of the assumptions of the cross-bridge theory was that each cross-bridge would act as an independent force generator, and that the force developed during a muscle contraction would depend on the number of simultaneous interactions between the cross-bridges and the actin filaments (Huxley, 1957). Experimentally, it has been shown that stretching the muscle fibre can vary the number of cross-bridge attachments, by altering the amount of filament overlap (Gordon et al., 1966). Therefore, if the cross-bridge theory of skeletal muscle contraction, as mathematically formulated by Huxley (1957) and Huxley and Simmons (1971) was correct, the tetanic, isometric force produced by a muscle would depend solely on the length of the sarcomeres, fibre, or whole muscle, but not on the history-dependent properties of contraction. A history-dependent property refers to the properties, such as force enhancement and force depression (discussed in detail later), which are dependent on the action of the muscle, such as stretching or shortening, prior to the steady-state isometric contraction. Force enhancement following active muscle stretching, and force depression following active muscle shortening are not part of the current theoretical description of the cross-bridge theory, and the mechanisms for these phenomena remain a topic of debate in the field of skeletal muscle biomechanics.

2.2 Skeletal Muscle Force-Length Relationship

The skeletal muscle force-length relationship, first described by Gordon et al. in 1966, provides evidence that supports the cross-bridge theory of muscle contraction (Huxley, 1957). Force-length relationships describe the relation between the maximal isometric force a muscle (or fibre, or sarcomere) can produce and its length (Herzog and ter Keurs, 1988). Gordon et al. (1966) examined the tension generated by isolated fibres from frog

skeletal muscle, using a photoelectric spot follower, at different degrees of stretch covering the whole range of lengths within which the thick and thin filaments overlap. The sarcomere force-length relationship, when plotted, has an ascending limb, a plateau, and a descending limb (Figure 2-2). This relationship, between the force a muscle can exert and its length, is typically obtained under isometric conditions and for maximally activated muscle. Isometric refers to a muscle contraction in which length remains constant, where length may refer to the length of the entire muscle, the length of a fibre, or the length of a sarcomere, depending on the system that is studied. In the study of Gordon et al. (1966), a photoelectric spot follower was used to control length and ensure uniformity and isometricity of sarcomere length during contraction.

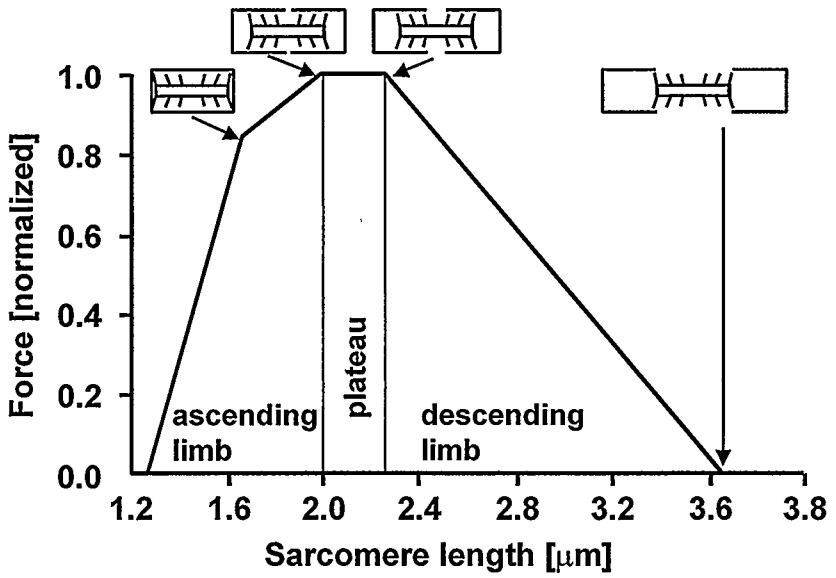

Figure 2-2. Force-length relationship adapted from Gordon et al. (1966). Active force producing regions include the ascending limb, the plateau, and descending limb region. Illustration of the myofilament overlap at the various sarcomere lengths along the force-length relationship is shown above.

The locations of cross-bridge interactions on the thick and thin filaments are positioned at uniform distances along the length of the two filaments. The cross-bridges on the thick filament are believed to be 14.3 nm apart in the longitudinal direction and offset relative to one another by 60 degrees (Figure 2-3). This means that two cross-bridges with the same orientation (i.e. a cross-bridge pair offset by 180 degrees) are 43nm apart (Figure 2-3).

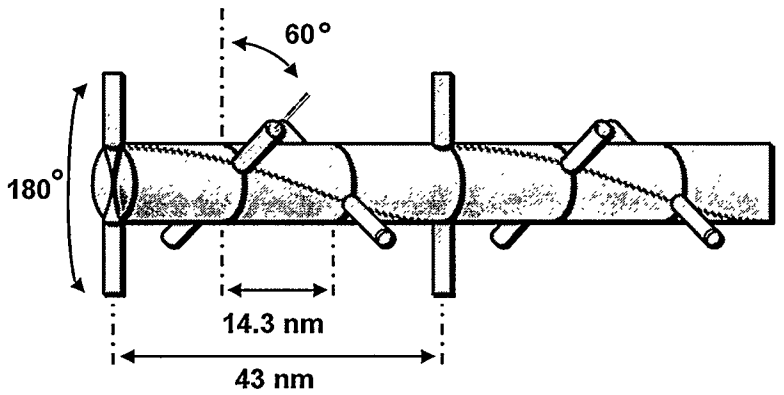

Figure 2-3. A schematic illustration of the cross-bridge arrangement on the thick myosin filament (Adapted from Pollack, 1990).

Each cross-bridge attachment that is made is believed to act independently but each produces the same average force (Huxley, 1957). Therefore the overlap between the myosin and actin filaments determines the number of possible cross-bridge formations and, thus, the total force that may be produced. At long sarcomere lengths (lengths > 3.65μm in frog skeletal muscle), no overlap exists between the myofilaments, therefore no cross-bridges can be formed and the resulting force produced is zero (Figure 2-2). Sarcomere shortening increases the number of potential cross-bridge formations in a linear manner with sarcomere length and therefore myofilament overlap, until a maximum number of cross-bridge formations are possible (Gordon et al., 1966). The

force produced at optimal actin-myosin overlap corresponds to the peak or plateau region of the force-length curve (approximately 2.05-2.25μm in frog skeletal muscle). Shortening of the sarcomeres below the plateau region (lengths < 2.05μm in frog muscle) is associated with a decrease in force production. Although the exact mechanism for this force decrease is not known, it has been presumed to be due to an interference of the thin myofilaments at the centre of the sarcomere, deformation of thick filaments, or the formation of an overlap between the thin filaments as they slide past each other at the centre of the sarcomere.

Probably the most important support for the cross-bridge theory was the linear relationship between force and length beyond the plateau region at sarcomere lengths greater than 2.2μm (for frog skeletal muscle fibres) (Figure 2-2). Gordon et al. (1966) concluded that in the plateau region and at lengths greater than the plateau region, the tension on each thin filament is made up of forces from cross-bridges that it overlaps on adjacent thick filaments. From the end of the descending limb region to the beginning of the plateau of the force-length curve (sarcomere length of 3.65μm to 2.0μm, as described by Gordon et al. (1966)), filament overlap increases in direct proportion to shortening, and the development of isometric tension is directly proportional to the number of cross-bridges overlapped. However, the linearity of the relationship has been questioned previously by investigators, who have shown a non-linear force-length behaviour on the descending limb region of the force-length relationship (e.g. ter Keurs et al., 1978). ter Keurs et al. (1978) constructed length-tension relationships from the levels of tension and sarcomere length measured during the plateau phase of isometric tetanus in single fibres of frog semitendinosus. Their results showed that the plateau tension was independent of

sarcomere length between 1.9 and 2.6μm, declined to 50% maximal at 3.4μm, and declined further at longer sarcomere lengths (ter Keurs et al., 1978 figure 9a). This difference may be due to the fact that Gordon et al. (1966) imposed a length clamp over a segment along the fibre to help maintain constant striation spacing during contraction, whereas ter Keurs et al. (1978) initially selected fibres, which did not show large non-uniformities allowing for the entire length of the fibre to be clamped.

The classical force-length relationship for skeletal muscle, as described by Gordon et al. (1966), is nothing more than a link between a series of static observations, each derived from an independent contraction. Therefore, the force-length relationship does not necessarily describe the force as a function of length in dynamic contractions. This concept is important in understanding dynamic muscle function in vivo. Many studies have shown that active muscle shortening reduces the steady-state isometric force following shortening (e.g. Abbott and Aubert, 1952; Délèze, 1961; Maréchal and Plaghki, 1979). These experiments led to the proposal that shortening influences the force-length characteristics of skeletal muscles (Meijer et al., 1997). Meijer et al. (1997) conducted experiments to systematically test how shortening prior to isometric contraction affects the steady-state isometric force-length curve of skeletal muscle. They found that the force-length curves following shortening, derived from post-shortening characteristics, were different from the isometric force-length curve obtained for purely isometric contractions, and they concluded that, as a consequence, the traditional isometric force-length relationship is a poor estimator of the force-length curve during dynamic contractions of muscle (Figure 2-4). The experiments of Meijer et al. (1997) were performed primarily on the ascending limb and plateau regions of the force-length

relationship, and only shortening contractions were considered. Therefore, their conclusions regarding the whole force-length relationship were incomplete.

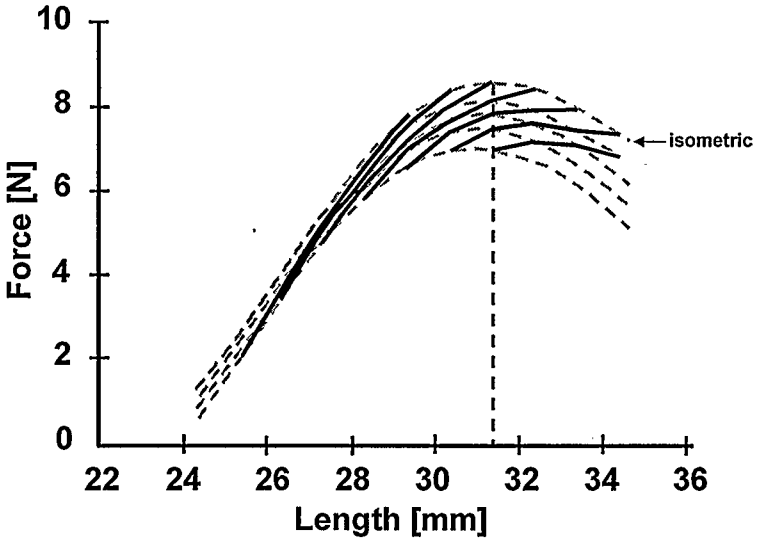

Figure 2-4. A schematic representation of the derived force-length relationships following the shortening experiments of Meijer et al. (1997). The curves shown are for different starting lengths and were performed at a shortening speed of 10mm/s. The dashed lines represent the isometric force-length relationships following shortening for isokinetic contractions with different shortening amplitudes. The top dashed line represents the fully isometric force-length curve (adapted from Meijer et al., 1997).

2.3 Force Enhancement

History-dependent properties of skeletal muscle force production have been demonstrated in abundance in both in vitro and in situ muscle preparations (Abbott and Aubert, 1952; Délèze, 1961; Edman et al., 1978, 1993; Granzier and Pollack, 1989; Herzog and Leonard, 1997; Julian and Morgan, 1979; Maréchal and Plaghki, 1979), and it is well accepted that there is long lasting force enhancement following muscle and fibre stretch (Abbott and Aubert, 1952; Edman et al., 1978, 1982; Herzog, 1998) (Figure 2-5).

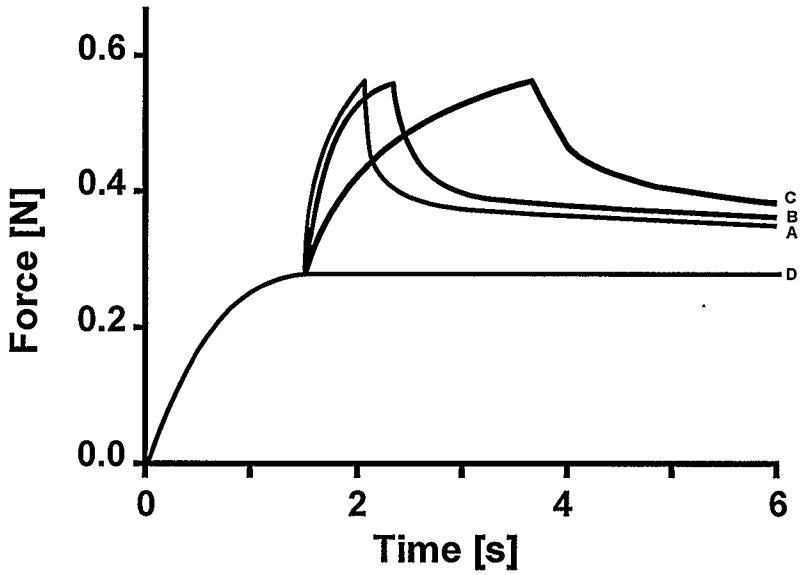

Figure 2-5. Results of force-enhancement following active muscle stretch of the toad sartorius muscle from the experiments of Abbott and Aubert (1952). Force enhancement was found to be dependent on the speed of contraction (i.e. force enhancement was larger for slower contraction speeds; curve C was performed at 1.9 mm/s as compared to curve A at 8 mm/s, and B at 5 mm/s). Curve D represents the purely isometric contraction at the final muscle length. Peak force following the active stretch remained that same for all conditions (Adapted from Abbott and Aubert, 1952).

Force enhancement can be defined as the total increase in steady-state isometric force above the force attained in a purely isometric tetanus at the corresponding muscle length following stretching of an actively stimulated muscle. This increase in force after stretch is maintained, at a steady-state, for a period of several seconds of tetanic stimulation (i.e. force enhancement has been shown to be long lasting (Abbott & Aubert, 1952) (Figure 2-5). Force enhancement has been shown to increase with increasing stretch amplitudes (Abbott & Aubert, 1952; Délèze, 1961; Edman et al., 1978; Edman et al., 1982; Rassier et al., 2002). Force enhancement has also been shown to be dependent on the rate of stretch in whole muscle, in some preparations (Abbott & Aubert, 1952)) (Figure 2-5), but is mostly found to be independent of the rate of stretch (Edman et al., 1978; Edman et al., 1982; Rassier et al., 2002; Sugi & Tsuchiya, 1988).

2.4 Proposed Mechanisms of Force Enhancement

Force enhancement following muscle stretching has typically been explained in the literature by the sarcomere length non-uniformity theory. Hill (1953) believed that the "creep" phase, or the slow rise component of the force-time curve, observed in his fixed-end contraction experiments on the descending limb of the force-length curve in frog and toad sartorius muscle, was associated with non-uniform length changes in sarcomeres.

The sarcomere length non-uniformity theory is based on the idea that muscle fibres are composed of a large number of sarcomeres in series, and due to differences in the initial sarcomere length, cross-sectional area, or activation, an inequality in force production between sarcomeres occurs (Edman and Reggiani, 1984). Any differences in sarcomere force production would result in some sarcomeres lengthening and some sarcomeres shortening during active muscle stretching (Allinger et al., 1996). Therefore, two sarcomeres in a fibre located at different initial lengths on the descending limb of the force-length relation diverge in length following active muscle stretching (Figure 2-6). The sarcomere length non-uniformity theory has been primarily observed in single fibre preparations during fixed-end contractions (Huxley and Peachey, 1961; Gordon et al., 1966; Julian and Morgan, 1979; Morgan et al., 1982; Sugi and Tsuchiya, 1988; Granzier and Pollack, 1990).

