Item Number: 6   **Publisher:** 2002.

9612

Description: Factors affecting the passive mechanical properties of skeletal contractions [manuscript]/Whitehead, N

Line Item Note: AMIS H24/3523 Thesis (Ph.D.)--Monash University, 2002.

Vendor Title/Number:

Copies: 1   **Cost:**   0.00 **Adjustments:**   0.00 **Less Pre**

Factors affecting the passive mechanical properties of skeletal muscle: thixotropy and eccentric contractions.

by

Nicholas P. Whitehead

B.Sc. (Hons)

A thesis submitted for the degree of Doctor of Philosophy

Department of Physiology

Monash University

July 2002

Declaration

I hereby declare that all work presented in this thesis is my own, and to the best of my knowledge, it contains no material previously published or written by any other person, except where due reference or acknowledgement is made in the text.

Nicholas P. Whitehead

Acknowledgements

This thesis was completed under the supervision of Professor Uwe Proske and Dr. David Morgan. I am very grateful for their continued support and guidance, and the invaluable intellectual contribution they have provided towards my research.

Special thanks to Dr. Ed Gregory, for his technical expertise and many useful discussions. Thanks also to Dr. James Fallon, Dr. Camilla Brockett and Emily Mifsud for their help and advice.

I would like to acknowledge Nivan Weerakkody, who collaborated with me on the experiments in Chapter 3, and Dr. Camilla Brockett, who was involved in the tendon organ experiments described in Chapter 4.

Thanks to James, Camilla and Andrew, my good friends and colleagues, for the many memorable social occasions.

Finally, I would like to offer my warmest appreciation to my family, for their love and support.

Publications

GREGORY, J. E., BROCKETT, C. L., MORGAN, D. L., WHITEHEAD, N. P. & PROSKE, U. (2002). Effect of eccentric muscle contractions on Golgi tendon organ responses to passive and active tension in the cat. *Journal of Physiology* **538**, 209-218.

WHITEHEAD, N. P., GREGORY, J. E., MORGAN, D. L. & PROSKE, U. (2001). Passive mechanical properties of the medial gastrocnemius muscle of the cat. *Journal of Physiology* **536**, 893-903.

WHITEHEAD, N. P., WEERAKKODY, N. S., MORGAN, D. L., GREGORY, J. E. & PROSKE, U. (2001). Changes in passive tension of muscle in humans and animals after eccentric exercise. *Journal of Physiology* **533**, 593-604.

Summary

It is known that the mechanical properties of a passive muscle are dependent on its previous history of movement and contraction, called thixotropy. Here, the history-dependence of the muscle at rest and during a slow, passive stretch was examined in the medial gastrocnemius muscle (MG) of the anaesthetised cat. It was hypothesised that the thixotropic effects could be interpreted, at least in part, by the presence of stable cross-bridges in resting muscle. The results provided support for this hypothesis. It was found that both the resting tension at short muscle lengths, and the initial tension response during a passive stretch, over a wide range of lengths, could be explained by the mechanical properties of cross-bridges.

In the past, muscle damage from eccentric contractions has been commonly assessed by changes to active mechanical properties of muscle. However, in more recent years, it has been shown that eccentric contractions can also affect the passive mechanical properties of muscle, by increasing passive tension and stiffness. In a series of experiments carried out on human triceps surae muscle, it was hypothesised that localised regions of contracture in damaged muscle fibres would lead to a rise in passive tension immediately after a period of eccentric exercise, independent of any neural activity. These experiments also explored whether or not muscle swelling contributed to the increase in passive tension several days after eccentric exercise. It was found that passive torque increased immediately after eccentric exercise and remained higher than control values for the next 4 days, while concentric exercise had no effect on passive properties, suggesting that the rise in passive tension was not simply due to exercise *per se*. The time-course of muscle swelling did not correspond with that of the changes in passive torque, thus it was proposed that swelling does not significantly contribute to the rise in passive tension after eccentric exercise.

The mechanism underlying the rise in passive tension immediately after eccentric contractions was further examined in experiments performed on cat MG. It was proposed that the presence of localised contracture in some damaged muscle fibres would show up as a rise in whole muscle passive tension and work absorbed by the passive muscle. In addition, it was envisaged that the increase in passive tension

would be signalled by Golgi tendon organs, receptors that monitor muscle tension. The results were consistent with the hypothesis. Immediately after a period of eccentric contractions on cat MG, there was a significant rise in passive tension and work absorption, as well as a change in the tendon organ threshold for firing during passive stretch, suggesting that tendon organs were signalling the rise in passive tension from damaged muscle fibres.

When eccentric contractions are carried out at progressively longer lengths, muscle damage becomes more extensive. It was decided to examine the effects of this length-dependence of eccentric contractions in terms of changes to passive mechanical properties. Eccentric contractions were carried out over various regions of the active length-tension relationship of cat MG, in which the motor supply of the muscle had been divided into three parts. Initially, there was found to be a smaller rise in passive tension following eccentric contractions carried out at long compared to short muscle lengths. Subsequent experiments, however, showed that changes to passive mechanical properties after eccentric contractions were influenced by a number of complicating factors, including history-dependent and temporal effects. When these factors were taken into account, the rise in passive tension tended to be higher following eccentric contractions at longer muscle lengths.

Abbreviations

DOMS .... delayed onset muscle soreness

EMG ....electromyogram

imp s^{-1} .... impulses per second

L$_{max}$ ....maximum physiological length

L$_{opt}$ .... optimum length

MG ....medial gastrocnemius

mm s^{-1} .... millimetres per second

ms ....millisecond

N ....newton

Nm .... newton-metre

pps .... pulses per second

s .... second

S.E.M. .... standard error of the mean

SREC .... short-range elastic component

TS..triceps surae

μm .... micrometre

Table of Contents

C h a p t e r   O n e

General Introduction

The basic function of skeletal muscle is to generate force, and through force to produce movement. This enables an individual to perform a diverse range of activities. Skeletal muscles are attached to bones, via tendons, and by changing their length, act to rotate joints and thereby move parts of the body. Voluntary movements are made possible by the active mechanical properties of muscle, in which the contractile machinery of the muscle is activated by nerve impulses. However, in many situations, force is produced during a movement while the muscle remains completely relaxed, or passive. This underlines the passive mechanical properties of muscle, which serve important functional roles in every day activities.

This chapter is divided into two sections. It begins with a description of skeletal muscle function, in relation to its active and passive mechanical properties. It then discusses eccentric exercise, a specific type of exercise that can lead to muscle damage. In particular, this section focuses on the proposed mechanisms of muscle damage from eccentric exercise, and the resultant changes to the mechanical properties of the muscle.

Skeletal muscle function

Active mechanical properties of muscle

Skeletal muscle consists of thousands of specialised cells, the muscle fibres, which are enveloped by a thin membrane, the sarcolemma (Figure 1.1). A muscle fibre comprises an orderly array of myofibrils, each consisting of thousands of repeating units called sarcomeres, the basic force generating units of muscle contraction. Each sarcomere consists of overlapping thin (actin) and thick (myosin) contractile filaments. The thin filaments attach at each end of the sarcomere to the Z-line, while the thick filaments are positioned in the centre of the sarcomere (see Figure 1.1). When a muscle fibre is viewed under a microscope using polarised light, the alternating bands of thick and thin filaments are responsible for its characteristic striated appearance, with the dark region at the centre of the sarcomere

Figure 1.1 *Schematic representation of a muscle fibre and sarcomere.*

A section of a muscle fibre comprised of myofibrils and surrounded by the sarcolemma. A sarcomere spans the distance between two Z-lines (Z), and consists of interdigitating thin filaments (actin) and thick filaments (myosin). The location of the other membrane systems, T-tubules and sarcoplasimic reticulum (SR) are also shown. Adapted from Bern & Levy (1993).
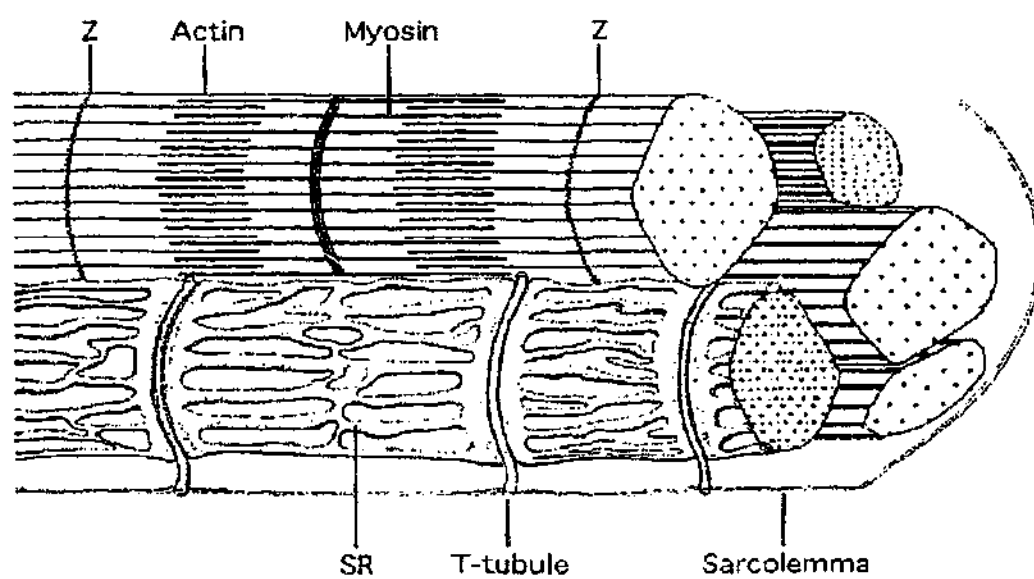
corresponding to the thick filaments (A-band) and the lighter part at the ends of the sarcomere corresponding to the thin filaments (I-band).