Many investigators believe that the descending limb of the force-length relationship is unstable based on these observed non-uniform sarcomere length changes along the length of the muscle fibre, and due to the negative slope in this region (Hill, 1953; Huxley and Peachey, 1961; Gordon et al., 1966; Julian and Morgan, 1979).

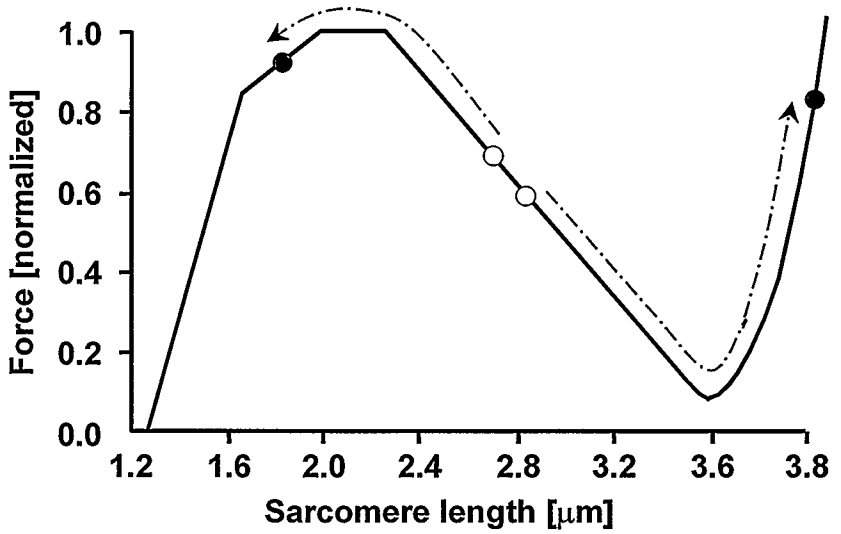

Figure 2-6. A schematic representation of the total force-length relationship for skeletal muscle (Gordon et al. 1966) and the expected unstable sarcomere behaviour on the descending limb (based on Hill, 1953). Two sarcomeres at different initial lengths (open circles) diverge in length until a stable situation is reached (solid circles on the ascending limb region and the passive force curve) (Adapted from Allinger et al., 1996).

From the sarcomere length non-uniformity theory, three different predictions based on the proposed mechanisms for force enhancement have been put forward. First, it is believed that force enhancement cannot occur on the ascending limb of the force length relationship, as non-uniformities are thought not to occur in this region. However, this hypothesis was rejected when force enhancement was observed on the ascending limb of the force-length relationship (e.g. De Ruiter et al., 2000; Herzog and Leonard, 2002). The second hypothesis put forward was that the steady-state isometric force following active muscle stretching on the descending limb of the force-length relationship should not exceed the purely isometric force at the initial muscle length and should not exceed the isometric forces at the plateau region. This feature of the sarcomere length non-uniformity theory has been debated for decades (Edman et al., 1978; Edman et al., 1982). Edman et al. (1982) found that early after the end of stretch, the muscle fibre was capable

of producing considerably higher force than was found during an isometric contraction at the plateau of the force-length relationship. This finding was further investigated, to see whether the steady-state force following stretching could exceed the isometric force at the plateau region. It was concluded that with the stretches used, forces "clearly" above the plateau region of the force-length curve were not found (Edman et al., 1982).

The third hypothesis developed based on the sarcomere length non-uniformity theory was the idea that fibre stiffness following active muscle stretch decreases compared to the fibre stiffness following the isometric contraction at the initial muscle length. This premise was questioned based on experimental evidence, which showed that stiffness in single fibres following active muscle stretching remained approximately the same as the amount of stiffness for the purely isometric contraction at the corresponding muscle length, suggesting that force enhancement was caused by an increase in the average force per cross-bridge (Herzog and Leonard, 2000; Sugi and Tsuchiya, 1988). In contrast to these previous observations, Linari et al. (2000) found that stiffness of actively stretched single frog fibres was greater than that observed for the purely isometric contractions at the corresponding fibre length. Based on these results, steady-state force enhancement could possibly be explained either by an increase in the number of attached cross-bridges or by an increase amount of force produced by each cross-bridge.

Another proposed mechanism of force enhancement, and of particular interest here, is the idea that there is an engagement of an elastic structure during active stretching (De Ruiter et al., 2000; Edman et al., 1982; Edman & Tsuchiya, 1996; Forcinito et al., 1998; Herzog and Leonard, 2002; Noble, 1992). This proposed non-cross-bridge based mechanism, is a possible explanation for force enhancement, which has recently been mentioned more

frequently than any of the other possible mechanisms. However, this theory has not yet been demonstrated by direct experimental evidence and warrants further investigation.

2.5 Force Depression

As with force enhancement, it is well acknowledged that there is a long lasting depression of force production following active muscle shortening (Figure 2-7). This observation was first described systematically in 1952 by Abbott and Aubert, and has been demonstrated in many different studies since (e.g. Edman et al., 1993; De Ruiter et al., 1998; Herzog and Leonard, 1997; Julian and Morgan, 1979; Maréchal and Plaghki, 1979; Meijer et al., 1997; Sugi and Tsuchiya, 1988). In the study of Abbott and Aubert (1952), it was experimentally demonstrated, using sartorii of frog and toad, and coracomandibulars of dogfish, that the steady-state isometric force following active muscle shortening was lower than the force produced for the purely isometric contraction at the corresponding length (Figure 2-7). Force depression has been shown to increase with increasing magnitudes of shortening and to be inversely related to the speed of shortening (Abbott and Aubert, 1952; De Ruiter et al., 1998; Herzog and Leonard, 1997; Maréchal and Plaghki, 1979; Sugi and Tsuchiya, 1988) (Figure 2-7).

Herzog and Leonard (1997) demonstrated force depression following active muscle shortening and the corresponding properties in in-situ cat soleus. They tested the influence of the magnitude and speed of shortening using different shortening ranges and different speed ranges at a constant distance of shortening. The results of their experiments showed that the amount of force depression increased with increasing amounts of shortening preceding the isometric contraction, decreased with increasing

speeds of shortening, and increased with increasing force during the shortening phase. These results were in agreement with the results of previous studies.

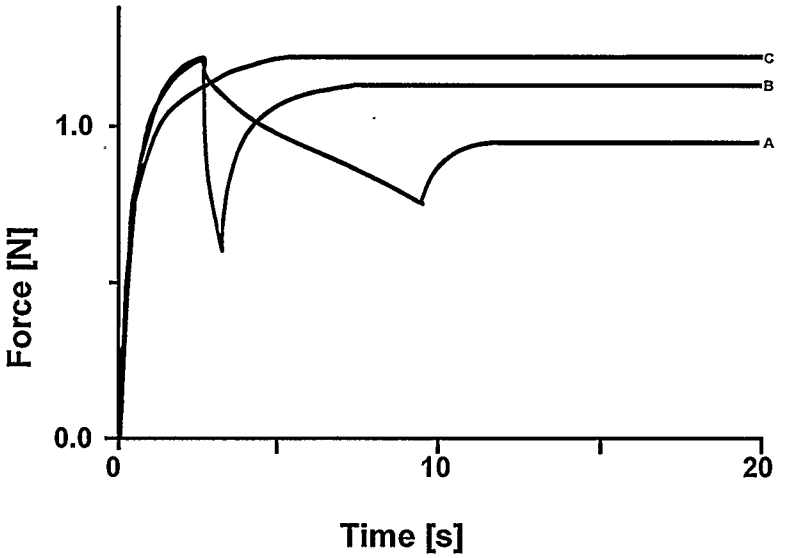

Figure 2-7. Representative results of force-depression following active shortening of the Dogfish jaw muscle from the experiments of Abbott and Aubert (1952). Force depression was found to be dependent on the speed of the shortening contraction (i.e. force depression was greater following slower shortening speeds; curve A was performed at 0.5 mm/s compared to curve B at 8 mm/s). Peak force following active muscle shortening is greater for the slower shortening contraction (A) compared with the active muscle shortening at the faster speed (B). Curve C represent the isometric contraction 37 mm (Adapted from Abbott and Aubert, 1952).

In experiments of De Ruiter et al. (1998), it was demonstrated that force depression occurred following muscle shortening in human skeletal muscle. They also showed that force depression following shortening was directly related to the angular displacement (i.e. the amount of muscle shortening), and was inversely related to the speed of muscle shortening.

Sugi and Tsuchiya (1988) showed that force depression is associated with a proportional decrease in fibre stiffness. It was therefore believed that the observed force depression

was caused by a decrease in the number of attached cross-bridges and not a decrease in the average force per cross-bridge.

It has been observed in isolated cases that force depression following active muscle shortening is brief and transient (Edman, 1996). However, this idea was met with controversy when results were published showing that force depression reaches a steady-state and is long lasting (Granzier & Pollack, 1989; Herzog, 1998). Other controversies surrounding the history-dependent phenomenon of skeletal muscle force depression have emerged. In 1989, Granzier and Pollack conducted experiments in which they studied the effects of shortening history on isometric force in single intact frog fibres using a fixed-end preparation and a segment clamp method. The object of their experiments was to have the muscle undergo shortening against a constant force while subsequently holding the muscle fibre length constant. Their results showed that force depression persisted without the occurrence of sarcomere non-uniformity. However, four years later, Edman et al. (1993) found contradictory results in similar experiments where the length of the intact muscle fibre was controlled throughout a tetanized shortening contraction. They found that the force deficit after loaded shortening was correlated with the variation of the sarcomere length along the muscle fibre. Edman et al. (1993) concluded, that force depression following active shortening of a muscle fibre is due to the development of sarcomere length non-uniformities along the length of the fibre.

An abundance of studies have been conducted, over the last 50 years, to investigate force depression following active muscle shortening, all leading to the development of different proposed mechanisms for this history-dependent phenomenon.

2.6 Proposed Mechanism of Force Depression

The primary mechanism of force depression following active muscle shortening remains unknown, however several mechanisms have been put forth in the past (Edman et al., 1982; Edman et al., 1993; De Ruiter et al., 1998; Granzier and Pollack, 1989; Herzog and Leonard, 1997; Maréchal and Plaghki, 1979; Morgan et al., 2000).

One such proposed mechanism of force depression is the idea of actin myofilament deformation when entering the actin-myosin overlap zone during sarcomere shortening, termed "stress-induced cross-bridge inhibition", first proposed by Maréchal and Plaghki in 1979. With this theory as a basis, many different aspects and ideas regarding force depression have been examined. As stated previously, force depression increases with increasing shortening amplitude (Abbott & Aubert, 1952; De Ruiter et al., 1998; Maréchal & Plaghki, 1979; Morgan et al., 2000). Force depression has also been found to reach a long-lasting steady-state (Abbott & Aubert, 1952; Herzog, 1998) but is abolished immediately when the tension is completely released (Abbott & Aubert, 1952; Herzog & Leonard, 1997; Maréchal & Plaghki, 1979). And finally force depression was found to be proportional to decreased fibre stiffness (Sugi & Tsuchiya, 1988).

Another proposed mechanism to explain the observed force depression following active muscle shortening is based on the idea that the shortening action of muscle reduces the affinity for calcium at the regulatory sites located on the actin myofilament. This idea proposed in 1996 by Edman, if correct, would mean that force depression would be brief and that force would be restored following the end of the shortening phase due to the

recovery of the calcium concentration. However, this idea and proposed mechanism have been rejected as there are many studies which show, as mentioned previously, that force depression is in fact a long-lasting phenomenon (Abbott & Aubert, 1952; De Ruiter et al., 1998; Edman et al., 1982; Herzog et al., 1998; Maréchal & Plaghki, 1979; Morgan et al., 2000).

A third mechanism proposed to explain force depression is based on the idea that protons and inorganic phosphate accumulate during active shortening (Granzier & Pollack, 1989). This molecular accumulation would have a long lasting effect, as it is similar to what occurs in muscle fatigue. However, it has been shown that force depression following shortening can be abolished instantaneously following a short and sudden pause in muscle activation, therefore it is not likely that this mechanism is the primary, or sole mechanism, to explain force depression (Abbott & Aubert, 1952; Herzog & Leonard, 1997).

The most commonly proposed mechanism of force depression is the sarcomere length non-uniformity theory (Edman et al., 1993; Julian and Morgan, 1979; Morgan et al., 2000; Sugi and Tsuchiya, 1988). Edman et al. (1993) demonstrated that sarcomere length shortening caused a decrease in the isometric force, and resulted in the development of sarcomere length non-uniformity. It was also shown that a positive correlation existed between the amount of force depression and the degree of sarcomere length non-uniformity. Sugi and Tsuchiya (1988) further stated that the development of sarcomere

length non-uniformities was more pronounced during slow compared to fast speeds of shortening.

The sarcomere length non-uniformity theory is based on the idea that during muscle contraction there is a redistribution of sarcomere lengths along the length of the fibre (Hill, 1953; Gordon et al., 1966). Edman et al. (1982) provided results showing that sarcomere length is not generally uniform along the length of an isolated muscle fibre, either at rest, or during activation. In fact, non-uniformity has been well demonstrated during tetanic contractions on the descending limb of the force-length relationship (Gordon et al., 1966b; Huxley and Peachey, 1961; Julian and Morgan, 1979), where sarcomeres near the end of the muscle fibre were found to shorten while the sarcomeres located in the centre were stretched. During muscle shortening, sarcomeres along the length of the fibre reach different lengths due to differences in shortening speed along the fibre. According to the sarcomere length non-uniformity theory, some sarcomeres will shorten much more than average, moving onto the ascending limb of the force-length relationship, while other sarcomeres will shorten less than average and remain on the descending limb region. The resultant isometric force measured following this active shortening contraction is lower than the true isometric force for the purely isometric contraction at the corresponding length (i.e. force depression).

There is, however, evidence that suggests that the sarcomere length non-uniformity theory cannot be the main mechanism of force depression. Granzier and Pollack (1989) conducted experiments on single frog semitendinosus fibres, where in one experiment the sarcomere lengths were kept uniform, and in the other experiment the sarcomere length

was not controlled. Their results demonstrated that force depression was similar following shortening regardless of the sarcomere length non-uniformity, thus indicating that this proposed theory for force depression may not be correct. Nevertheless, Edman et al. (1993) performed similar sarcomere length control experiments with conflicting results. It was found that the force depression following active muscle shortening disappeared following a fibre shortening with uniform sarcomere length.

It has also been proposed, based on the sarcomere-length non-uniformity theory, that force depression cannot occur on the ascending limb of the force-length relationship. However, force depression has been observed in this region in a number of studies (De Ruiter et al., 1998; De Ruiter et al., 2000; Herzog and Leonard, 1997; Meijer et al., 1997). Morgan et al. (2000) proposed an additional hypothesis based on the idea that force depression should be associated with a decrease in fibre stiffness. Again, this proposed hypothesis, founded on the sarcomere length non-uniformity theory, was met with conflicting evidence that showed that the stiffness following active shortening was the same as that measured for purely isometric reference contractions (Herzog and Leonard, 2000).

Based on the observations made opposing the sarcomere length non-uniformity theory and its assumptions, it is proposed that the sarcomere length non-uniformity theory cannot be the sole mechanism of force depression following active muscle shortening.

CHAPTER 3: Force Enhancement Above the Initial Isometric Force on the Descending Limb of the Force-Length Relationship

3.1 Introduction

If a skeletal muscle is actively stretched and then held isometrically at some final length, the steady-state isometric force following the active stretch will be greater than the corresponding force at the same final length obtained during a purely isometric contraction (Figure 3-1). This phenomenon, which we will call force enhancement following stretch, has been observed consistently in whole muscle (e.g. Abbott and Aubert, 1952; Maréchal and Plaghki, 1979; Morgan et al., 2000) and single fibre preparations (e.g. Edman et al., 1978, 1982, 1984, 1993; Sugi and Tsuchiya, 1988; Edman and Tsuchiya, 1996). Typically, force enhancement has been observed on the descending, but not the ascending limb of the force-length relationship (e.g. Morgan et al., 2000). This result has prompted a series of theoretical considerations aimed at explaining the mechanism underlying force enhancement (Morgan 1990; 1994). Combined with Hill's notion of the instability of sarcomere lengths on the descending limb of the force-length relationship, the development of sarcomere length non-uniformities during active stretch of a muscle on the descending limb of the force-length relationship was put forward as a possible cause for the observed force enhancements (Morgan, 1990; 1994; Edman and Tsuchiya, 1996; Morgan et al., 2000; Julian and Morgan, 1979).

It has been observed that during stretching of a single fibre, all segments of the fibre are stretched, albeit to different extents (Edman et al., 1982). This observation, in combination with the mathematical formulation of the sarcomere length non-uniformity

theory allows for the prediction of testable hypotheses. One such hypothesis is that the steady-state force following active muscle stretch on the descending limb of the force-length relationship cannot exceed the purely isometric force at the initial length of the muscle (the length from which stretching was started) (e.g. Morgan, 1990; Zahalak, 1997; Morgan et al., 2000). However, Edman et al. (1978; 1982) showed, for single fibres of frog tibialis anterior, that the steady-state force enhancement exceeded the purely isometric force at the initial length for some stretch conditions. For these conditions, the negative slope of the descending limb of the force-length relationship became positive throughout (Figure 3-1). To our knowledge, such a result has not been demonstrated systematically for whole mammalian skeletal muscle.

The purpose of this study was to test if the steady-state forces following active stretch of cat semitendinosus were greater than the corresponding purely isometric forces at the muscle length from which the stretch was started.

3.2 Methods

3.2.1 Preparation

The experiments were carried out on cat semitendinosus (n = 10). Animals were anaesthetized initially using a mask and a 5% halothane gas mixture; then they were intubated and maintained during the experiment using 1.0-1.5% halothane. At the end of each experiment, animals were sacrificed by euthanol injection.

The semitendinosus was separated from the surrounding muscles, for a clear view of the nerve and its two branches to the muscle. A silicon cuff was surgically implanted on the semitendinosus nerve, high enough to stimulate both compartments of the muscle. The insertion of semitendinosus on the tibia was removed with a remnant piece of bone on the

tendon. The cat was secured in a prone position in a hammock, with a heating pad, and the pelvis, thigh, and shank of the experimental hind limb were fixed with bilateral steel pins to a stereotaxic frame. The stereotaxic frame was fixed to a metal ground plate that was rigidly attached to an experimental table. This setup prevented movement of the hind limbs during the experimental contractions. The remnant piece of bone at the distal end of the semitendinosus tendon was attached with sutures to a muscle puller (MTS, Eden Prairie, MN, natural frequency > 10 kHz) in an orientation as close to physiological as possible.

3.2.2 Protocol

Semitendinosus forces and displacements were measured continuously by the muscle puller. In order to attain fused tetanic contractions, a stimulation rate between 40 to 60 Hz was chosen. The stimulation voltage was set at a supramaximal value of three times the alpha motor unit threshold (Herzog et al., 2000) and ranged from 1.5 to 5 volts. Stimulation times ranged from 3s to 17s depending on the time required for the specific tests and in order to attain steady-state force production following the stretch. A 4-minute rest period was enforced between contractions to allow for full recovery. First, the optimal length was identified as the length at which semitendinosus produced the maximal active isometric force. All lengths greater than optimal were considered the descending limb of the force-length relationship (Morgan et al., 2000). Second, stretches of 4, 8, 12 and 16 mm amplitude at 8 and 24 mm/s were applied from three different starting lengths on the descending limb of the force-length relationship. Isometric reference contractions were performed at the initial and the final lengths of the stretch, before and after the stretch tests. Only tests in which the isometric reference contractions

were the same (± 5.0 %) were considered for analysis. All starting lengths were greater than the optimal length of the muscle. All data were sampled on a 486 PC using CODAS software.

The order of the stretches for each series of contractions was kept constant. The isometric reference contractions at the initial and final lengths were performed first for a given starting length of the stretch. Next, the stretches at a speed of 8mm/s were performed, followed by corresponding passive stretches at the same speed. Then, the isometric reference contractions were repeated, and all procedures were redone at a stretching speed of 24mm/s. The next series of contractions was then performed at a new starting length. Stretch contractions were always completed in the same order; 8, 4, 12, and 16 mm, and the starting lengths always increased from one set of contractions to the next on the descending limb of the force-length curve.

The above protocol was performed exactly as described for the last 4 animals. For animals 1-6, specific parts of the above protocol were tested, and additional stretch amplitudes (i.e. 2, 6, 14 mm) were used in selected cases.

3.2.3 Analysis

A total of 131 different stretch contractions were used for analysis from 10 muscles. Data were analysed using Matlab software.

Raw force traces for each set of contractions were plotted with the corresponding passive stretch contraction force trace. The passive stretch force was then subtracted from the total stretch force to attain the active stretch force. This active force was then used for the force enhancement analysis.

Steady-state force enhancement measurements, defined here once the force-time trace following active stretch was parallel to the corresponding isometric reference contractions, were taken 1 second prior to the cessation of stimulation for every trial. This corresponded to a range of approximately 2 – 10 seconds following the end of the stretch, when near steady-state force conditions had been achieved.

All statistical analyses were performed using non-parametric tests at a preset level of significance of $\alpha = 0.05$. All experimental procedures were approved by the Life Sciences Animal Ethics Committee of the University of Calgary.

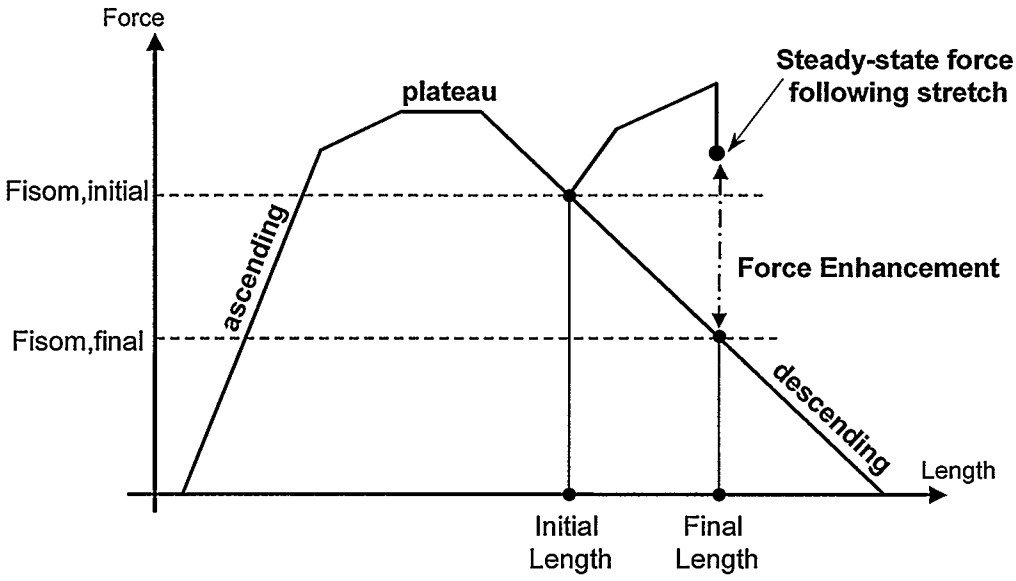

Figure 3-1. Schematic representation of the force-length relationship, illustrating the ascending limb, the plateau region, and the descending limb. The bold line represents a schematic muscle stretch, and the steady-state force reached following the stretch. Force enhancement occurs when the steady-state force following stretch remains above the isometric force at the final muscle length. Here, we tested specifically whether the steady-state force following stretch would also exceed the isometric force at the initial muscle length.

3.3 Results

The steady-state, active forces following muscle stretches were greater than the purely isometric forces at the final (long) muscle length in 126 out of 131 tests (Figures 3-2, 3-4 and Table 3-1). Four of the five cases in which force enhancement was not observed occurred for 2 and 4 mm stretch amplitudes. The amount of force enhancement above the purely isometric forces at the final (long) muscle length reached a peak value of 7.2N, which corresponded to a 39% greater force than the final isometric force. The steady-state active forces following muscle stretching were greater than the purely isometric forces at the initial (short) length in 109 out of the 131 tests analyzed (Figure 3-3, 3-4 and Table 3-1). The peak force enhancement above the isometric force at the initial length was 6.5N or 35% of the corresponding isometric reference force. In 22 cases, the forces following muscle stretch were smaller than the steady-state isometric forces produced at the initial length. Eighteen of these cases occurred for stretch distances of 8mm or less, and they were more frequent at the short compared to the long initial muscle lengths (Table 3-1).

Table 3-1. Summary table for all stretch tests performed.

Stretch Amplitude (mm)	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**20**	**24**	**Total**
FE (above initial isometric force)	*3*	*14*	*6*	*48*	*2*	*21*	*2*	*13*	*0*	*0*	*109*
FE (above final isometric force)	*7*	*18*	*8*	*52*	*2*	*21*	*2*	*14*	*1*	*1*	*126*
Passive FE	*1*	*3*	*3*	*10*	*1*	*12*	*2*	*14*	*1*	*1*	*48*
Total	*9*	*20*	*8*	*52*	*2*	*21*	*2*	*15*	*1*	*1*	*131*

Italicised numbers represent the numbers of occurrences for each specific case. Total numbers of stretch tests performed for each stretch amplitude are indicated at the bottom. Total occurrences observed for each measurement (i.e. Force enhancement (FE)) are shown in the far right column.

The residual force enhancement following stretch increased with increasing magnitudes of stretch (Figure 3-4) relative to the isometric forces at the final and the initial muscle lengths. Force enhancement following stretch was statistically independent of the speed of stretching but tended to be slightly greater for the slow compared to the fast speed of stretch (Figures 3-4 and 3-5).

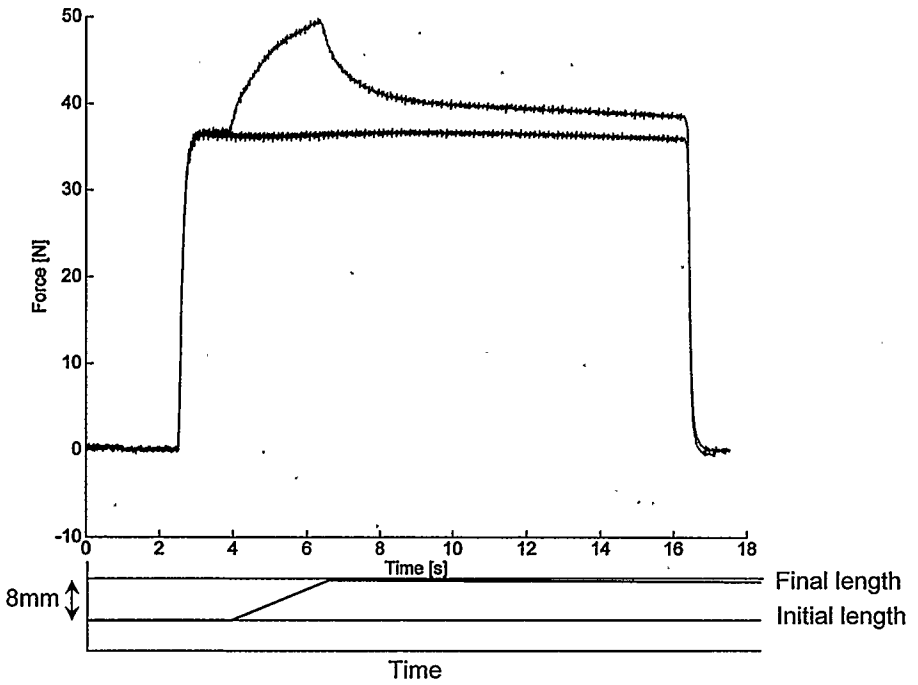

Figure 3-2. Raw data of the force-time history of a representative isometric reference contraction at the final muscle length, and the corresponding force-time history for an 8mm stretch to that final length. Note, the amount of force enhancement following the active stretch, ΔF. Note further, that all passive forces were subtracted, so that only active force production is shown.

In 48 tests, the passive forces following active muscle stretching were considerably greater than the passive forces at the corresponding length for purely isometric contractions, or after stretching of the passive muscle (Table 3-1, Figure 3-6). This passive force enhancement, following stimulation, for the active stretch was always

higher than the passive force following stimulation for the purely isometric force at the final muscle length (i.e. the passive force was not the same and not below).

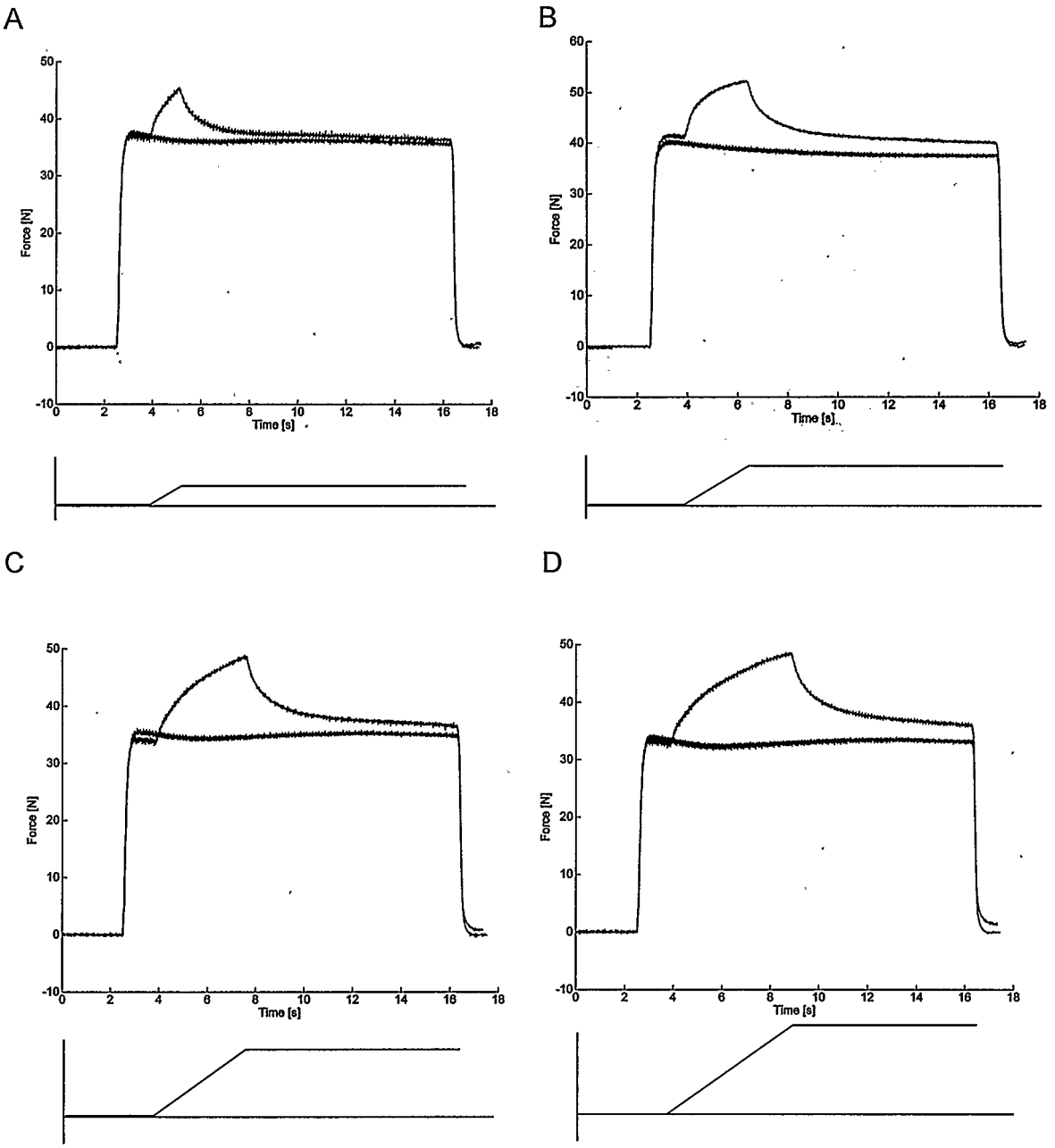

Figure 3-3. Representative force-time histories of isometric contractions at the initial isometric reference length and the corresponding force-time histories for a 4, 8, 12, and 16 mm stretch test to the final length (A, B, C, and D, respectively). In each graph, the upper line represents the stretch contraction, and the lower line represents the isometric contraction at the initial length.