In addition to the sarcolemma, a muscle fibre has two membrane systems that are responsible for the control of muscle contraction, the transverse (T) tubules, and the sarcoplasmic reticulum (SR) (see Figure 1.1). The T-tubules are invaginations of the sarcolemma and so are filled with extracellular fluid. They form a system of branched tubules that lie perpendicular to the long axis of the fibre, running deep into the fibre to encircle myofibrils at regular intervals. In mammalian muscle, the T-tubules are located at the A-I junction of the sarcomere, while in amphibian muscle they reside at the level of the Z-line (McCallister & Hadek, 1970; Flucher, 1992). The SR is an internal membrane system of closed channels, which runs parallel to the long axis of the fibre and surrounds myofibrils. The SR has specialised calcium storage compartments, the terminal cisternae. These form junctions with the T-tubules called triads, which have an important functional role in activating the contractile filaments during muscle contraction (see below).

Excitation-contraction coupling

To initiate a contraction, nerve impulses from the central nervous system lead to the initiation of action potentials in muscle fibres, via neuromuscular transmission. Each motoneurone, and the many muscle fibres it activates, is called a motor unit. Thus, all muscle contractions occur through the activation of motor units rather than individual muscle fibres (for a review see Freund, 1983).

The sequence of events leading to a muscle contraction, which begins with activation of a muscle fibre membrane, and ends with tension produced by the contractile filaments, is referred to as excitation-contraction coupling (E-C coupling). The process starts at the neuromuscular junction, where an action potential arriving at the nerve ending of a motoneurone triggers the release of the neurotransmitter acetylcholine (ACh). This acts to depolarise the muscle fibre membrane (sarcolemma), initiating an action potential that propagates along the membrane and into the interior of the fibre via the T-tubules. The excitation of the T-tubules leads to calcium release from the terminal cisternae of the SR. While the details of this process remain unclear, it is thought to involve an electrochemical coupling between voltage sensors located on the T-tubule membrane and calcium release channels on

the SR (see Flucher, 1992; Melzer *et al.* 1995). The increased intracellular calcium concentration, as a result of E-C coupling, is the trigger for muscle contraction (see below).

Sliding filament theory

An important insight into the mechanism by which a muscle is able to generate force during a contraction, and change its length, was provided by the results of two independent studies from the early 1950s (Huxley & Niedergerke, 1954; Huxley & Hanson, 1954). Using the interference microscope on living frog muscle fibres, A.F. Huxley & Niedergerke (1954) observed that during passive movements or contractions where the fibre shortened, the length change was taken up entirely by the I-band region of the sarcomere, while the length of the A-band remained unchanged. Similar findings were reported by H.E. Huxley & Hanson (1954), who showed, using phase contrast microscopy, that except at very short lengths, the width of the A-band was independent of sarcomere length. The results of both studies were explained in terms of the sliding of thick and thin filaments past each other, without the filaments themselves changing length. This is commonly referred to as the 'sliding filament theory'. An important clue as to the underlying mechanism by which the filaments slide past each other and produce force during a contraction, was provided by the work of H.E. Huxley (1957). Using electron microscopy of thin longitudinal sections of muscle, small projections were found that extended from the thick filament at regular intervals along its length. These projections connected or 'interdigitated' with the thin filament, in the region of overlap between the two sets of filaments, and were termed 'cross-bridges'. Huxley postulated that the points of attachment of these cross-bridges to the thin filament, were the sites of force generation during a contraction.

Mechanism of force production by cross-bridges

Since the discovery of cross-bridges, their mechanical and biochemical properties during muscle contraction have been a topic of extensive research (see Huxley, 2000). The first step in the process is the diffusion of calcium, released from the SR during E-C coupling, into the vicinity of the myofilaments. The binding of calcium to the protein troponin on the thin filament changes its structure and exposes 'active' sites on the thin filament. A cross-bridge consists of a tail region and two globular heads, each having an actin binding site and an ATPase site. Cross-bridge

attachment occurs because of the affinity of the cross-bridge-ADP-Pi complex for actin. The release of ADP-Pi causes rotation of the cross-bridge head, referred to as the 'working stroke', which generates force and causes sliding of the thin filament towards the centre of the sarcomere. The cross-bridge then detaches from the thin filament by binding a molecule ATP, before re-attaching further along the thin filament, and repeating the process. Once activation ceases, calcium is rapidly pumped back into the SR and the muscle relaxes.

Types of muscle contraction

The term 'contraction' refers to the tension and subsequent movement produced by an active muscle, which is dependent on the external force acting on it. During activation, the cycling cross-bridges generate force and attempt to shorten the muscle. If the isometric force generating capacity of the muscle exceeds the external force the muscle will shorten, which is referred to as a concentric contraction. For some actions, though, the external force acting on the muscle equals or exceeds the force produced by the muscle. Where the forces are balanced, the length of the muscle remains constant. This is an isometric contraction. Where the external force is greater than the isometric force, the active muscle is forcibly lengthened, which is called an eccentric contraction.

All three types of muscle contraction are routinely used in every day activities. Concentric contractions predominate in activities such as cycling, swimming, walking uphill and lifting objects, where the muscle actively shortens to perform work on the environment. Isometric contractions occur, for example, when attempting to lift an immovable object or when muscles are used for postural stability. Eccentric contractions are performed during activities such as during downhill walking, skiing, and lowering an object, where the muscle acts as a brake to control body movements. Here, work is done on the muscle as it is being actively lengthened. An interesting characteristic of an eccentric contraction is that despite producing higher forces than isometric or concentric contractions, the net energy use is considerably less (Asmussen, 1953; Curtin & Davies, 1973). In order to explain this finding, Curtin & Davies (1973) postulated that during active lengthening of a muscle, some cross-bridges are forcibly detached without the breakdown of any ATP.

Active length-tension relationship

An important prediction of the simplest version of the sliding filament theory of muscle contraction, was that each cross-bridge would act as an independent force generator. Thus, the force generated during an isometric contraction would be proportional to the total number of attached cross-bridges, which would vary depending on the degree of overlap between the thick and thin filaments. This underlies a fundamental mechanical property of muscle, known as the active length-tension relationship. Gordon *et al.* (1966) performed a detailed examination of the length-tension relationship of single frog fibres, by recording the tension generated during isometric contractions over a wide range of sarcomere lengths (Figure 1.2 A). At the optimum sarcomere length, between 2.05 to 2.2 μm, the overlap between the thick and thin filaments is such that all cross-bridges are able to attach to the thin filaments, and maximum tension is produced. This region is referred to as the plateau of the length-tension relationship. At sarcomere lengths shorter than the optimum, known as the ascending limb of the length-tension relationship, tension decreases in two stages. In the first stage, down to a sarcomere length of 1.67 μm, tension falls because the thin filaments from opposite sides of the sarcomere overlap with each other, thereby reducing the number of cross-bridges that can attach and generate tension. In the second stage, tension decreases more steeply down to a sarcomere length of 1.27 μm, at which point tension equals zero. This is thought to reflect the collision of the ends of the thick filaments with the Z-lines, which adds resistance to sarcomere shortening, and also, such crumpling of the thick filaments is likely to reduce the number of attached cross-bridges. As the fibre is stretched beyond the optimum length, tension falls in direct proportion to the amount of overlap between the thick and thin filaments, until at a sarcomere length of 3.65 μm, tension is again reduced to zero because there is now no longer any overlap between the filaments. This region is referred to as the descending limb of the length-tension relationship and its linearity provides an important piece of evidence in support of the sliding filament hypothesis, that is, each cross-bridge acts as an independent force generator (Gordon *et al.* 1966). The general shape of the length-tension relationship for frog muscle is the same as for mammalian muscle. However, the entire relationship is shifted to longer sarcomere lengths, with optimum lengths ranging from 2.5 to 3.1 μm for various mammalian muscles (Close, 1972). The main

reason for this species difference is that despite a similar thick filament length, the thin filament in mammalian muscle is longer than that in amphibian muscle (Page & Huxley, 1963).

Force-velocity relationship

It has been known for many years that the tension generated by a fully activated muscle is dependent on the velocity of the movement the muscle produces (Fenn & Marsh, 1935; Hill, 1938; Katz, 1939). This underlies another fundamental characteristic of the contractile properties of skeletal muscle, known as the force-velocity relationship (Figure 1.2 B). During shortening (concentric) contractions, Hill (1938) showed that the relationship between active force and velocity was approximately hyperbolic, with zero velocity at the maximum isometric force (P_0), and maximum shortening velocity (V_{max}) at zero force. The force-velocity relationship of active lengthening muscle was first described by Katz (1939). He found that when the applied force exceeded the isometric capability of the muscle, it would lengthen slowly as tension increased steeply until the applied force exceeded 1.8 P_0, at which point the muscle yielded and lengthened at a very high velocity, with tension remaining independent of velocity.

The force-velocity relationship of muscle has been interpreted in terms of the cross-bridge theory of muscle contraction by A.F. Huxley (1957). Under isometric conditions, the force is proportional to the number of attached cross-bridges. As the shortening velocity increases, tension is thought to decline because there is less time for cross-bridges to attach and, an increased number of cross-bridges exert a pushing (negative) force while fewer exert a pulling force. The maximum shortening velocity of a muscle has been shown to reflect the rate of cross-bridge cycling, which is determined by the myofibrillar ATPase activity of the muscle (Bárány, 1967).