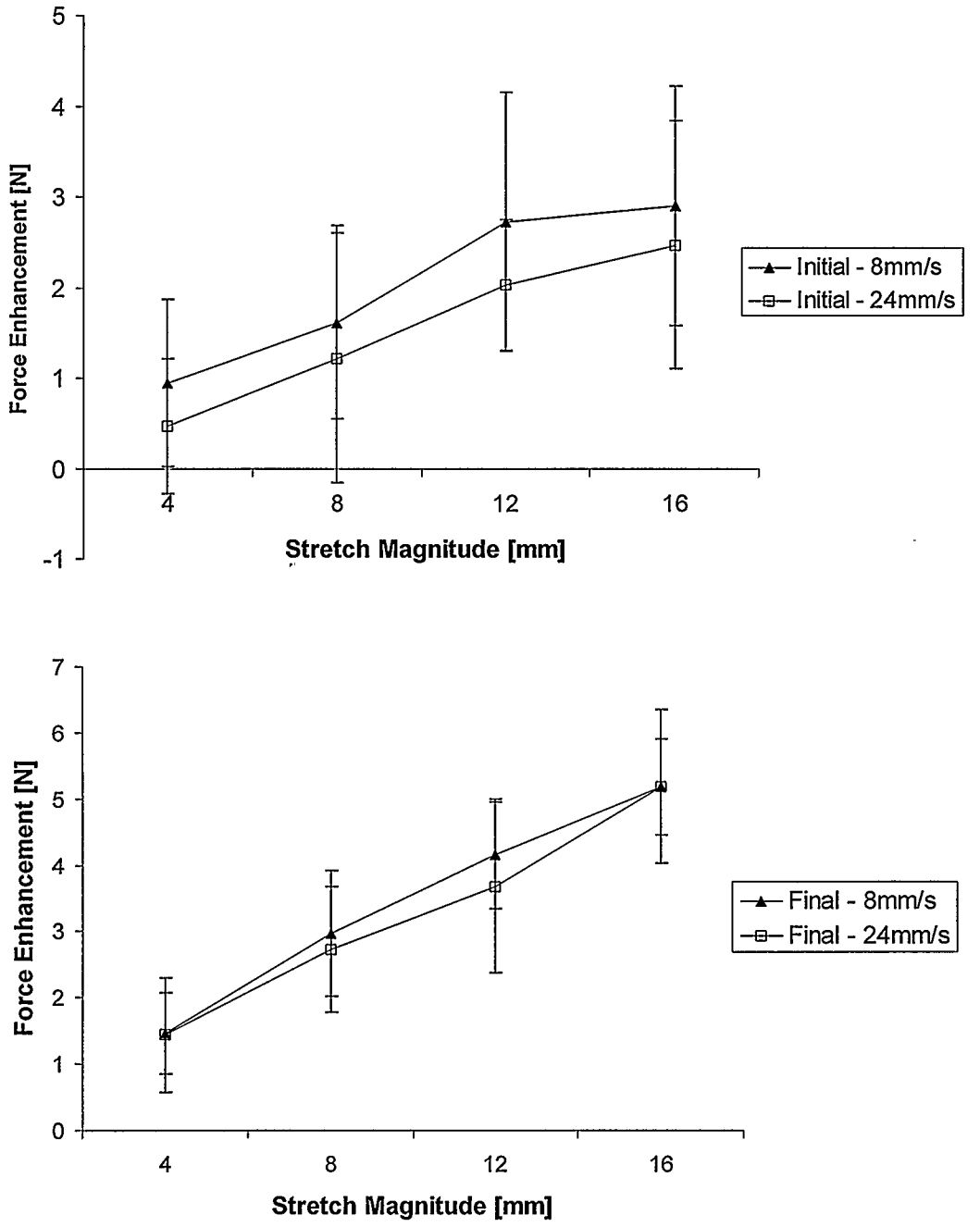

Figure 3-4. Relationship between the amount of steady-state force enhancement (means ± 1 SD) as a function of the stretch amplitude. Data from all 131 experimental tests were combined to illustrate the difference in steady-state force enhancement and the initial (top graph) and final (bottom graph) isometric force for both speeds tested. Note that the force enhancement above the isometric force at the initial and final lengths is dependent on the amount of stretch. Note further, that force enhancement was found to be statistically the same for both speeds of stretch. Error bars represent standard deviation between trials.

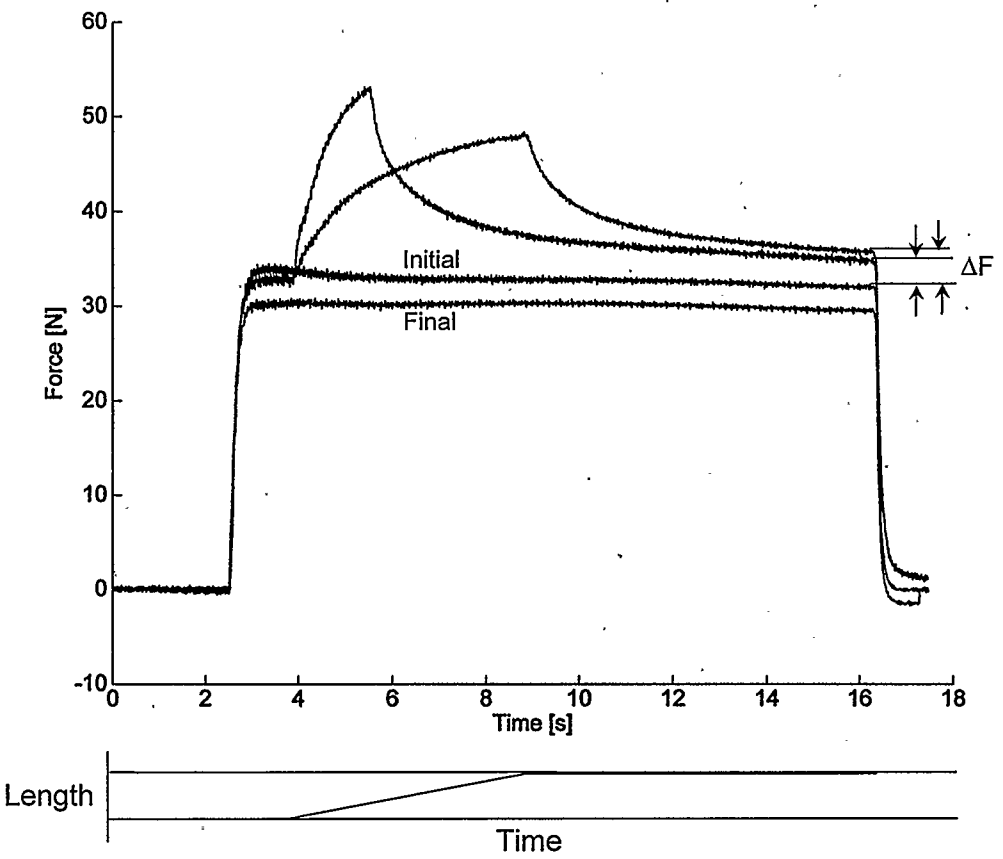

Figure 3-5. Force-time history of a representative isometric reference contraction at the final muscle length, the initial muscle length, and the corresponding force-time history of a 16 mm stretch at 8 mm/s and 24 mm/s. Note, that the amount of force enhancement following active stretch at both speeds, ΔF, is similar, and although the slow stretch appears to be associated with a greater ΔF, the difference observed for the slow and fast speeds of stretching did not reach statistical significance. Note further, that the steady-state force following stretch remained above the initial isometric reference force.

This passive force was obtained consistently at the greatest muscle lengths and tended to increase with increasing stretch amplitudes. The amount of passive force enhancement observed following the cessation of stimulation ranged from 0.2N – 6.0N.

3.4 Discussion

Force enhancement was defined here as the difference between the steady-state forces following an active muscle stretch and the isometric forces at the corresponding muscle length (Abbott and Aubert, 1952). Force enhancement was observed in 126 out of 131 tests (Table 3-1). This observation is consistent with force enhancement observed in a variety of other muscle preparations (Abbott and Aubert, 1952; Délèze, 1961; Edman et al., 1978; Edman et al., 1982; Morgan et al., 2000).

In 109 out of 131 tests, the steady-state active force following active muscle stretch was also greater than the steady-state active isometric force at the initial muscle length (i.e. the length at which active stretching was started) (Table 3-1). This result is consistent with that observed by Edman et al. (1978; 1982) in single fibres of frog. In his preparations, Edman et al. (1982) checked carefully whether there was a development of gross sarcomere length non-uniformities. They observed that during stretching all segments of the fibres elongated. Therefore, when working on the descending limb of the force-length relationship, and accepting the sarcomere length non-uniformity theory as the mechanism for the observed force enhancement (Morgan, 1990; 1994; Morgan et al., 2000), force enhancement should not exceed the purely isometric reference force at the length from which the stretch was initiated.

However, for most tests, particularly those involving great muscle lengths and great amplitudes of stretching, the steady-state forces following stretching exceeded the initial isometric forces. In view of the sarcomere length non-uniformity theory, this result can only be explained in two ways: (1) either the sarcomere length non-uniformity theory is

not complete, or (2), some segments of the fibres must have shortened during the stretching of the muscle.

It is quite conceivable that the sarcomere length non-uniformity theory is not the complete explanation for the observed force enhancement following muscle stretch, because force enhancement has been observed in "sarcomere-clamped" preparations (Granzier and Pollack, 1989), it has been observed on the ascending limb of the force-length relationship (e.g. de Ruiter et al., 2000), and has been shown to exceed the peak isometric force at fibre optimal lengths (Rassier et al., 2002). All these observations are inherently incompatible with the sarcomere length non-uniformity theory.

However, there exists the possibility that the results observed here are caused by local, segmental shortening of muscle fibres during the stretching of the whole muscle. More precisely, the greater the amount of stretch, the greater this shortening of some segments would have to be since force enhancement above the initial isometric force increased with increasing distances of stretch (Figure 3-4). Such a scenario seems unlikely, although it cannot be excluded based on the measurements made here.

A more likely scenario to explain the results observed here is the idea that when a muscle is stretched an additional force beyond that corresponding to the isometric force, is recruited. We found evidence for the recruitment of a passive force in about one third of all active stretch tests (Table 3-1, Figures 3-3, 3-5, 3-6). Specifically, such passive forces were observed more frequently at great muscle lengths and great amplitudes of stretch (Table 3-1). Although, it has been speculated that a passive element might be involved in force enhancement following active muscle stretch (Edman et al., 1982; Edman and

Tsuchiya, 1996; Forcinito et al., 1997; De Ruiter et al., 2000; Noble, 1992), we believe that our results are the first to document these passive forces directly (Figure 3-6). Since we only became fully aware of the possible role of these passive forces in the data analysis phase of the study, no systematic tests were performed to elaborate on the origin and the mechanical properties of this extra passive force. However, this extra passive force was not associated with muscle damage, and it did not disappear as long as the muscle was held (passively) at the stretched length. Relaxing the muscle to a short length on the descending limb of the force-length relationship abolished the extra passive force immediately.

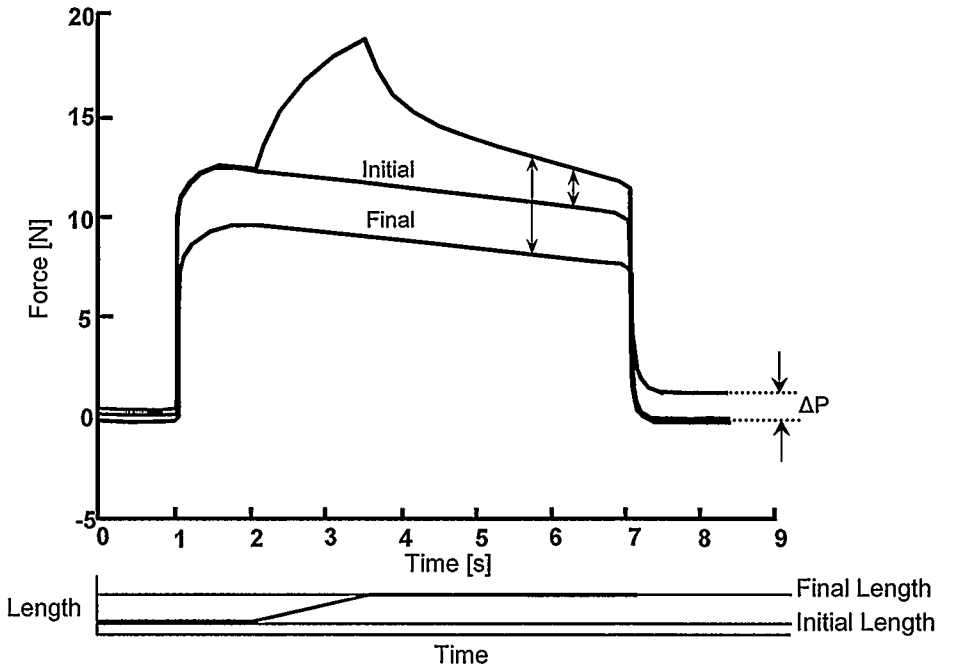

Figure 3-6. Force-time history of a representative isometric reference contraction a the final muscle length, the initial muscle length, and the corresponding force-time history of a 12 mm stretch to that final length. Note, the amount of force enhancement above the initial and final isometric forces (arrows). Note further, the amount of passive force enhancement at the end of stimulation, ΔP.

Summarizing, we propose that cat semitendinosus force production following active stretch is greater than the corresponding isometric force at the muscle length from which the stretch was started for many contractile conditions on the descending limb of the F-L relationship. This force enhancement appears to have an active and a passive component, and the passive component seems to be particularly significant at great muscle lengths and great distances of active stretch. Therefore, one might speculate that this passive force is specifically designed to prevent over-stretching and damage of an actively contracting muscle. It will be necessary to identify the mechanism, and the underlying structure, that produces this passive force enhancement, and to elaborate on the detailed interplay between active and passive force enhancement of skeletal muscle on the descending limb of the force-length relationship.

CHAPTER 4: The Effects of Muscle Stretching and Shortening on Isometric Forces on the Descending Limb of the Force Length Relationship

4.1 Introduction

History-dependent properties of force production in skeletal muscle have been characterized for over half a century (Abbott and Aubert, 1952). These properties have been observed on all structural levels investigated to date ranging from myofibrillar and single fibre preparations (Edman et al., 1978; 1982; 1993; Edman and Tsuchiya, 1996; Sugi and Tsuchiya, 1988) to whole muscles (Abbott and Aubert, 1952; Délèze, 1961; Maréchal and Plaghki, 1979; Herzog and Leonard, 1997; Morgan et al., 2000). Specifically, the steady-state isometric force is depressed following active muscle shortening, and is enhanced following active muscle stretching compared to the corresponding purely isometric forces.