During active lengthening, the steep increase in force reflects a greater force per attached cross-bridge. This is due to the initial high resistance to stretch from the elastic linkages of the cross-bridges, which has been termed the 'short-range stiffness' (Rack & Westbury, 1974; Flitney & Hirst, 1978). At the elastic limit, cross-bridges are forcibly detached and with further stretch, the tension produced by the muscle is dependent on the imposed velocity of the movement, with tension continuing to rise at low velocities and remaining constant at intermediate to high

Figure 1.2 *Length-tension and force-velocity relationships.*

(A) The length-tension relationship of a sarcomere as shown for a single frog fibre by Gordon *et al.* (1966). (B) The force-velocity relationship of frog muscle, shown for both shortening (Hill, 1938) and lengthening (Katz, 1939) velocities. The maximum shortening velocity (V_{max}), maximum isometric tension (P_0) and yield point of tension during lengthening are indicated (arrows).

A
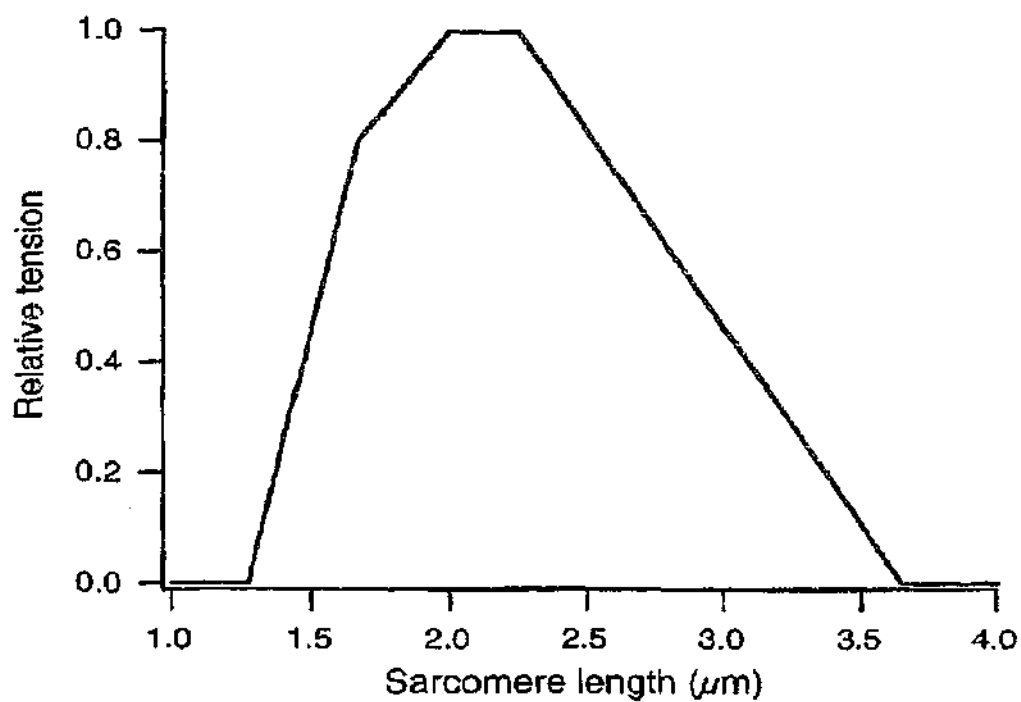
B
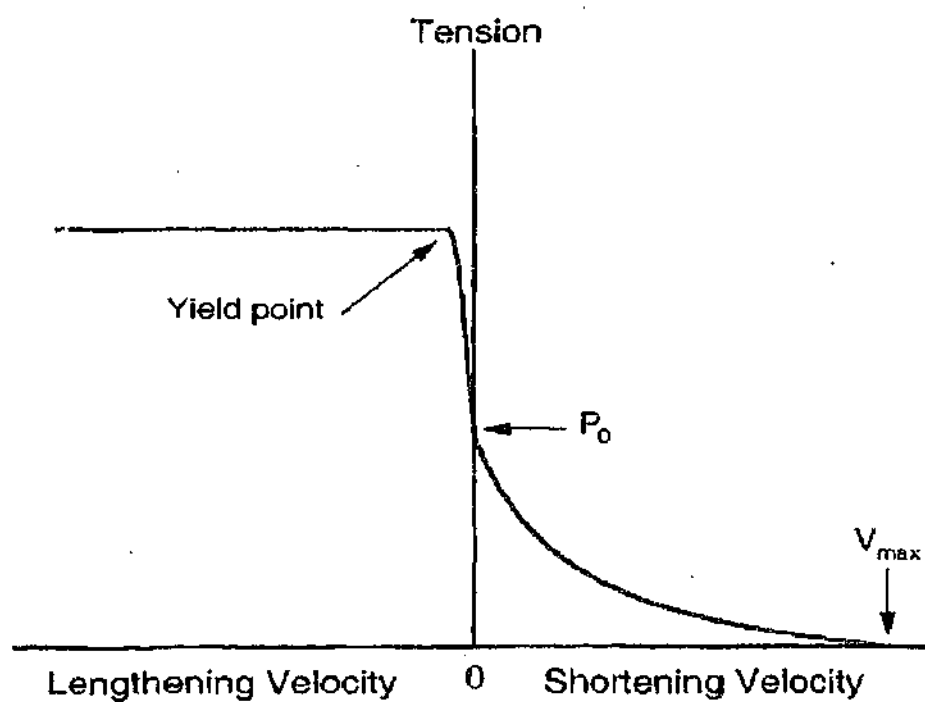
velocities (Edman *et al.* 1978). These differences are thought to reflect a balance of the force per cross-bridge, and the number of attached cross-bridges as a function of time, which becomes less as the speed of myofilament sliding increases.

Passive mechanical properties of muscle

Passive properties of muscle refer to the tension generated when a non-activated muscle is at rest (static) or undergoing movement. In every day activities, passive properties serve many important functional roles. For example, when an agonist muscle contracts and shortens to rotate a joint, the inactive, antagonist muscle is passively stretched. Upon relaxation, both muscles return to their original length before the contraction, one, by passive stretch (agonist), the other, by passive shortening (antagonist). Furthermore, many voluntary contractions require the activation of only a small number of motor units, so that a considerable fraction of the agonist muscle will be passive.

Although research has been carried out on passive mechanical properties of muscle for over a century, significant advances have occurred only recently, over the past two decades or so. This section outlines how our understanding and knowledge of passive properties has developed over time, and it also addresses some modern day controversies surrounding this topic.

Passive length-tension relationship

Connective tissue and sarcolemma

The length-tension relationship of a passive muscle is approximately exponential, with negligible tension at short muscle lengths, and a steeply rising tension at long lengths (Close, 1972). Traditionally, the source of passive tension in muscle was attributed to structures in 'parallel' with the contractile filaments, such as connective tissue and the sarcolemma. Skeletal muscle consists of three connective tissue layers, the epimysium, covering the muscle belly, the perimysium, surrounding bundles of fibres (fascicles) and the endomysium, which connects with individual fibres and the perimysium (Borg & Caulfield, 1980). These connective tissues provide the muscle with structural support and also assist in transmitting forces produced by muscle fibres to adjacent fibres and the tendon (Purslow & Trotter, 1994; Huijing, 1999). In an early study, Banus & Zetlin (1938) reported that the tension produced by a relaxed muscle was similar before and after surgical removal

of the muscle fibres, and on this basis, proposed that connective tissue provided a major contribution to passive tension. However, this idea has been challenged by a more recent biomechanical model (Purslow, 1989). Here, it was suggested that perimysial connective tissue contributed to the production of passive tension only at sarcomere lengths longer than about 3.6 μm for mammalian muscle, a length at which there is already significant passive tension in the muscle (see below).

In a study investigating the active and passive length-tension properties of single frog muscle fibres, Ramsey & Street (1940) originally assigned all of the passive tension to the sarcolemma. However, one of the authors later retracted this proposal, based on uncertainties of their method of measuring the segment length of fibres with contraction clots and empty sarcolemmal tubes (Street, 1983). Subsequent research, also carried out on single frog fibres, suggested that the sarcolemma contributed to passive tension only at relatively long sarcomere lengths, above 3 to 3.2 μm (Podolsky, 1964; Rapoport, 1972). At these lengths, the sarcolemma has been shown to lose its folded appearance, which is evident at shorter lengths (Dulhunty & Franzini-Armstrong, 1975). This might provide a structural explanation for the generation of tension by the sarcolemma. The passive length-tension properties of single fibres were found to be remarkably similar to those of whole frog muscle, as reported by Magid & Law (1985). Based on their findings, the authors concluded that non-contractile structures within myofibrils generated most of the passive tension at lengths within the normal physiological range, with contributions from connective tissue and sarcolemma only at long sarcomere lengths, greater than 3.8 μm (Magid & Law, 1985).