Despite the general acceptance of force depression and force enhancement in dynamic contractions, these properties are rarely, if ever, considered in biomechanical models of skeletal muscle. Meijer et al. (1997) pointed out that the isometric force-length properties of rat medial gastrocnemius following shortening contractions differ greatly from those observed for purely isometric contractions. If correct, this observation could have far-reaching implications for the modeling of dynamic muscle behaviour. Unfortunately, the study by Meijer et al. (1997) was performed primarily on the ascending limb and plateau region of the force-length relationship where history-dependent properties are thought to be small at best, and non-existent at worst (Edman et al., 1978; 1982; Morgan et al., 2000). Furthermore, they only investigated the force-

length properties following shortening but not following stretch contractions, thereby ignoring any possible force enhancement effects on the force-length properties. Finally, the isometric forces following shortening were evaluated 350 ms after the end of the shortening contractions, a time that proved to be too short to reach "steady-state" isometric force values (e.g. Meijer et al., 1997, their figures 3B and 4B).

The purpose of this study was to systematically quantify the effects of active muscle shortening and stretching on the isometric force-length properties of mammalian skeletal muscle. Shortening and stretching was performed for magnitudes ranging from 3-9 mm, and for speeds ranging from 3-27 mm/s in cat soleus. The steady-state isometric force-length properties obtained following the dynamic (history-dependent) contractions were then compared to those obtained for force-length properties obtained in the classical way; purely isometric. All experiments (N = 10) were conducted on the plateau and descending part of the force-length relationship to maximize history-dependent properties. We hypothesized that dynamic contractions preceding isometric contractions would systematically influence the force-length properties. Specifically, we thought that the history-dependent effects would be related to the magnitude and speed of the dynamic stretch / shortening contractions and the position on the force-length relationship where the dynamic contractions finished.

4.2 Methods

Experiments were carried out on cat soleus (N = 10). The Life Sciences Animal Ethics Committee of the University of Calgary approved all experimental procedures.

4.2.1 Preparation

Outbred, adult, male cats (mass $\geq$ 3.5 kg) were anaesthetized using a 5% halothane gas mixture; then they were intubated and maintained during the experiment using 1.0 – 1.5% halothane. At the end of each experiment, animals were sacrificed by euthanol injection. The sciatic nerve with its partition into the tibial and common peroneal nerve was exposed underneath the biceps femoris using a single cut on the postero-lateral aspect of the thigh. A silicone cuff electrode was then surgically implanted on the tibial nerve, distal to the junction with the common peroneal nerve for soleus stimulation. Following nerve cuff implantation, the soleus was freed from the surrounding muscles, and the insertion of the soleus on the calcaneus was removed with a remnant piece of bone. Animals were secured in a prone position in a hammock, with the pelvis, thigh, and shank of the experimental hind limb fixed with bilateral bone pins in a stereotaxic frame. Body temperature was maintained at 36 degrees Celsius (± 1 °C) using an infrared heat lamp and a heating pad. The stereotaxic frame was fixed to a metal ground plate that was rigidly attached to an experimental table. This set-up prevented movement of the hind limbs during the experimental contractions. The remnant piece of bone at the distal end of the soleus tendon was rigidly attached to a muscle puller (MTS, Eden Prairie, MN, natural frequency > 10 kHz) in its physiological orientation.

4.2.2 Protocol

Soleus forces and length changes were continuously measured by the muscle puller at a sampling frequency of 200 Hz, and were stored on a 486 PC using CODAS data collection software. In order to attain fused tetanic contractions, without causing fatigue, 0.1 ms square wave pulses were administered to the tibial nerve at 30 Hz (Herzog and

Leonard, 1997). The stimulation voltage (1.2-2.0 volts) was set at a supramaximal value of three times the alpha motor unit threshold to ensure activation of all motor units (Herzog and Leonard, 1997). The stimulation duration was 9 seconds. A 1:30-minute rest period was enforced between contractions to allow for full recovery (Herzog and Leonard, 1997).

Before testing, the optimal length of the soleus was determined as the length at which soleus produced the maximal active isometric force. This length became the reference length, and is hereafter referred to as 0mm. All lengths greater than optimal are on the descending limb of the force-length relationship and are given as positive (Gordon et al, 1966; Morgan et al., 2000). For example, a muscle length of 9 mm refers to a length that is 9 mm greater than the 0 mm reference length.

After optimal length had been determined, stretches of 3, 6, and 9 mm amplitude (i.e. about 9, 18, and 27 % of the muscle fibre optimal length; AlAmood & Pope, 1972) were applied from three different starting lengths. Following the three sets of stretch tests, the muscle underwent shortening contractions of 3, 6, and 9 mm amplitudes from three different starting lengths on the descending limb of the force-length relationship. Two different speeds, 3 and 27 mm/s (i.e. about 9 and 81 % fibre length per second), were applied for the stretch and shortening contractions of 9 mm amplitude. Isometric reference contractions were performed at the initial and the final lengths of the dynamic contractions, before and after each experimental stretching and shortening test. Only tests in which the isometric reference contractions were the same (± 1.0 %) were considered for analysis.

The order of the stretch and shortening contractions for each series of tests were kept constant. For each series of tests (i.e. one data point), six contractions were performed: (i) two isometric reference contractions, one each at the initial and final lengths; (ii) two experimental stretch or shortening contractions (one active, one passive); and (iii), the two isometric reference contractions at the initial and final muscle lengths were repeated. Following one set of tests, isometric reference contractions at the new initial and final lengths were performed, and all procedures were repeated for the next stretch amplitude. Stretch contractions were always completed in the same order: 3, 6, and 9 mm, and the starting lengths always increased from one set of contractions to the next on the descending limb of the force-length curve. The active forces were calculated by subtracting the passive trial forces from those of the active trials in the last six experiments. For the first four experiments, passive trials were not performed, and the passive forces were estimated, by linear interpolation, from the relaxed muscles prior to and following the active stretch / shortening experiments.

4.2.3 Analysis

A total of 12 stretch and 12 shortening contractions from each of the muscles tested in this study were used for analysis. Data were analysed using Matlab software. Force enhancement and force depression measurements were taken 0.5 seconds prior to the cessation of stimulation for every trial (Figure 4-1a and 4-1b, respectively). This corresponded to a time point of 4.5 seconds following the end of the stretch or shortening contraction, when steady-state force conditions had been achieved, defined here once the force-time trace following active stretching or shortening was parallel to the corresponding isometric reference contractions. Force enhancement was calculated as

the difference between the steady-state, isometric, force following stretch and the purely isometric force at the corresponding muscle length (ΔF_E – Figure 4-1a). Passive force enhancement was calculated as the difference between the steady-state, isometric, passive force of the muscle following the experimental stretch contraction, and the corresponding passive force following the purely isometric reference contraction at the same muscle length (ΔF_P – Figure 4-1a). Force depression was calculated 4.5 seconds following the end of the shortening contraction as the difference between the steady-state, isometric, force following muscle shortening and the purely isometric force at the corresponding muscle length (ΔF_D – Figure 4-1b). In order to evaluate force depression and force enhancement across muscles, the recorded force values were normalized relative to the isometric reference force at the optimum length for each muscle.

Statistical analyses were performed using non-parametric tests, and linear regression analysis at a preset level of significance of $\alpha = 0.05$.

4.3 Results

4.3.1 Force Enhancement

Force enhancement was observed for all stretch conditions and all muscles tested in this study (Table 4-1, Figures 4-1a, 4-2, and 4-3). The isometric, steady-state forces following muscle stretching also remained greater than the purely isometric forces at the initial (short) length for all stretch conditions and muscles tested in this study (result not shown). Force enhancement was found to increase with increasing stretch magnitudes and increasing final muscle lengths, but was independent of the speed of stretch (Table 4-1, Figures 4-3 and 4-4).

The steady-state force enhancement following active muscle stretching was associated with a passive component (ΔF_P - Figures 4-1a and 4-2). The passive force enhancement increased with increasing stretch magnitude and final muscle length (Table 4-1, Figures 4-4 and 4-5). The average active force enhancement (that is the force enhancement following subtraction of the passive force enhancement) remained approximately the same for increasing stretch amplitudes for the stretches to 3 and 6 mm final lengths but decreased with increasing stretch amplitudes for the stretches to a final length of 9 mm (Table 4-1 and Figure 4-6).

4.3.2 Force Depression

Force depression was observed following all shortening contractions and in all muscles tested in this study (Table 4-2, Figures 4-1b, 4-7 and 4-8). Force depression was found to increase with increasing magnitudes of shortening and with decreasing final muscle lengths (Table 4-2 and Figure 4-8). Force depression observed at shortening speeds of 27 mm/s was less than that observed for shortening contractions of the same magnitude at 3 mm/s (Table 4-2).

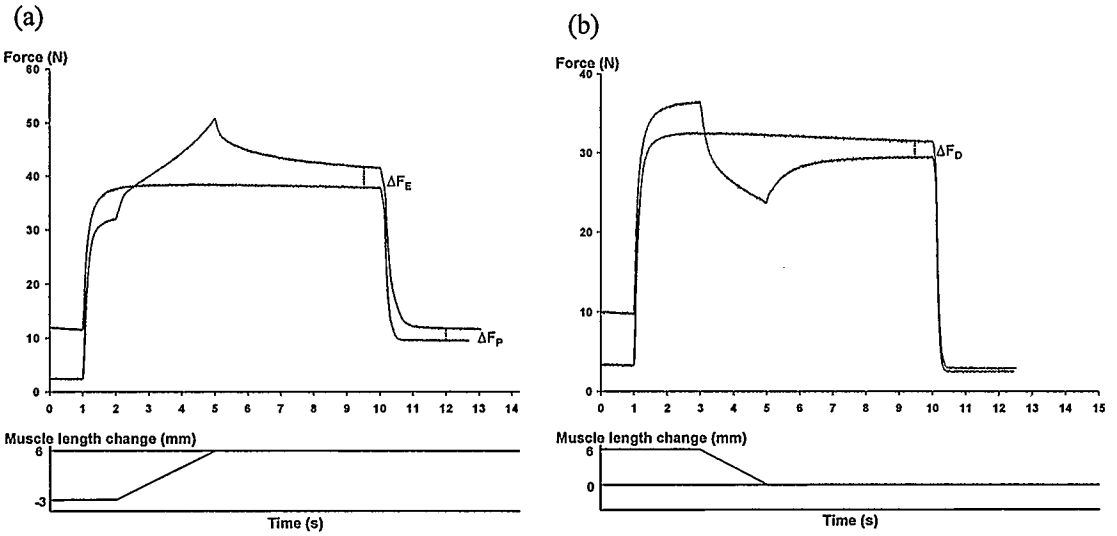

Figure 4-1. (a) Force enhancement, ΔF_E, was determined 4.5 seconds following the end of the stretch contraction as the difference between the steady-state, isometric, force following stretch and the purely isometric force at the corresponding muscle length. Passive force enhancement, ΔF_P, was determined as the difference between the steady-state, isometric, passive force of the muscle following the experimental stretch contraction, and the corresponding passive force following the purely isometric reference contraction at the same muscle length. Example result. (b) Force depression, ΔF_D, was determined 4.5 seconds following the end of the shortening contraction as the difference between the steady-state, isometric, force following shortening and the purely isometric force at the corresponding muscle length. Example result.

Table 4-1. Force enhancement, passive force enhancement, and active force enhancement for different stretch conditions following active muscle stretching of the cat soleus muscle on the descending limb of the force-length relationship.

Final muscle length (mm)	Force Enhancement (N)								
	3			6			9		
	ΔF_E	ΔF_P	AF_E	ΔF_E	ΔF_P	AF_E	ΔF_E	ΔF_P	AF_E
Magnitude of stretch (mm)									
3	2.0±0.7	0.4±0.7	1.6±0.9	1.9±0.9	0.6±0.3	1.3±0.6	3.3±2.5	1.0±0.6	2.3±2.0
6	2.8±0.7	1.1±0.4	1.7±0.5	3.2±1.0	1.7±0.7	1.5±0.5	4.7±2.8	3.6±3.8	1.1±1.3
9	3.1±0.7	1.3±0.5	1.8±0.5	3.8±1.1	2.3±0.9	1.5±0.5	5.8±3.5	4.9±4.7	0.9±1.3
9 (27mm/s)	3.3±0.9	1.7±0.7	1.6±0.5	3.9±1.3	2.7±1.2	1.2±0.4	5.2±3.4	4.6±4.7	0.6±1.5

Values are means ± 1 S.D. (N = 10)
Force enhancement and passive force enhancement increase significantly ($P < 0.05$) with increasing magnitudes of stretch and final muscle length but were not dependent on the speed of stretching.
ΔF_E = Force enhancement
ΔF_P = Passive force enhancement
AF_E = Active force enhancement (Force enhancement – Passive force enhancement)

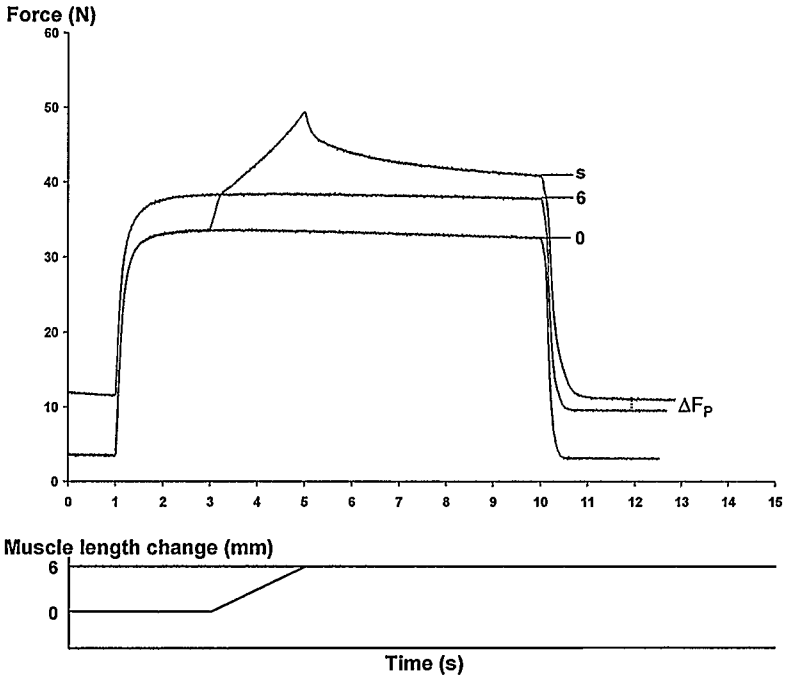

Figure 4-2. Representative force-time histories of two isometric and one experimental stretch contraction. The isometric contractions were performed at lengths of 0 and +6 mm (0 and 6, respectively). The stretch test (s) was performed from 0 to 6 mm at a constant speed of 3 mm/s. The steady-state isometric force following stretch was greater than the corresponding isometric reference force at the initial and final lengths. Note further, that the passive force following active stretch is greater than the corresponding passive force following the isometric contraction (ΔF_P).