Titin filaments

The discovery of the elastic filament titin, or connectin (Wang *et al.* 1979; Maruyama *et al.* 1981) established the existence within sarcomeres of a non-contractile structure capable of generating passive tension. There is now a wealth of experimental evidence in support of titin's role as a major source of passive tension in both skeletal and cardiac muscle (for recent reviews see Horowits, 1999; Wang *et al.* 2001). As its name implies, titin is a large polypeptide, which constitutes about 10% of the total muscle protein mass (Labeit & Kolmerer, 1995). It provides an elastic, mechanical link between the Z-line and thick filament, spanning the distance of each half sarcomere (Z-line to M-line) (Fürst *et al.* 1988). An early method of

investigating the role of titin in the generation of passive tension was to selectively degrade it using protease digestion (Funatsu *et al.* 1990) or low-dose ionising radiation (Horowits *et al.* 1986). In both studies, there was a strong correlation between the degree of titin fragmentation and the resultant drop in passive tension. Many studies have now shown that the generation of passive tension is associated with stretch of the extensible I-band region of titin, between the Z-line and the end of the thick filament (see Horowits, 1999). Here, titin consists of three structurally distinct but serially linked segments; the immunoglobulin (Ig) and fibronectin (Fn3) domains, the PEVK region, and the N2-A segment (Labeit & Kolmerer, 1995). The A-band region of titin, consisting of Ig and Fn3 domains, is strongly bound to the thick filament, making it inextensible under normal physiological conditions (Labeit & Kolmerer, 1995). An explanation for the tension produced by each I-band segment of titin during passive length-tension measurements, the segmental extension model, was proposed by Wang *et al.* (1993) (see also Linke *et al.* 1996). At short sarcomere lengths, between 2.2 and 2.6 μm for skinned rabbit psoas fibres, a low level of passive tension is produced, which is thought to reflect straightening of the Ig segment of titin. With further stretch, tension rises exponentially due to extension of the PEVK and N2-A segments (Figure 1.3). Recent support for this model comes from a study using an immuno-staining technique, which showed that each of the I-band regions of titin extend as distinct segments (Granzier *et al.* 2000). At longer lengths, approximately 3.8 μm, titin reaches its elastic limit and yields, whereby a part of the titin segment originally anchored to the end of the thick filament dislodges and becomes extensible. Passive tension then remains at a constant level as the fibre is stretched further, until at 4.5 μm, tension again rises because of the contribution of the intermediate filament network (see Figure 1.3). This includes the protein desmin, which provides a mechanical link between the Z-line and M-line of sarcomeres in adjacent myofibrils (Lazarides, 1980). Wang *et al.* (1993) commented that stretch of a muscle fibre to these extreme lengths would be unlikely to occur under normal physiological conditions. However, they suggested that in the case where a sarcomere became damaged, intermediate filaments might act as a mechanical bypass structure to stabilise the sarcomere. There is experimental evidence to suggest that sarcomere overextension and disruption can occur during repeated eccentric contractions, as described later in the chapter.
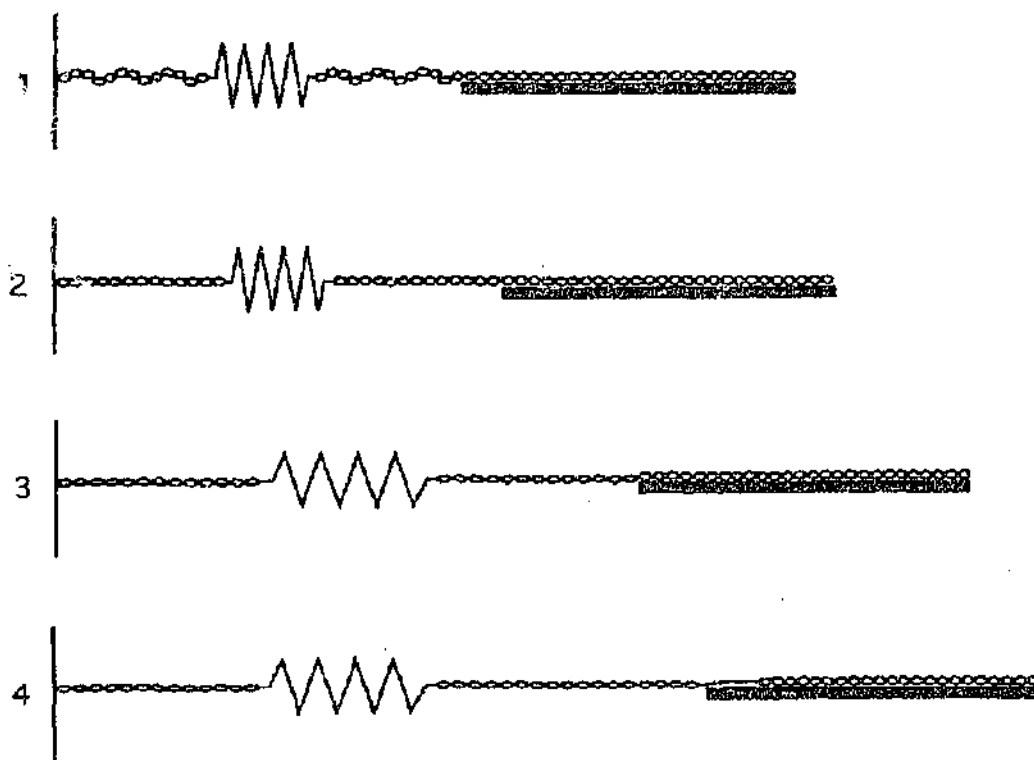
Figure 1.3 *Passive-length tension relationship of titin.*

(A) The passive length-tension relationship of a single sarcomere, based on measurements made using single skinned mammalian muscle fibres (Wang *et al.* 1993). (B) The proposed extension of titin filaments during passive lengthening of a sarcomere, as proposed by Wang *et al.* (1993). The I-band region of titin (blue) is attached to the Z-line and thick filament, while the A-band titin (red) is anchored to the thick filament. Each phase of sarcomere extension (numbered) corresponds to a region of the length-tension relationship shown in (A). At very short sarcomere lengths, passive tension is near zero and titin is slack (1). With extension up to ~2.6 µm, part of the I-band titin extends and there is a small increase in passive tension (2). Beyond this length, passive tension increases exponentially (3) as titin is extended. At a sarcomere length of 3.8 µm, the region of the titin filament attached to the thick filament dislodges and becomes extensible (4). With further extension, tension levels off until at ~4.5 µm, tension again rises due to strain of the intermediate filaments (dotted line in A).

A
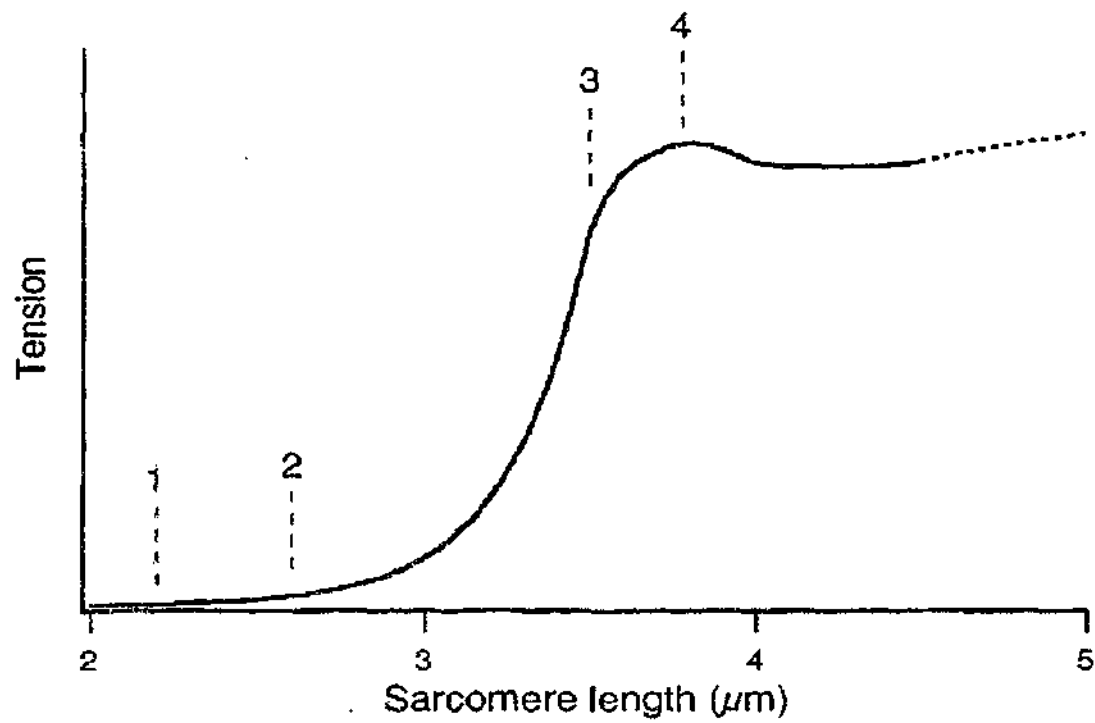
B

It has been proposed that differences in the passive length-tension relationships between muscles, both within and across species, can be attributed to various titin isoforms. Wang *et al.* (1991) reported significant correlations between the size of the titin isoform and both the sarcomere length at which passive tension began to rise exponentially, and the length at which titin yielded. It has been proposed that variations in the length of the PEVK region, and the number of Ig domains, provide an explanation for such differences (Labeit & Kolmerer, 1995). Linke *et al.* (1996) showed differences in the yield point and slack sarcomere lengths for isolated myofibrils from rabbit psoas and soleus muscles. They proposed that the length of the PEVK region determined passive stiffness, while the length of the Ig domain set the slack sarcomere length.

Visco-elastic properties of passive muscle

Like many biological tissues, the mechanical properties of passive muscle are visco-elastic, that is, they are dependent on the velocity of movement (viscous) and changes in length (elastic). Two commonly investigated visco-elastic properties of passive muscle are stress relaxation and hysteresis. Stress-relaxation is the exponential decay of tension towards a steady level, when a muscle is held at a fixed length after being stretched. Hysteresis refers to a higher tension produced during stretch than during release, over the same length range (Wang *et al.* 1993). Recent studies have reported that such visco-elastic behaviour of passive muscle, evident in experiments carried out on whole muscles (Magid & Law, 1985; Malachy *et al.* 1992), can be explained by the mechanical properties of titin filaments (Wang *et al.* 1993; Bartoo *et al.* 1997; Mutungi & Ranatunga, 1998; Kellermayer *et al.* 2001; Minajeva *et al.* 2001).

Recently, it was shown that when isolated myofibrils were passively stretched and then held at a fixed length, the amplitude of the tension decay progressively increased with higher stretch velocities and longer sarcomere lengths up to 3.7 μm, but then became less at longer lengths (Minajeva *et al.* 2001). This stress relaxation was proposed to reflect the unfolding of a few Ig domains in the I-band region of the titin filament, while sarcomere length remained relatively constant. Force hysteresis during stretch-shortening movements over a physiologically relevant length range was also attributed to the folding and unfolding of Ig domains (Minajeva *et al.* 2001). However, when the yield point of titin is exceeded during the stretch, the amount of

hysteresis becomes much greater, and this is thought to reflect an increase in the extensible length of the titin filament (Wang *et al.* 1993).

Mechanical properties of cross-bridges in passive muscle

An interesting feature of a passive muscle is that during stretch, the mechanical response is biphasic; an initial steep rise in tension followed by a more gradual change. A detailed examination of this phenomenon was first reported by D.K. Hill (1968). Hill noted that during stretch of a frog sartorius muscle, the initial high stiffness persisted over only a small fraction of the length change, about 0.2% of the fibre length, and so he referred to this as the short-range elastic component (SREC). Hill postulated that the SREC was due to the spring-like, mechanical properties of a small number of long-lived cross-bridges between actin and myosin, present in the resting muscle. Once the cross-bridges reached their 'elastic limit' and detached, tension dropped slightly and then levelled off with further stretch. Hill also proposed that a component of the resting tension, which he termed the filamentary resting tension (FRT), was attributable to these cross-bridges.

Since the work carried out by Hill, (1968), several studies have examined, under a variety of experimental conditions, whether or not the mechanical properties of passive muscle are consistent with the presence of such cross-bridges. This topic is currently the subject of debate, with some studies supporting the cross-bridge hypothesis (see Campbell & Lakie, 1998; Proske & Morgan, 1999) and others opposing it and providing alternative explanations (see Mutungi & Ranatunga, 2000; Ranatunga, 2001).

Velocity-dependence

The biphasic tension response during stretch of a passive muscle, as shown by Hill (1968) was observed when the muscle was stretched at velocities ranging from 1×10^{-4} to 0.2 fibre lengths per second (L_0 s^{-1}). It was shown that while the amplitude of the initial tension rise up to the elastic limit remained relatively constant at low stretch velocities, it became progressively greater at higher velocities. A similar velocity-dependence of both the tension and stiffness of the SREC was recently reported by Campbell & Lakie (1998), in which single frog fibres were stretched from very low speeds (5×10^{-4} L_0 s^{-1}) up to the maximum shortening speed for frog muscle (2 L_0 s^{-1}). The authors suggested that at low velocities of stretch, the

mechanical behaviour of the SREC was due to an elastic structure, consistent with slowly cycling cross-bridges, while at higher speeds, a viscous, velocity-dependent element was likely to dominate the response. Using much faster stretches on frog single fibres, up to 250 L_0 s^{-1}, Bagni et al. (1995) separated the initial tension rise during stretch into three components. An initial rapid rise in tension was found to increase proportionally with stretch velocity and so was attributed to viscous resistance to sliding of the contractile filaments. This component had a finite value at zero velocity, which was attributed to the SREC. A second less steep tension increment, which was proportional with velocity up to a plateau value, was proposed to represent the visco-elastic properties of structural filaments, presumably the cytoskeleton. At the end of the stretch, tension decayed to a steady value, the amplitude of which was independent of velocity. This was attributed to an elastic structure, possibly titin filaments. Similar findings and conclusions were reported by Mutungi & Ranatunga (1996b), on bundles of mammalian fibres. Here, though, it was postulated that the visco-elastic component of the tension rise might also represent the mechanical properties of titin filaments.

While it seems likely at high stretch velocities that viscous resistance and titin filaments provide a large contribution to the measured tension, there is, however, no convincing evidence that titin can produce the biphasic tension response during slow stretch of passive muscle (see Campbell & Moss, 2002). Furthermore, an argument put forward by Campbell & Lakie (1998) against the contribution of titin to the SREC and FRT, was that their experiments were carried out at a short initial sarcomere length of 2.2 μm, which is close to the slack sarcomere length for frog muscle. They proposed that at this length, the contribution of titin to resting tension was likely to be negligible and that to produce the biphasic tension response of the SREC, titin would have to be 'disproportionately stiff for small movements', which was not supported by previous experimental evidence (Tskhovrebova et al. 1997). Thus, an important factor that needs to be taken into account when considering the mechanical response of passive muscle during stretch, is the initial sarcomere length of the fibre.

Length-dependence

If cross-bridges are responsible for the SREC, then, in theory, the initial short-range tension and stiffness would be expected to reach a peak at a length corresponding to

optimum overlap between thick and thin filaments for cross-bridge attachment. However, it has been shown that the stiffness of the SREC continues to increase with length beyond the optimum (Hill, 1968; Moss & Halpern, 1977; Haugen & Sten-Knudsen, 1981). In support of a cross-bridge origin of the SREC, though, was the finding that the short-range stiffness and tension fall to zero when there is no myofilament overlap (Haugen & Sten-Knudsen, 1981). It has been suggested that at lengths beyond the optimum, an increased number of cross-bridges might still be able to form in resting muscle, due to a reduced interfilament spacing (Proske & Morgan, 1999). This idea was originally put forward by Hill (1968), as an explanation for an increase in the size of the SREC and FRT in hypertonic solutions. He proposed that fibre shrinkage from the hypertonic solution had brought the thick and thin filaments closer together, allowing more cross-bridges to attach. It has, however, since been shown that hypertonic solutions can partially activate the contractile filaments (Lannergren, 1971), which could provide an alternative explanation for Hill's findings. Proske & Morgan (1999) have also suggested that the length-dependence of the SREC might be due to an increase in the sensitivity of the myofilaments to calcium, at lengths beyond optimum overlap, as shown by Endo (1973). However, in a more recent study on single mammalian muscle fibres, it was found that while calcium sensitivity increased at lengths beyond the optimum, it then decreased at longer lengths (Balnave & Allen, 1996). Thus, it remains unclear as to whether or not changes in calcium sensitivity, particularly under resting conditions, can explain the length-dependence of the SREC.

Calcium-dependence

It is now generally accepted that if cross-bridges exist in passive muscle, they must cycle slowly or not at all, and can not correspond to the proposed weakly attached, rapidly cycling cross-bridges thought to occur in resting muscle (see Campbell & Lakie, 1998; Ranatunga, 2001 for references). There has been some indirect evidence for the presence of slowly cycling cross-bridges in resting muscle and their role in the mechanical response to passive stretch. Studies carried out on single skinned muscle fibres showed that there was no SREC in relaxing solutions containing low calcium concentrations. However, as the calcium concentration of the bathing solution was raised to a level just below that required for contractile activation, the tension response during stretch was similar to that observed in a

normal resting muscle (Moss *et al.* 1976). More recent studies have reported similar findings, and have proposed that the tension response to stretch of a passive muscle is qualitatively similar to that of actively contracting muscle, suggesting that both are attributable to the tension produced by cross-bridges, which varies according to the intracellular calcium concentration (Campbell & Moss, 2000; Campbell & Moss, 2002). An important difference is the rate of cross-bridge cycling, which is about 10 times faster for actively contracting muscle (Campbell & Moss, 2000) than passive muscle (Campbell & Lakie, 1998).

Thixotropy

It has been known for many years that the onset of the initial steep tension rise observed during the stretch of a passive muscle is delayed more, and is less steep, for the second of two stretches (Denny-Brown, 1929). This underlies a property of passive muscle referred to as thixotropy (Proske *et al.* 1993), in which the initial high stiffness during stretch is temporarily reduced by previous movement or contraction. Thixotropic behaviour for passive muscle has been reported for single frog fibres (Lannergren, 1971; Campbell & Lakie, 1998), whole frog muscle (Herbst, 1976; Lakie & Robson, 1988a; Lakie & Robson, 1988c; Lakie & Robson, 1988d) and human muscle (Lakie *et al.* 1984; Hufschmidt & Schwaller, 1987; Lakie & Robson, 1988b).

One of the main arguments in favour of the presence of cross-bridges in resting muscle is that both the SREC and FRT are dependent on the muscle's previous mechanical history (Campbell & Lakie, 1998; Proske & Morgan, 1999). The history-dependence of the SREC and FRT has been recently demonstrated on single frog fibres subjected to two consecutive stretch-shortening movements at a constant velocity (Campbell & Lakie, 1998). It was found that the SREC tension and stiffness fell during the second stretch, and the FRT was less at the end of the first movement than it had been beforehand. These findings were proposed to reflect the strain and detachment of cross-bridges during the first stretch, so that the population of attached cross-bridges was now smaller at the onset of the second stretch. It was also shown that by delaying the onset of the second movement, the SREC and FRT recovered with time to their initial values, but the time constant of recovery was slower for the SREC tension (12.5 s) compared with the FRT (less than 1 s). The recovery rate of the SREC tension reported by Campbell & Lakie (1998) compares well with other

studies on frog single fibres (Lannergren, 1971), amphibian whole muscle (Proske & Stuart, 1985) and human ankle extensor muscles (Hufschmidt & Schwaller, 1987). Hufschmidt & Schwaller (1987) also reported a similar time-course of FRT re-development to that shown by Campbell & Lakie (1998).