4.3.3 Force-Length Relationship

The force-length relationships obtained using the steady-state isometric contractions following muscle shortening and stretching differ substantially from the force-length relationship obtained in the classical way; i.e., by purely isometric contractions (Figure 4-9). The relationships form a family of curves that are distinctly different depending on the magnitudes of the dynamic contractions. For the tests performed here, the maximal deviation from the isometric reference curve occurred for the 9 mm shortening contractions finishing at the 0 mm length (10.8 %), and for the 9 mm stretch conditions finishing at the 9 mm muscle length (19.8 %). At muscle lengths of 3 and 6 mm, where we obtained values for force depression and force enhancement, the maximal difference between the smallest and greatest isometric values following dynamic contractions were 17.5 % and 20.6 %, respectively.

Novel to the literature is the finding that the force enhancement following some stretch conditions was greater than the isometric reference force at the optimum muscle lengths (Figure 4-9). This result was found for the 3, 6, and 9 mm stretches finishing at the 3 mm length, the 6 and 9 mm stretches finishing at the 6 mm length, and the 9 mm stretch finishing at the 9 mm length.

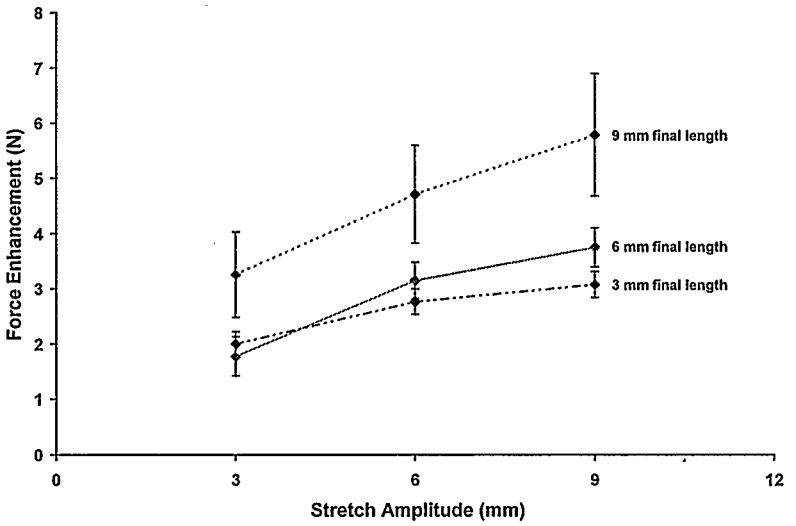

Figure 4-3. Average (N = 10, ± 1 SE) force enhancement as a function of stretch amplitude and final muscle length. All stretches were performed at a speed of 3 mm/s. Force enhancement increases with increasing stretch magnitude and with increasing final muscle length. The amount of force enhancement is statistically independent of the speed of muscle stretching (result not shown).

4.3.4 Active Force Enhancement

Subtraction of the passive force enhancement from the total force enhancement still produced active force enhancement for all stretch conditions and muscles tested in this study (Figure 4-10). However, the force enhancement above the isometric forces at optimum muscle length was lost. Also, the active force enhancements for the 3 and 6mm final muscle lengths were similar for all stretch magnitudes (Figure 4-6), indicating that the increase in total force enhancement with increasing stretch magnitudes was caused by the passive component of the

force enhancement, while the active component was unaffected. For the 9 mm final muscle length, the active force enhancements were similar for the 6 and 9 mm stretch amplitudes, but were greater than the 6 and 9mm stretches for the 3 mm stretch amplitude.

Table 4-2. Force depression following active shortening of cat soleus muscle on the descending limb of the force-length relationship as a function of amplitude of stretch and final muscle length.

	Force Depression (N)		
Final muscle length (mm)	6	3	0
Magnitude of shortening (mm)			
3	1.1±0.7	1.5±0.9	1.9±1.0
6	2.2±1.5	2.8±2.0	3.7±3.2
9	3.1±2.6	4.5±4.1	6.2±6.0
9 (27mm/s)	2.3±2.3	3.5±3.9	5.3±5.8

Values are means ± 1 S.D. (N=10)
Force depression increases significantly ($P<0.05$) with increasing magnitudes of shortening and decreasing final muscle length.

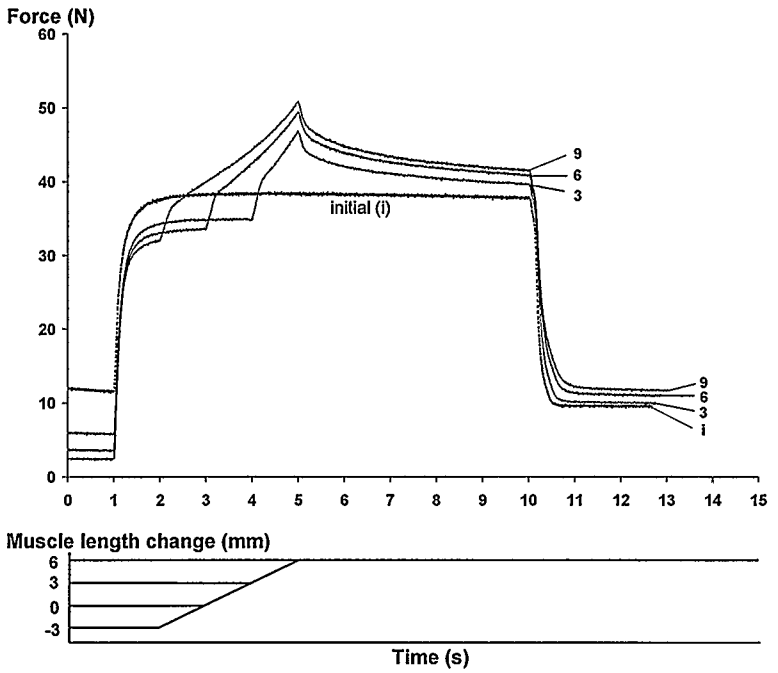

Figure 4-4. Force enhancement and passive force enhancement increased with increasing stretch magnitudes. Exemplar result for a single muscle and stretches of 3, 6, and 9 mm (3, 6, and 9 respectively) magnitudes executed at a speed of 3 mm/s (i = isometric reference contraction at the corresponding final muscle length of 6 mm).

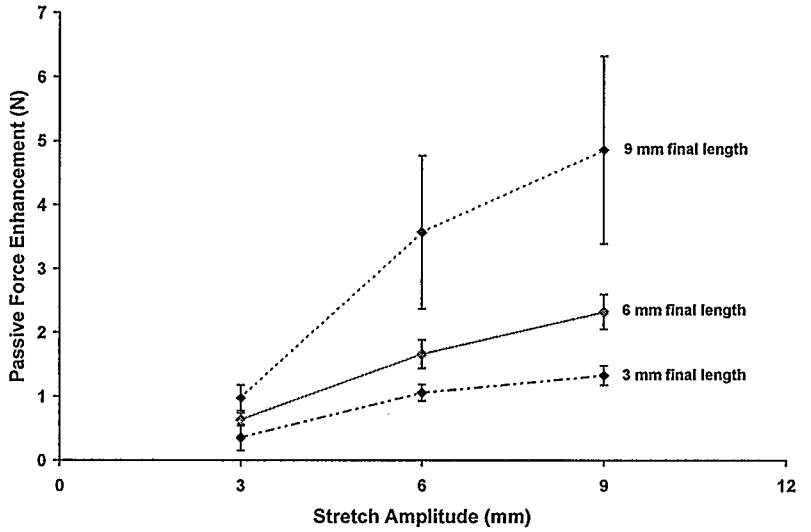

Figure 4-5. Average (N = 10, ± 1 SE) passive force enhancement as a function of stretch amplitude for final muscle lengths of 3 mm, 6 mm, and 9 mm (3, 6, and 9, respectively). Passive force enhancement increased with increasing stretch amplitudes and with increasing muscle lengths, but was not dependent on the speed of stretching (result not shown).

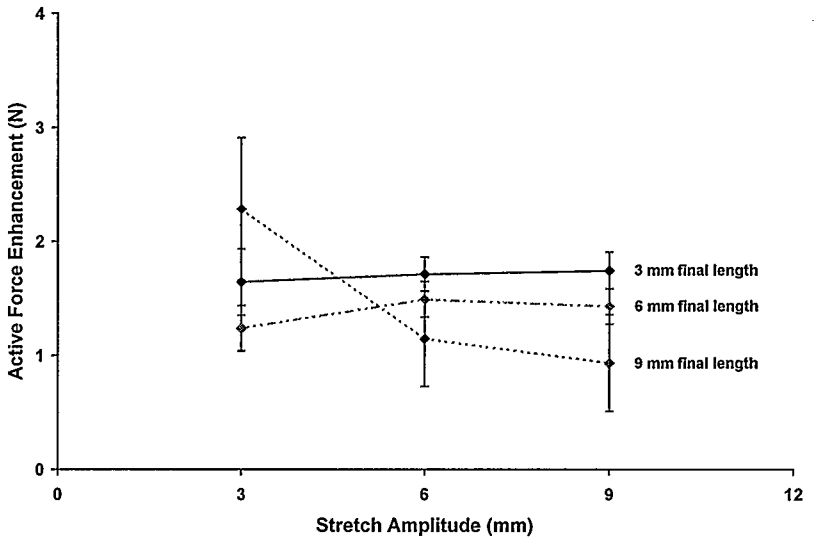

Figure 4-6. Average (N = 10, ± 1 SE) active force enhancement (the force enhancement remaining following subtraction of the passive force enhancement from the total force enhancement) as a function of stretch amplitude for final muscle lengths of 3 mm, 6 mm, and 9 mm, respectively. Active force enhancement remains approximately the same with increasing stretch amplitude for stretches to a final muscle length of 3 mm and 6 mm. Active force enhancement for stretches to a final muscle length of 9 mm decreases with increasing amplitudes of stretch.

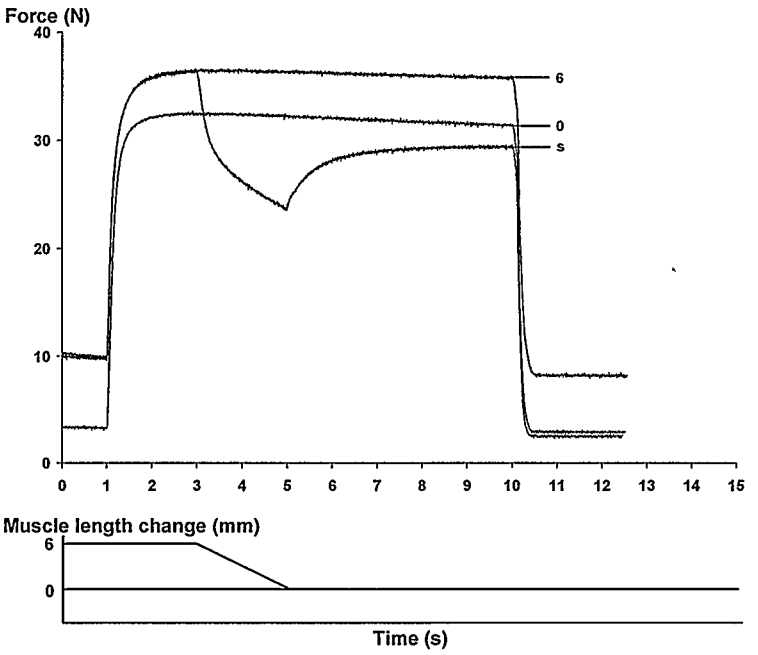

Figure 4-7. Representative force-time histories of two isometric and one experimental shortening contraction. The isometric contractions were performed at lengths of +6 and 0 mm. Shortening was performed from 6 mm to 0 mm at a constant speed of 3 mm/s. The 6 mm shortening contraction produced a steady-state active force that remained below the corresponding active isometric force at the final length (force depression).

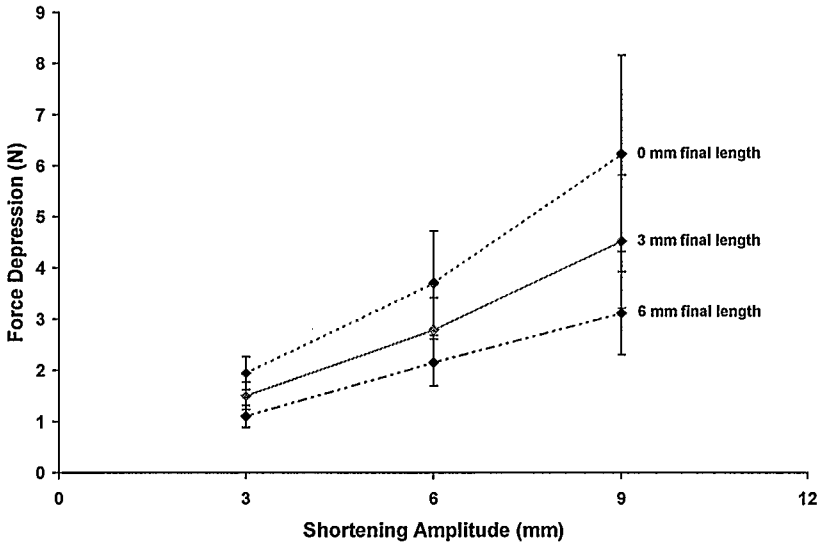

Figure 4-8. Average (N = 10, ± 1 SE) force depression as a function of shortening amplitude for final muscle lengths of 0 mm, 3 mm, and 6 mm. Force depression increases with increasing shortening magnitudes. Force depression was statistically smaller for 9 mm shortening at 27 mm/s compared with shortening of the same magnitude at 3 mm/s (result not shown).

4.4 Discussion

The isometric force-length relationship is arguably the most basic property of skeletal muscle force production, and it has been known for well over a century (Blix, 1893; 1894). Furthermore, the sarcomere force-length relationship found by Gordon et al. (1966) was one of the cornerstones for confirming predictions of the cross-bridge theory of muscle contraction (Huxley, 1957).

Typically, the force-length relationship is taken as an invariant property of muscle. However, Meijer et al. (1997) demonstrated that force-length properties are shifted following muscle shortening on the ascending limb compared to those obtained for purely isometric contractions. Here, we extend those findings to the plateau region and descending limb, and further demonstrate a shift of the force-length property opposite to those observed by Meijer et al. (1997) for muscle stretching. Specifically, we found that the force-length relationship following muscle shortening fell below the corresponding isometric relationship (Figure 4-9). Differences increase with increasing amounts of shortening preceding the isometric phase, and were more pronounced in the plateau region compared to the descending limb. Although not specifically tested, one might expect that the differences in the force-length relationships become even greater than those shown here (Figure 4-9) if the amplitude of shortening was increased, and / or if shortening was extended onto the ascending limb of the force-length relationship.

In contrast to the results by Meijer et al. (1997), the family of force-length relationships obtained following the shortening contractions typically showed a negative slope of the force-length relationship. The exclusively positive slopes found by Meijer et al. (1997) might have occurred because they worked on the plateau and ascending part of the force-

length relationship, or because their isometric contraction times following shortening were too short for forces to reach a true steady-state.

As for the shortening contractions, muscle stretch shifted the force-length relationship, but in this case to greater values than those obtained for the purely isometric force-length property. The steady-state forces following stretching increased with the magnitude of stretch, as had been shown previously (Abbott and Aubert, 1952; Edman et al., 1978; Edman and Tsuchiya, 1996; Edman, 1999; Schachar et al., 2002), but also increased as a function of the final muscle length.