The fact that the recovery of the SREC tension after movement took many seconds, implied that the rate of cross-bridge attachment in the static muscle was low. However, such a low cross-bridge attachment rate seemed at odds with the fact that tension does not drop to zero beyond the yield point of the SREC, but remains at a relatively constant level. In order to overcome this difficulty, Campbell & Lakie (1998) developed a model, in which the cross-bridge attachment rate increased during movement. This prevented tension from falling to zero beyond the yield point. They suggested that when the movement had ended, the cross-bridge attachment rate became less, so that the SREC took many seconds to fully recover. While this remains a plausible explanation, it is inherently unsatisfactory in terms of the A.F. Huxley (1957) model. That is, it requires a departure from the idea that rate-constants for cross-bridge attachment are determined by energy levels. An alternative explanation has been proposed by Proske & Morgan (1999). They suggested that cross-bridges attach at the same, slow rate whether the muscle undergoes movement or is at rest. During a slow stretch, it was postulated that sarcomeres in the passive muscle would lengthen non-uniformly. That is, not all sarcomeres would resist the stretch with the same strength, so that some would stretch more than others. Thus, the weakest sarcomeres would be stretched until their cross-bridges detached. With further stretch, these sarcomeres would lengthen rapidly until passive tension, from non cross-bridge sources, had risen to a level where cross-bridges in the next weakest sarcomeres became detached. This process would continue throughout the stretch, in order from the weakest to strongest sarcomere, in a similar way to stretch of active muscle (Morgan, 1990). With such a model, the level of tension would not be expected to fall to zero with stretch beyond the yield point of the SREC, yet the re-formation rate of cross-bridges could remain uniformly low (Proske & Morgan, 1999).

One potential difficulty with a slow attachment rate of cross-bridges in the resting muscle is that it seems inconsistent with the more rapid recovery of the FRT. Campbell & Lakie (1998) found a way around this problem by proposing that at the

end of a stretch-shortening movement, most attached cross-bridges are compressed and negatively strained. This would cause a drop in FRT. Immediately after the movement had ended, these compressed cross-bridges would rapidly detach, leading to an early rise in FRT. The more gradual phase of FRT re-development is due to the slow attachment rate of cross-bridges in the resting muscle, which is also responsible for the recovery of the SREC tension.

An important piece of evidence in support of the role of cross-bridges in the history-dependence of passive mechanical properties, is that muscles fall slack under certain circumstances. At short lengths, if a muscle is shortened sufficiently it will lie slack, while if it is stretched to long enough lengths, slack will not develop after any form of mechanical conditioning. However, at intermediate lengths, the incorporation of slack depends on the muscle's previous mechanical history (Proske *et al.* 1993). When a muscle is stretched, contracted isometrically, and then held for a few seconds, upon being shortened back to its original length it will fall slack. It has been proposed that following the isometric contraction at the longer length, stable cross-bridges will form, so that when the muscle is shortened, some cross-bridges will detach while others will remain attached and generate a compressive force on the sarcomeres. Thus, the muscle fibres are unable to fully accommodate the length change and fall slack. The presence of slack shows up as a drop in resting tension and a delay in the tension rise during a slow stretch (see Proske & Morgan, 1999; Fig. 2). In order for slack to fully develop, at least for the intrafusal fibres of muscle spindles, it is proposed that the muscle needs to be held at the longer length for several seconds before being shortened to the initial length (Morgan *et al.* 1984). In a whole intact muscle, the tendon might fall slack. This has been observed at short lengths for cat medial gastrocnemius muscle during treadmill locomotion (Elek *et al.* 1990) and over a range of muscle lengths for the relaxed human brachialis muscle (Herbert & Gandevia, 1995). Thus, slack appears to be present under normal physiological conditions.

While the thixotropic behaviour of passive muscle provides strong supporting evidence for the involvement of cross-bridges, it has been recently suggested that history-dependent properties of titin filaments might underlie the thixotropic behaviour of the SREC (see Mutungi & Ranatunga, 2000; Ranatunga, 2001). This proposition was based on studies in which single molecules of titin were shown to

display 'mechanical fatigue' during repeated stretch-shortening movements over a physiologically relevant force range (Kellermayer *et al.* 1998; Kellermayer *et al.* 2001). The response was characterised by a progressive shift of the extension-tension relationship of titin to longer lengths. The authors suggested that this reflected an increase in the extensible segment length of titin, a process that was reversible if the molecule was held at a short length for approximately 4 minutes. While it seems likely that titin possesses long-range history-dependent properties, it is less likely, based on the experimental evidence presented above, that the mechanical properties of titin can explain the short-range, thixotropic behaviour of passive muscle, particularly at short lengths, where it is known that titin contributes little to the resting tension.

In Chapter 2 of this thesis, the effects of muscle thixotropy on the passive mechanical properties of mammalian muscle will be examined, the aim being to explore some of the unresolved issues surrounding this topic.

Muscle damage from eccentric contractions

The sensations of muscle stiffness and soreness the day after a period of unaccustomed exercise is a common experience. The first experimental evidence of such delayed muscle pain was reported by Hough (1902), in subjects performing repetitive exercise of their middle finger muscles, using both concentric and eccentric contractions. Hough noted that the delayed soreness was experienced in untrained individuals, who had not performed this activity for several months. It first became evident about 8 hours after the exercise and reached maximum intensity a day or two later. He commented that this type of soreness was different to the pain experienced during intense exercise, and proposed that it was due to a type of 'rupture' within the muscle. These were rather insightful findings and they foreshadowed what are now known to be important characteristics of this type of muscle soreness. It was, however, more than half a century later before the observations of Hough were more formally investigated. An important step towards understanding the cause of this delayed muscle soreness, was the discovery that it occurred after exercise involving eccentric contractions (eccentric exercise) but not concentric contractions (concentric exercise) (Talag, 1972; Newham *et al.* 1983b). There is now a wealth of experimental evidence to show that delayed onset muscle

soreness (DOMS) and the associated sensations of muscle stiffness and weakness are manifestations of muscle damage caused by eccentric contractions.

Histological evidence of muscle damage

Histological evidence of structural damage to muscle from eccentric exercise was first shown in muscle biopsy samples from human subjects, who had experienced DOMS in the affected muscle (Fridén et al. 1981). Two days after the exercise, there was evidence of structural changes to sarcomeres, as viewed under the electron microscope. In particular, the Z-band appeared to be affected, showing signs of broadening, streaming (wavy appearance) and disruption. Adjacent sarcomeres were often hypercontracted or structurally disorganised. The damage was distributed randomly throughout the muscle sample and could be restricted to a few or even one half sarcomere in a myofibril. In some cases, though, the damage had spread transversely across parts of the fibre, characterised by non-uniform register of sarcomeres in adjacent myofibrils. Similar ultrastructural changes to human muscle were reported by Newham et al. (1983a) after eccentric but not concentric exercise. Here, it was shown that the incidence of disrupted sarcomeres increased in samples taken 1 to 2 days after exercise compared to samples taken immediately after the exercise.

Eccentric contraction protocols carried out on animals have shown similar patterns of sarcomere disruption. Ogilvie et al. (1988) reported various structural abnormalities in rat soleus muscle after downhill exercise on a treadmill. They showed disruption and widening of the A-band, Z-line dissolution and 'clotted' muscle fibres, characterised by regions of hypercontracted sarcomeres. The presence of overextended sarcomeres or half sarcomeres, randomly scattered throughout the fibre, is commonly observed after repeated eccentric contractions (Wood et al. 1993; Balnave et al. 1997; Macpherson et al. 1997). Some animal studies have also reported more extensive muscle damage after eccentric contractions. The presence of enlarged or 'swollen' segments of muscle fibres have been described, in which the damage had spread across the fibre (Warren et al. 1995; Fridén & Lieber 1998; Komulainen et al. 1998). These 'swollen' regions of the fibre have been shown to consist of hypercontracted sarcomeres (Fridén & Lieber 1998).

Mechanisms of damage from eccentric contractions

Initial event

Early studies investigating the cause of muscle damage following repeated eccentric contractions proposed that metabolic factors such as depletion of ATP, lower pH and the accumulation of metabolic waste products might be responsible (see Armstrong, 1984; Byrnes & Clarkson, 1986). However, it is now widely accepted that such metabolic factors are not the initiating event leading to structural damage in the muscle, since for a given level of force production, eccentric contractions are known to be metabolically 'cheaper' than isometric or concentric contractions, which do not cause muscle damage (see Armstrong et al. 1991). Furthermore, a recent study has shown that an increased oxidative capacity does not protect the muscle from eccentric contraction-induced damage (Patel et al. 1998).

It is known that the force generated during an eccentric contraction can be almost twice that of an isometric contraction (Katz, 1939). This led some researchers to propose that high forces might cause structural disruption of sarcomeres, as is commonly observed after eccentric contractions (Fridén et al. 1981; Armstrong et al. 1983; Newham et al. 1983b; Fridén et al. 1984; McCully & Faulkner, 1986). Experiments carried out on mouse extensor digitorum muscle in situ, showed that the peak force developed during the first of a series of eccentric contractions was correlated with the reduction in isometric force measured 3 days after the exercise, which, in turn, was related to the degree of muscle damage measured histologically (McCully & Faulkner, 1986). However, in the same study, it was also reported that damage occurred following eccentric contractions but not concentric or isometric contractions, in which the peak force level was the same in each case (85% P_0). Based on this finding, the authors suggested that a factor other than peak force also contributed to the initial damage after eccentric contractions.

Newham et al. (1988) showed evidence of more extensive damage to human elbow flexors following eccentric exercise carried out at long compared to short muscle lengths, despite the fact that the contractions at shorter lengths produced higher peak forces. Experimental evidence, based on changes to the active length-tension properties of the muscle, lend support to the idea that the amount of damage increases as the eccentric contractions are carried out at longer muscle lengths (Katz, 1939; Talbot & Morgan, 1998).

Sarcomere 'popping' hypothesis

An explanation for the initial event leading to muscle damage from eccentric contractions, which is able to account for most of the known facts, was proposed by Morgan (1990). The model was based on the assumption that sarcomeres within a myofibril will have some random variation in their strengths, due to small differences in sarcomere spacings or cross-sectional area along the myofibril. As a result of these sarcomere inhomogeneities, lengthening of an active muscle would occur non-uniformly. During an eccentric contraction, tension would rise until the weakest sarcomere reached the yield point of its force-velocity relationship, causing it to lengthen more rapidly than the other sarcomeres without increasing tension. If the weakest sarcomere was on the descending limb of its length-tension relationship, its ability to generate active tension would be reduced with further stretch, and so it would be unable to maintain the existing fibre tension at any velocity. It would continue to lengthen rapidly until the tension from non-contractile, passive structures within the sarcomere was equal to the fibre tension. At this point, the sarcomere would stop lengthening. As the stretch continued and tension rose further, the same process would then be repeated for the next weakest sarcomere in the myofibril. In this way, it was proposed that lengthening of an active muscle occurs by the rapid overextension or 'popping' of sarcomeres, one at a time, in order from weakest towards strongest. This would continue until the stretch ended or tension in the fibre was reduced (Morgan, 1990). An important prediction of the model was that the number of overextended sarcomeres in fibres would increase as the stretch was carried out at lengths further down the descending limb of the length-tension relationship.

Recent experimental support for the non-uniform lengthening of sarcomeres during an eccentric contraction was provided by Morgan *et al.* (2000). This study investigated a phenomenon known as 'permanent extra tension', in which the maintained isometric tension at the end of an active stretch remains higher than the equivalent isometric tension at that length. It was found that the permanent extra tension only occurred on the plateau or descending limb of the length-tension relationship, and became greater at longer lengths. This was interpreted as being due the non-uniform lengthening of sarcomeres during the stretch, so that most of the length change was taken up by a few 'popped' sarcomeres while the majority of

sarcomeres lengthened little. In this way, the tension at the end of the stretch would be closer to the isometric tension at the initial rather than final length.

Ultrastructural damage from sarcomere non-uniformities

The mechanical consequences of sarcomere 'popping' provide an explanation for many ultrastructural changes to muscle after a series of eccentric contractions (Figure 1.4). Morgan (1990) proposed that when the muscle relaxed after a single eccentric contraction, most of the overstretched sarcomeres, that is, weak sarcomeres that had been stretched to beyond myofilament overlap, would be able to reinterdigitate and resume normal function. However, a few would become disrupted, so that they could not realign their normal overlapping pattern. This might be because the thick and thin filaments butt up against one another, or non-contractile filaments become detached, leading to disturbance of the spatial alignment between the myofilaments. With repeated eccentric contractions, these sarcomeres would rapidly stretch, placing extra tension on sarcomeres in adjacent myofibrils, possibly leading to further sarcomere disruption. Morgan postulated that spread of the damage across the fibre in this way could also cause damage to muscle membranes and the subsequent loss of calcium homeostasis, which has been proposed to trigger a cascade of events leading to further damage to the muscle (see Armstrong *et al.* 1991).

While the presence of overextended sarcomeres has been shown histologically after repeated eccentric contractions (see above), according to the hypothesis of Morgan (1990), most of the overextended sarcomeres that develop during an eccentric contraction would resume their normal interdigitating pattern and so not be evident in a muscle that was fixed after relaxation. In order to overcome this problem, Talbot & Morgan (1996) used a rapid fixation technique developed by Brown & Hill (1991). They showed that if a muscle was fixed while still contracting, at the end of a single eccentric contraction on the descending limb of its length-tension relationship, the number of overextended sarcomeres found in muscle fibres could account for more than half of the imposed length change during the stretch. Importantly, such overextended sarcomeres were absent when the muscle was fixed during a single isometric contraction at the same length, and only a few were found if a single active stretch was carried out on the ascending limb (Talbot & Morgan, 1996). This was taken as evidence of non-uniform lengthening of sarcomeres during

Figure 1.4 *Proposed mechanical consequences of sarcomere non-uniformities during an eccentric contraction.*
Schematic representation showing four sarcomeres from two adjacent myofibrils of a mammalian muscle fibre. Titin filaments (blue) are shown, as well as desmin intermediate filaments (red) and the T-tubule system (black). Before an eccentric contraction, the sarcomeres are passive and have a uniform length (upper panel). During an eccentric contraction, the sarcomeres in the top myofibril lengthen uniformly, whereas in the bottom myofibril, the half-sarcomere to the right becomes overextended, causing its titin filament to yield. It also results in loss of myofibrillar registry, placing strain on desmin (centre) and T-tubules (left sarcomere), and the possible rupture of some T-tubules (right sarcomere). Based on Allen (2001).

Before eccentric contraction

T-tubule

Titin

Desmin

During eccentric contraction
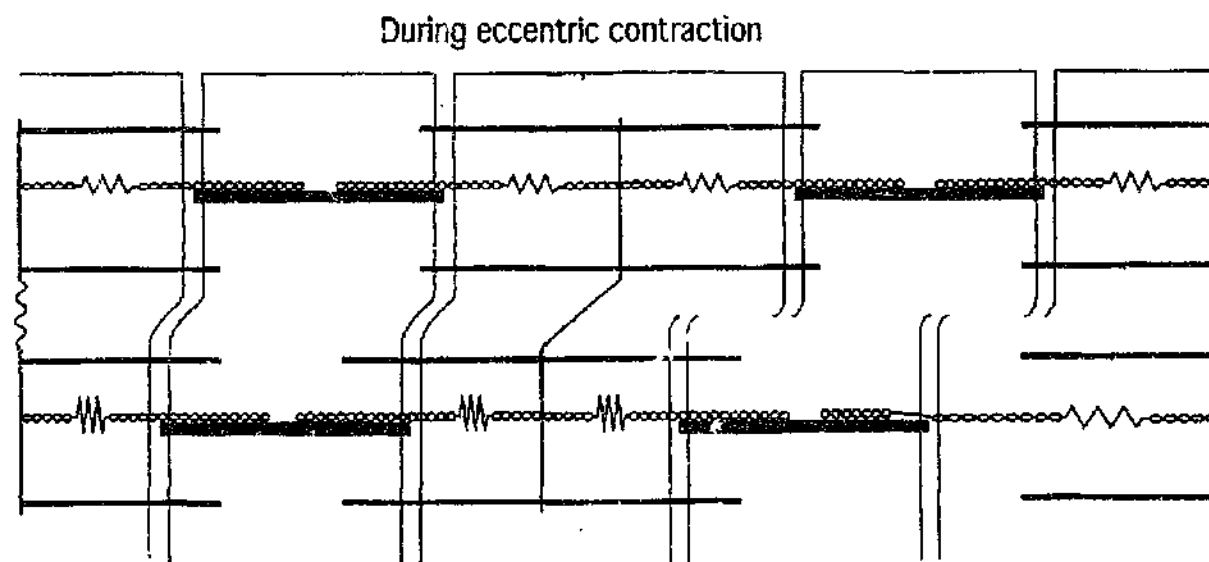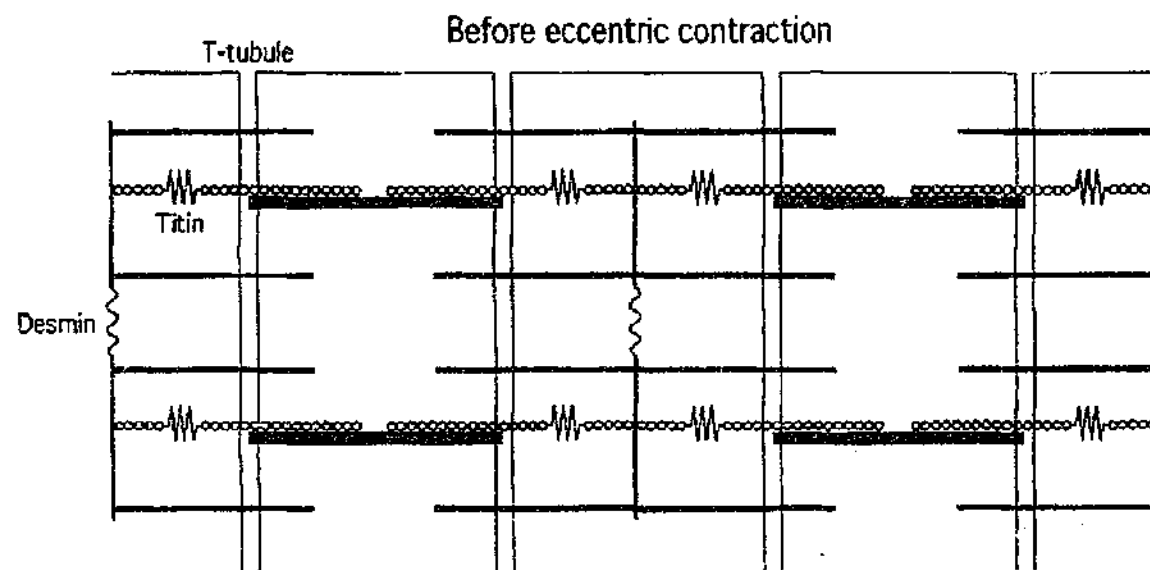
an eccentric contraction, but only when the muscle was stretched onto the descending limb of its length-tension relationship.