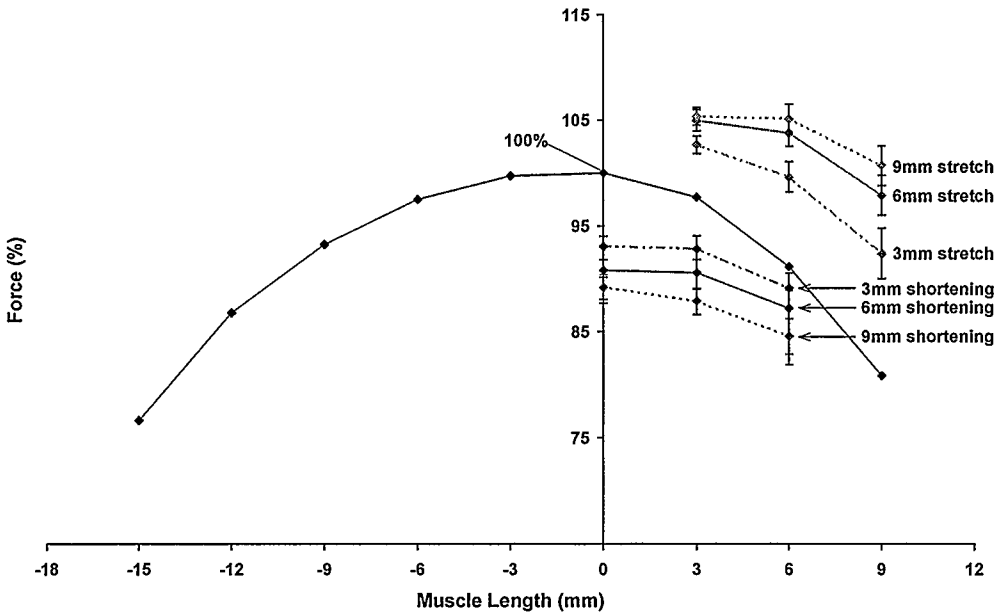

Figure 4-9. Mean (N = 10, ± 1 SE) force-length relationship and the corresponding steady-state, isometric, force-length curves following stretching and shortening on the descending limb. (a) Maximal deviation of force depression from isometric was 10.8% (9mm shortening, final muscle length 0 mm), (b) maximal deviation of force enhancement from the isometric curve was 19.8% (9 mm stretch, final muscle length 9 mm) and , (c) maximal deviation between force enhancement and force depression was 20.6% (difference between 9 mm stretch and 9 mm shortening at a final muscle length of 6 mm). Force enhancement following many of the stretch conditions exceeded the maximal isometric force at the optimum muscle length (100%).

In summary, there appears to be a family of steady-state isometric force-length relationships. These relationships differ depending on the contractile history preceding the isometric contractions. Shortening shifts the force-length relationship to smaller force values, and stretching shifts the force-length relationship to greater force values, than those obtained in the classical way; for purely isometric contractions.

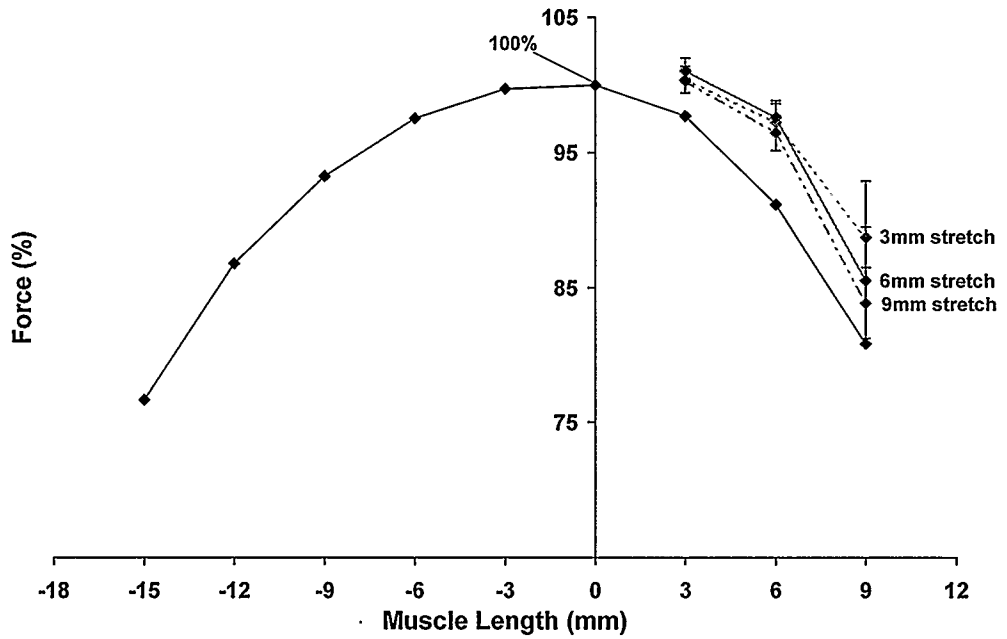

Figure 4-10. Mean (N = 10, ± 1 SE) force-length relationship and the corresponding steady-state isometric force-length curves following stretching on the descending limb after subtraction of the passive force enhancement. Forces are always at about, or below, 100% following subtraction of the passive force enhancement.

Although not the focus of this study, two surprising results need to be addressed. First, for some stretch conditions, the isometric, steady-state forces following the stretch exceeded the purely isometric forces on the plateau region (or optimum muscle length) (Figure 4-9). For decades, it had been argued that force enhancement following stretch could not exceed the

isometric plateau (Edman et al., 1982), because sarcomere length non-uniformities following stretching were thought to cause the force increases following muscle stretching. Independent of how one views the sarcomere length non-uniformity theory, forces can never be greater than those of a sarcomere at optimum length (Edman et al., 1982; Morgan et al., 2000).

Second, and as it will turn out, somewhat related to the first point, we found in this study that a passive component contributes to the force enhancement following stretch. Although it had been hypothesized previously that force enhancement might be caused by the engagement of a passive structure at the length of muscle activation (Edman et al., 1982; Edman and Tsuchiya, 1996), we have provided first systematic proof of the existence of such a passive force enhancement (Table 4-1, Figures 4-1a, 4-2, 4-4, and 4-5). When subtracting the passive component of force enhancement from the total force enhancement (Figure 4-10), the average force enhancement reaches maximal values of about 100 % (i.e. maximal force at optimum length) but not beyond. We interpret these results as follows: there is an active and a passive component of force enhancement. The active component allows for force enhancement following muscle stretch up to the isometric plateau, but not beyond. The passive component allows for an extra force, so that the isometric plateau forces can be exceeded. Finally it appears that the active component of the force enhancement is not influenced much by the magnitude of stretching (Figure 4-6). The increased force enhancement with increasing stretch magnitudes seems to be primarily produced by increases in the passive component of force enhancement (Table 4-1, Figures 4-9 and 4-10). The detailed interaction between passive and active force enhancement, the structural origin of the passive component of force enhancement, and a qualitative interpretation of force

enhancement above the isometric plateau force need to be investigated carefully in future studies.

4.5 Conclusion

The results of this study led to the conclusion that (a) force enhancements following active muscle stretch are composed of an "active" and a "passive" component, (b) that the observed "passive" component of force enhancement allows for forces, following some stretch conditions, to be greater than the purely isometric forces on the plateau region, and (c) that the magnitude of force enhancement and depression are dependent on the final muscle length.

CHAPTER 5: SUMMARY AND CONCLUSION

History-dependent effects have been observed consistently in a variety of muscle preparations, including whole muscle (e.g. Abbott and Aubert, 1952; Maréchal & Plaghki, 1979; Morgan et al., 2000), single fibre (Edman et al., 1978, 1982, 1984, 1993; Sugi and Tsuchiya, 1988; Edman and Tsuchiya, 1996) and in vivo human muscle (Lee et al., 2001; Lee et al., 2002). To date there is no generally accepted explanation for the observed force enhancement following active muscle stretching. Although many theories have been proposed, the sarcomere length non-uniformity theory has received the greatest support over the past twenty years (Edman et al., 1982, Edman and Tsuchiya, 1996; Julian and Morgan, 1979; Morgan, 1990; Morgan, 1994; Morgan et al., 2000). The sarcomere length non-uniformity theory has the advantage that it allows for specific predictions; however, many of these predictions are not supported by experimental evidence. One such prediction is that the steady-state forces following active muscle stretching on the descending limb of the force-length relationship cannot exceed the purely isometric force at the initial muscle length (e.g. Morgan, 1990, Zahalak, 1997; Morgan et al., 2000). However, it has been shown, for single frog fibres, that the steady-state force enhancement exceeds the purely isometric force at the initial muscle length for some stretch conditions (Edman et al., 1978; 1982). These observations were the initial motivation for this thesis work.

Therefore, the purpose of this research was:

1) To test for force enhancement as a function of muscle length, amount of stretch, and speed of stretch on the descending limb of the force-length relationship in an in-situ whole muscle preparation and,

2) To test the effects of history-dependent properties of skeletal muscle, force depression and force enhancement, on the isometric forces on the descending limb of the force-length relationship.

5.1 Summary of Results

In chapter 3, the existence of force enhancement above the initial isometric force following active muscle stretching on the descending limb of the force-length relationship in an in-situ whole muscle preparation was addressed. Steady-state force enhancement above the force produced at the initial muscle length was observed in 109 of 131 tests and was more likely to occur following stretches of great compared to small amplitude. Force enhancement increased with increasing amount of stretch. Following active stretching the passive muscle forces were observed to be significantly greater than the passive forces for the isometric contractions or passive stretches. This passive force enhancement was observed at the longest muscle lengths tested and ranged from 0.2N-6.0N.

In chapter 4, the focus was on investigating the effect of active muscle stretching and shortening on isometric forces on the descending limb of the force-length relationship. A shift in the force-length relationship to greater force values following muscle stretching, and to smaller force values following muscle shortening, was observed. The upward and downward shifts crucially depended on the amount of stretching or shortening, as well as the final muscle length. Interesting, and novel to the literature, were the observations that the steady-state isometric force enhancement, for some stretch conditions, exceeded the maximal isometric forces at the optimal muscle length. As well, force enhancement was associated with an increase in passive force, and when this passive force enhancement

was subtracted from the total force enhancement the forces following the stretch were always about equal to or smaller than the isometric force at optimum muscle length. Force enhancement above the initial (and therefore also the final) muscle length was observed for all stretch conditions and all muscles tested in this study. As found in other studies, force enhancement increased with increasing stretch magnitudes and increasing final muscle length on the descending limb of the force-length relationship, but was independent of the speed of stretch (Edman et al., 1978; Edman et al., 1982; Sugi and Tsuchiya, 1988). Force depression was observed for all shortening contractions and all muscles tested in this study. The observed force depression increased with increasing magnitudes of shortening and decreasing final lengths, and was found to be less for fast (27mm/s) compared to slow speeds of shortening (3mm/s) for the same magnitude of shortening.

5.2 General Discussion and Conclusion

Force enhancement following active muscle stretching and force depression following active muscle shortening are well defined and well accepted properties of skeletal muscle (Abbott and Aubert, 1952; Edman et al., 1978; Edman et al., 1982; Herzog and Leonard, 1997; Maréchal and Plaghki, 1979; etc). Although these phenomena seem to be well defined, a conclusion regarding the mechanism of force enhancement and force depression has yet to be agreed upon. The sarcomere length non-uniformity theory has been repeatedly proposed as the primary mechanism for both force enhancement following muscle stretching and force depression following muscle shortening (Edman et al., 1982; Edman et al., 1993; Edman and Tsuchiya, 1996; Julian and Morgan, 1979; Morgan, 1990; Morgan, 1994; Morgan et al., 2000). However, based on the results of

this study it does not seem intuitively correct that the same mechanism can describe both phenomena. Force enhancement increased with greater magnitudes of stretching and with longer final muscle lengths, whereas force depression increased with greater magnitudes of shortening but with shorter final muscle lengths (i.e. lengths where there is increasing force during shortening). It was concluded that the observed increase in force enhancement with increasing stretch magnitudes was primarily produced by an increase in the passive component of force enhancement, as passive force enhancement was also seen to increase with greater magnitudes of stretching and with longer final muscle lengths. Passive forces did not seem to contribute to force depression following active muscle shortening.

Meijer et al. (1997) explained that the observed shifts in the force-length relationship and the phenomenon of force depression following active muscle shortening were likely related to the processes within the muscle fibres themselves. Although, the sarcomere length non-uniformity theory has been previously proposed to explain the post shortening force decrease (Edman, 1993; Granzier and Pollack, 1989; Julian and Morgan, 1979; Sugi and Tsuchiya, 1988), several observations have been made that are incompatible with the predictions of this theory. These include observations of force depression on the ascending limb of the force-length relationship, where sarcomere length non-uniformities have been said not to occur (Allinger et al., 1996; Morgan, 1990; Morgan et al., 2000; Zahalak, 1997); force depression in sarcomere clamped experiments where sarcomere length uniformity was enforced (Granzier and Pollack, 1989); and a decrease in fibre stiffness during force depression (Sugi and Tsuchiya, 1988).

A decrease in muscle fibre stiffness may indicate a decrease in the proportion of attached cross-bridges, which in turn can be explained by an inhibition of cross-bridge formation in the newly formed overlap zone between the actin and myosin filaments during active muscle shortening. Therefore, inhibition of cross-bridge formation may be a possible mechanism for the observed force depression. Based on this theory, force depression should increase with increasing amplitudes of shortening and increasing force during shortening (Abbott and Aubert, 1952; De Ruiter et al., 2000; Maréchal and Plaghki, 1979; Morgan et al., 2000). This increased force depression with increasing magnitudes of shortening and with decreasing final muscle lengths was observed in our study (Chapter 4, Figure 9). Specifically, shortening magnitudes of 3 mm always produced less pronounced force depression than 6 mm of shortening, which in turn produced less force depression than 9 mm shortening for the same final muscle length.

Interestingly, force depression increased with decreasing muscle lengths, meaning that force depression was seen to be greatest at about optimal (final) length. It is well known that force depression is very small on the ascending limb of the force-length relationship (Herzog and Leonard, 1997; Maréchal and Plaghki, 1979). Therefore, force depression seems to be maximal at about optimal length, but seems to decrease on the ascending and the descending limbs. This observation fits well into the theory of stress-induced force depression, first reported by Maréchal and Plaghki (1979), as those authors suggested that the force during shortening was directly related to the amount of force depression. Obviously, one might expect the force of shortening (for a given amount and speed of shortening) to be greatest around the plateau region of the force-length relationship, which is in agreement with our observations.

The novel force enhancement results from the two experiments performed in this thesis lead to the conclusion that force enhancement following active muscle stretching on the descending limb of the force-length relationship is associated with a passive component. It can be speculated that this passive component allows for the isometric steady-state force following stretching to reach values greater than the isometric values at optimal muscle length (Chapter 4, Figure 9). When a relaxed muscle is stretched, it develops a passive tension as a function of length. This passive tension is an important component of normal limb movements, and it is known that this passive force comes primarily from the muscle protein titin. However, when the whole muscle is actively stretched to long lengths on the descending limb of the force-length relationship, it is likely that a passive component, parallel to the actin-myosin cross-bridge interactions, engages, contributing to the increase in the observed force. To our knowledge, these are the first experiments where this passive force enhancement has been observed, however it is not known where this passive force enhancement comes from.

Titin is an extremely large connecting filament protein, which was first isolated, by Maruyama and colleagues in 1976 (cited from Pollack, 1990). Titin consists primarily of approximately 300 immunoglobulin (Ig) and related fibronectin type III repeats, and a unique proline (P), glutamate (E), valine (V) and lysine (K) rich (PEVK) domain (Kellermayer et al., 1997) (figure 5-1). Part of the titin molecule overlaps the myosin filament and is firmly attached to it at the M-line, while the remaining part extends through the I-band region connecting at the Z-line (figure 5-1). Due to its structure and position, titin is believed to function as a longitudinal stabilizer for the myosin filament,

keeping it in the middle of the sarcomere during contraction, when small asymmetries in pulling forces are produced on the two halves of the myosin filament (Herzog, 2000; Kellermayer et al., 1997; Labeit and Kolmerer, 1995). Titin has also been proposed to contribute to the elasticity of the muscle when it is stretched either actively or passively, particularly in the plateau and descending limb regions of the force-length relationship. The portion of the titin molecule located in the I-band region has been proposed to be highly flexible and elastic along its entire length (Labeit and Kolmerer, 1995). The flexibility and elasticity of titin is related to the coiling of the tandem Ig domain and the PEVK domain (figure 5-1). It has been shown that in skeletal muscle fibre preparations, passive tension first becomes apparent at sarcomere lengths at which the tandem Ig segment of titin has almost ceased to extend (Labeit and Kolmerer, 1995; Linke et al, 1996). With further sarcomere extension, there is a steady increase in passive tension, believed to be due to the PEVK region of the titin molecule (Labeit and Kolmerer, 1995; Linke et al, 1996; Linke and Granzier, 1998). Therefore, it has been proposed that the PEVK domain of titin represents a relatively stiff spring that is primarily responsible for the extensibility of skeletal myofibrils during large magnitudes of stretching. It is these characteristics of titin that make it a prime candidate for the provision of the passive force enhancement at long muscle lengths, and the force enhancement above the isometric plateau seen in these studies.

I would like to speculate that, as the muscle is actively stretched on the descending limb of the force-length relationship, forces are initially produced by the cross-bridges, however as the muscle continues to be stretched and there is less actin-myosin overlap, there is an increase in passive force enhancement production due to titin. This increase in

passive force enhancement must be attributed to an increase in stiffness of the titin molecule. It is not known what causes this increase in titin stiffness during active stretch, however several possibilities exist. It could be that there exists an interaction between the titin molecule and the actin filament, which upon active muscle stretch would resist increasing sarcomere lengthening resulting in increased stiffness and an increased production of passive force enhancement. This titin-actin interaction has been observed previously and is described to act to anchor titin to the Z-line, thereby increasing the structural integrity of the sarcomere (Granzier et al., 1997).

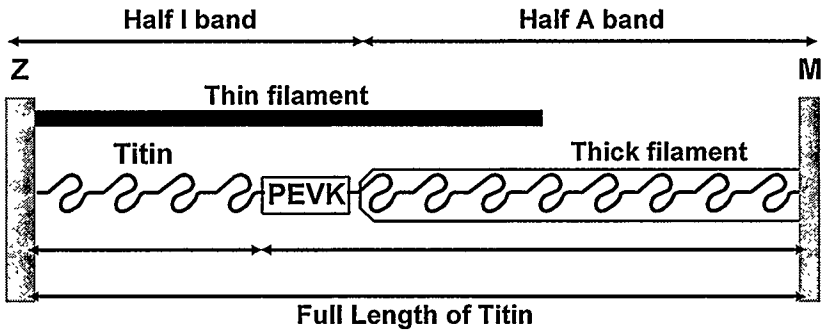

Figure 5-1. Schematic illustration of titin and its relationship with other structures within the half-sarcomere. Titin is a large protein consisting primarily of immunoglobulin, fibronectin type III repeats and a proline (P), glutamate (E), valine (V), and lysine (K)-rich (PEVK) domain. This molecule spans through the half-sarcomere from the M-band to the Z-line. (Adapted from Kellermayer et al., 1997).

Another possibility is that there exists a calcium-titin interaction. The titin filament has been observed to have a high affinity for calcium ions (Tatsumi et al., 2001; Wang and Greaser, 1985) therefore, it may be that when the muscle is actively stretched, calcium

binds to titin, either in the PEVK region or in the Ig region, resulting in an alteration to the configuration of these proteins that would cause an increase in titin filament stiffness. As seen in the results of the studies presented here, the production of passive force enhancement increases at longer muscle lengths and for great amplitudes of active stretching. This increase in passive force enhancement may be designed to act as a protective mechanism in order to prevent damage and injury to the muscle.

5.3 Significance

The research conducted for this thesis provides new information on the history-dependent phenomena of force enhancement and force depression on the descending limb of the force-length relationship. I expect that the novel observations made in this work will contribute, if only in a small way, to the history-dependent properties of skeletal muscle biomechanics. More importantly, I hope that these findings can provide some new insight into the possible mechanism of skeletal muscle force enhancement following active muscle stretching and force depression following active muscle shortening.

5.4 Future Directions

This thesis was aimed at 1) describing force enhancement following active muscle stretching in an in-situ whole muscle preparation in order to determine whether the steady-state forces produced could remain higher than the purely isometric forces for the initial isometric contraction and, 2) describing the history-dependent effects in an in-situ whole muscle preparation on the isometric forces on the descending limb of the force-length relationship. It is not easy to extrapolate possible mechanisms for the observed

force enhancement and force depression from the results of this thesis. Here we can only make speculations.

A limitation of these studies includes the use of an in-situ whole muscle preparation. While an in-situ preparation is important for understanding how the whole muscle works, working with a whole muscle preparation does not allow observations to be made regarding the possible contribution of titin, or other structural proteins, to the passive force enhancement.

Therefore, future work should be done with a primary focus on the development of passive force enhancement during active muscle stretching as a contributing mechanism for force enhancement. It would be important to determine the role titin may play during active muscle stretching of large amplitudes and to long muscle lengths on the descending limb of the force-length relationship in a single fibre preparation, if and how titin interacts with the actin filament, and how the stiffness of titin changes (i.e. how calcium binding and configuration changes may contribute to increased titin stiffness, if at all). I look forward to the future of skeletal muscle biomechanics. I believe that in the near future many new and interesting discoveries will be made.

REFERENCES

Abbott, B. C., Aubert, X. M., 1952. The force exerted by active striated muscle during and after change in length. Journal of Physiology 117, 77-86.

Allinger, T. L., Epstein, M., Herzog, W., 1996. Stability of muscle fibers on the descending limb of the force-length relation. A theoretical consideration. Journal of Biomechanics 29, 627-633.

Allinger, T.L., Herzog, W., ter Keurs, H.E.D.J., Epstein, M., 2000. Sarcomere length non-uniformities and stability on the descending limb of the force-length relation of mouse skeletal muscle. Herzog, W., Skeletal Muscle Mechanics: From Mechanisms to Function. John Wiley and Sons, Chichester, pp. 455-474.

Al-Amood, W. S., Pope, R., 1972. A comparison of the structural features of muscle fibres from a fast and slow-twitch muscle of the pelvic limb of the cat. Journal of Anatomy 113, 49-60

Blix, M., 1893. Die Länge und die Spannung des Muskels. Skandinavisches Archiv für Physiologie 4, 399-409

Blix, M., 1894. Die Länge und die Spannung des Muskels. Skandinavisches Archiv für Physiologie 5, 149-206.

Délèze, J. B., 1961. The mechanical properties of the semitendinosis muscle at lengths greater than its length in the body. Journal of Physiology 158, 154-164.

De Ruiter, C. J., de Haan, A., Jones, D. A., Sargeant, A. J., 1998. Shortening-induced force depression in human adductor pollicis muscle during stretch and the effects of fatigue. Journal of Physiology 526, 671-681.

De Ruiter, C.J., Didden, W.J.M., Jones, D.A., De Haan, A., 2000. The force-velocity relationship of human adductor pollicis muscle during stretch and the effects of fatigue. Journal of Physiology 526. 3, 671-681.

Edman, K. A. P., 1999. The force bearing capacity of frog muscle fibres during stretch: its relation to sarcomere length and fibre width. Journal of Physiology 519.2, 515-526.

Edman, K. A. P., Caputo, C., Lou, F., 1993. Depression of tetanic force induced by loaded shortening of frog muscle fibres. Journal of Physiology 466, 535-552.

Edman, K. A. P., Elzinga, G., Noble, M., 1978. Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. Journal of Physiology 281, 139-155.

Edman, K. A. P., Elzinga, G., Noble, M. I. M., 1982. Residual force enhancement after stretch of contracting frog single muscle fibers. Journal of General Physiology 80, 769-784.

Edman, K. A. P., Reggiani, C., 1984. Redistribution of sarcomere length during isometric contraction of frog muscle fibres and its relation to tension creep. Journal of Physiology 351, 169-198.

Edman, K. A. P., Tsuchiya, T., 1996. Strain of passive elements during force enhancement by stretch in frog muscle fibres. Journal of Physiology 490.1, 191-205.

Forcinito, M., Epstein, M., Herzog, W., 1998. Can a rheological muscle model predict force depression/enhancement? Journal of Biomechanics 31, 1093-1099.

Gordon, A. M., Huxley, A. F., Julian, F. J., 1966. The variation is isometric tension with sarcomere length in vertebrate muscle fibres. Journal of Physiology 184, 170-192.

Granzier, H., Kellermayer, M., Helmes, M., Trombitás, K., 1997. Titin elasticity and mechanism of passive force develipment in rat cardiac myocytes probed by thin-filament extraction. Biophysical Journal 73, 2043-2053.

Granzier, H. L. M., Pollack, G. H., 1989. Effect of active pre-shortening on isometric and isotonic performance of single frog muscle fibres. Journal of Physiology 415, 299-327.

Granzier, H. L. M., Pollack, G. H., 1990. The descending limb of the force-sarcomere length relation of the frog revisited. Journal of Physiology 421, 595-615.

Herzog, W., 1998. History dependence of force production in skeletal muscle: a proposal for mechanisms. Journal of Electromyography 8, 111-117.

Herzog, W., 2000. Cellular and Molecular Muscle Mechanics. Herzog, W., Skeletal Muscle Mechanics: From Mechanisms to Function. John Wiley and Sons, Chichester, pp. 33-52.

Herzog, W., Leonard, T.R., 1997. Depression of cat soleus forces following isokinetic shortening. Journal of Biomechanics 30, 865-872.

Herzog, W., Leonard, T. R., 2000. The history dependence of force production in mammalian skeletal muscle following stretch-shortening and shortening stretch cycles. Journal of Biomechanics 33, 531-542.

Herzog, W., Leonard, T. R., 2002. Force enhancement following stretching of skeletal muscle: a new mechanism. Journal of Experimental Biology 205, 1275-1283.

Herzog, W., Leonard, T.R., Wu, J. Z., 2000. The relationship between force depression following shortening and mechanical work in skeletal muscle. Journal of Biomechanics 33, 659-668.

Herzog, W., ter Keurs H. E.D. J., 1988. Force-length relation of in-vivo human rectus femoris muscles. European Journal of Physiology 411, 642-647.

Hill, A.V., 1938. The heat of shortening and the dynamic constants of muscle. Proceedings of the Royal Society London 126, 136-195.

Hill, A.V., 1953. The mechancis of active muscle. Proceedings of the Royal Society London 141, 104-117.

Huxley, A. F., 1957. Muscle structure and theories of contraction. Progress in Biophysics and Biophysical Chemistry. **7,** 255-318.

Huxley, A. F., 1974. Muscular contraction. Journal of Physiology 243, 1-43.

Huxley, A.F., Niedergerke, R. 1954. Structural changes in muscle during contraction. Interference microscopy of living muscle fibres. Nature 173, 971-973.

Huxley, A. F., Peachey, L. D., 1961. The maximal length for contraction in vertebrate striated muscle. Journal of Physiology. 165, 150-165.

Huxley, A. F., Simmons, R. M., 1971. Proposed mechanism of force generation in striated muscle. Nature 233, 533-538.

Huxley, H. E., Hanson, J. 1954. Changes in cross-striations of muscle during contraction and stretch and their structural implications. Nature 173, 973-976.

Julian, F. J., Morgan, D. L., 1979. The effect on tension of non-uniform distribution of length changes applied to frog muscle fibres. Journal of Physiology 293, 379-392.

Kellermayer, M. S. Z., Smith, S. B., Granzier, H. L., Bustamante, C., 1997. Folding-unfolding transitions in single titin molecules characterized with laser tweezers. Science 276, 1112-1116.

Labeit, S., Komerer, B., 1995. Titins: giant proteins in charge of muscle ultrastructure and elasticity. Science 270, 293-296.

Linari, M., Lucii, L., Reconditi, M., Vannicelli Casoni, M. E., Amenitsch, H., Bernstorff, S., Piazzesi, G., 2000. A combined mechanical and x-ray diffraction study of stretch potentiation in single frog muscle fibres. Journal of Physiology 526, 589-596.

Linke, W. A., Granzier, H., 1998. A spring tale: new facts on titin elasticity. Biophysical Journal 75, 2613-2614.

Linke, W. A., Ivemeyer, M., Olivieri, N., Kolmerer, B., Rüegg, J. C., Labeit, S., 1996. Towards a molecular understanding of the elasticity of titin. Journal of Molecular Biology 261, 62-71.

Maréchal, G., Plaghki, L., 1979. The deficit of the isometric tetanic tension redeveloped after a release of frog muscle at a constant velocity. Journal of General Physiology 73, 453-467.

Meijer, K., Grooternboer, H. J., Koopman, B. F. J. M., Huijing, P., 1997. Fully isometric length-force curves of rat muscle differ from those during and after concentric contractions. Journal of Applied Biomechanics 13, 164-181.

Morgan, D. L., Mochon, S., Julian F. J., 1982. A quantitative model of intersarcomere dynamics during fixed-end contractions of single frog muscle fibers. Biophysical Journal 39, 189-196.

Morgan, D. L., 1990. New insights into the behavior of muscle during active lengthening. Biophysical Journal 57, 209-221.

Morgan, D. L., 1994. An explanation for residual increased tension in striated muscle after stretch during contraction. Experimental Physiology 79, 831-838.

Morgan, D. L., Whitehead, N.P., Wise, A. K., Gregory, J.E., Proske, U., 2000. Tension changes in the cat soleus muscle following slow stretch or shortening of the contraction muscle. Journal of Physiology 522, 503-513.

Noble, M. I. M., 1992. Enhancement of mechanical performance of striated muscle by stretch during contraction. Experimental Physiology 77, 539-552.

Pollack, G. H., 1990. Muscles & Molecules: Uncovering the Principles of Biological Motion. Ebner & Sons Publishers, Seattle. pp. 43-44.

Rassier, D. E., Herzog, W., Wakeling, J., Syme, D., 2002. Stretch-induced, steady-state force enhancement in single skeletal muscle fibers exceeds the isometric force at optimal fibre length. Journal of Biomechanics (In Press)

Schachar, R., Herzog, W., Leonard, T., 2002. Force enhancement above the initial isometric force on the descending limb of the force-length relationship. Journal of Biomechanics 35, 1299-1306.

Sherwood, L, 1996. Muscle Physiology. Human Physiology: From Cells to Systems. Wadsworth, Belmont, CA., pp. 215.

Sugi H., Tsuchiya T., 1988. Stiffness changes during enhancement and deficit of isometric force by slow length changes in frog skeletal muscle fibres. Journal of Physiology 407, 215-229.

Talbot, J.A., Morgan, D.L., 1996. Quantitative analysis of sarcomere non-uniformities in active muscle following a stretch. Journal of Muscle Research and Cell Motility 17, 261-268.

Tatsumi, R., Maeda, K., Hattori, A., Takahashi, K., 2001. Calcium binding to an elastic portion of connectin / titin filaments. Journal of Muscle Research and Cell Motility 22, 149-162.

ter Keurs, H. E. D. J., Iwazumi, T., Pollack, G.H., 1978. The sarcomere length-tension relation in skeletal muscle. Journal of General Physiology 72, 565-592.

Zahalak, G.I., 1997. Can muscle fibers be stable on the descending limbs of their sarcomere length-tension relations? Journal of Biomechanics 30, 1179-1182

Wang, S. M., Greaser, M., 1985. Immunocytochemical studies using a monoclonal antibody to bovine cardiac titin on intact and extracted myofibrils. Journal of Muscle Research and Cell Motility 6, 293-312.