The overextension of sarcomeres during eccentric contractions can also provide an explanation for the observed damage to cytoskeletal structures within the muscle, in particular titin, and the intermediate filament desmin (see Figure 1.4). As discussed earlier in the chapter, when a sarcomere becomes overextended to a length beyond myofilament overlap, the rising tension borne by titin causes it to yield (Wang et al. 1993). With repeated eccentric contractions, it is possible that progressively more of the titin filament is dislodged from the thick filament. Given that titin has an important role in stabilising the thick filament in the centre of the sarcomere (Horowits, 1999), such structural disruption might lead to misalignment of the contractile filaments so that the sarcomere is no longer able to generate active tension. Recent experimental evidence in support of the above proposal was provided by the findings of (Fridén & Lieber, 1998). They showed that repeated eccentric contractions of rabbit extensor digitorum longus muscle led to a disturbance of the regular staining pattern of titin in some sarcomeres, scattered throughout the fibre.

There is some evidence that the structural integrity of the intermediate filament desmin is also affected by a series of eccentric contractions (Lieber et al. 1996; Fridén & Lieber, 1998). It was found, using immunohistochemisty, that 'desmin negative' fibres were evident as soon as 5 minutes after eccentric contractions, suggesting that disruption of desmin represented an early event in the damage process (Lieber et al. 1996). The authors proposed that a rise in intracellular calcium due to membrane damage would lead to the activation of calpain, a protease that selectively degrades the intermediate filament network (Belcastro, 1993). The proteolysis of desmin would then lead to further sarcomere disruption, exacerbated by additional eccentric contractions (Lieber et al. 1996). However, a recent study showed that in gene knockout mice, muscles lacking desmin were less vulnerable to injury than normal muscles with desmin (Sam et al. 2000). This finding suggested that desmin played a role in the damage process. Since desmin mechanically links adjacent myofibrils at the Z-line, the overextension of a sarcomere during an eccentric contraction would be expected to place extra strain on desmin, which, in turn, could transmit any structural disruption of sarcomeres across a fibre from one to

adjacent myofibrils. This process would provide an explanation for the loss of myofibrillar registry after eccentric contractions, and why damage appears to spread across the fibre.

Membrane damage from sarcomere non-uniformities

There is evidence to suggest that disruption of sarcomeres from eccentric contractions also leads to damage of muscle membranes (see Allen, 2001). Two recent studies have provided evidence that the T-tubules might be particularly vulnerable to damage from eccentric contractions (Takekura *et al.* 2001; Yeung *et al.* 2002a). Using electron microscopy, Takekura *et al.* (2001) reported that immediately after eccentric exercise on rats, there was a disorganisation of the T-tubular system, shown by the presence of longitudinally orientated T-tubules throughout muscle fibres. The authors proposed that this could be partly explained by sarcomere non-uniformity, leading to T-tubule damage. Yeung *et al.* (2002a) used confocal microscopy to observe the morphology of the T-tubule system after eccentric contractions on single mouse muscle fibres. They showed that eccentric contractions led to the development of T-tubule vacuoles and a slowed diffusion of the dye sulforhodamine out of the T-tubule system. The authors proposed that non-uniform lengthening of sarcomeres might strain adjacent T-tubules, causing them to rupture if the shear force became too great (see Figure 1.4). This, in turn, would lead to an influx of calcium and sodium into the cell. It was postulated that if the osmotic load of sodium and water exceeded the capacity of the T-tubule sodium pump, T-tubule vacuoles would develop (Yeung *et al.* 2002a). The possible functional consequences of such damage to fibre membranes will be discussed later in the chapter.

Secondary events in the damage process

It is generally thought that following the initial mechanical disruption to sarcomeres and subsequent membrane damage, the next step in the damage process is an influx of calcium into the cell (Armstrong *et al.* 1991). There has been direct (Balnave *et al.* 1997; Lynch *et al.* 1997; Ingalls *et al.* 1998) and indirect (Duan *et al.* 1990; Fridén & Lieber, 1996) evidence for an increase in resting calcium concentration soon after a period of eccentric contractions. If calcium homeostasis was not maintained, because calcium pumps were damaged by eccentric contractions, or calcium buffering systems such as the mitochondria became overwhelmed by the

excess calcium (Duan *et al.* 1990), it is possible that various calcium-dependent proteases and phospholipase A_2 would be activated. These enzymes would degrade the contractile filaments, cytoskeleton and muscle membranes (Armstrong *et al.* 1991; Belcastro *et al.* 1998).

The rate of protein degradation of damaged muscle has been shown to increase by a maximum of 60%, two to four days after eccentric contractions (Lowe *et al.* 1995). An increased level of myofibrillar proteins such as creatine kinase (CK) and myoglobin in the blood, has been commonly used as a marker of muscle damage from eccentric contractions (Byrnes *et al.* 1985b; Newham *et al.* 1986; Donnelly *et al.* 1992; Balnave & Thompson, 1993; Pizza *et al.* 1996). The appearance of these proteins in the blood is delayed, and the time-course is quite variable, with peak values found as early as a few hours after eccentric exercise, or up to 4 or 5 days post-exercise (see Warren *et al.* 1999b). Also, the magnitude of the increase in these proteins can vary considerably between subjects (Balnave & Thompson, 1993). Using elevated levels of muscle proteins as a marker of muscle damage is complicated by the fact that the circulating levels reflect both their release and clearance rates (Clarkson & Newham, 1995). Furthermore, it has been reported that the rise in creatine kinase after eccentric contractions does not correlate with another indicator of muscle damage, the reduction in the isometric force generated by the muscle (Fridén & Lieber, 2001).

Contracture

If the influx of calcium into the cell from damaged membranes was localised, sarcomeres in this region of the fibre would be expected to undergo a period of uncontrolled contraction, or contracture. There is experimental evidence in support of such an event. As described earlier, it has been shown by Ogilvie *et al.* (1988) that following eccentric exercise on rats, some muscle fibres contained hypercontracted sarcomeres or 'clotted' regions. Fridén & Lieber (1998) reported that sarcomeres in the enlarged 'swollen' regions of damaged muscle fibres were hypercontracted, with an average length of 0.9 µm compared to 2.5 µm for adjacent, normal sarcomeres in the same fibre. In addition, Warren *et al.* (1995), using calcium fluorescence techniques, found in their study that the 'swollen' regions of muscle fibres contained an elevated resting calcium concentration.

The development of a contracture could potentially either accelerate or slow down progression of the damage process in a muscle fibre. On one hand, the mechanical forces generated by the contracture might act to cause further disruption to sarcomeres and membranes in neighbouring regions, thereby exacerbating the damage. However, it has been proposed that a band of hypercontracted sarcomeres might prevent spread of calcium along the fibre and thereby protect adjacent sarcomeres from degradation (Armstrong et al. 1991). This protective effect might also be facilitated by the formation of a demarcating membrane on each side of a 'swollen' region, acting to 'wall off' the injury site (Warren et al. 1995; Komulainen et al. 1998).

Inflammation, swelling and regeneration

In response to muscle damage, an inflammatory response is initiated in order to remove the necrotic tissue as a first step towards repair and regeneration. This process is characterised by the migration of white blood cells to the injury site, with macrophages being the predominant cell type at 24 to 48 days after the eccentric contractions (Smith, 1991). Accompanying inflammation is muscle swelling, in which a protein-rich fluid, known as exudate, accumulates in and around damaged regions of the muscle, as a result of an increased vascular permeability (Stauber et al. 1988; Smith, 1991). In human subjects, swelling has been measured as an increase in limb circumference (Cleak & Eston, 1992; Howell et al. 1993; Brockett et al. 2001), limb volume (Whitehead et al. 1998) and muscle cross-sectional area using ultrasonography (Chleboun et al. 1998). These studies have shown that swelling does not become significant until the first or second day after eccentric exercise and usually reaches a peak value over the next two to three days.

The final step in the damage process is regeneration of the previously injured regions of muscle fibres. In both human (Fridén et al. 1983; Jones et al. 1986) and animal (Armstrong et al. 1983) studies, signs of regeneration have been shown to occur from 72 hours after eccentric contractions. During regeneration, satellite cells migrate from the periphery of the fibre to the injury site and initiate repair and regeneration of the damaged myofibers (Darr & Schultz, 1987). There is evidence that small diameter myofibers develop by 5 days after eccentric contractions (Darr & Schultz, 1987; Stauber et al. 1988), with complete regeneration of the fibre taking up to 30 days or more (McCully & Faulkner, 1985).

Adaptation and protection from muscle damage

An interesting consequence of eccentric exercise, first observed by Hough (1902) is that a single period provides a significant protective effect. That is, if similar eccentric exercise is performed a week or so after the initial bout, there is considerably less soreness and muscle damage, as indicated by changes to active mechanical properties of the muscle (Nosaka *et al.* 1991; Clarkson *et al.* 1992; Balnave & Thompson, 1993; Brockett *et al.* 2001). This suggests that some kind of adaptation occurs within the muscle in response to the initial period of eccentric exercise, and while various hypotheses have been put forward to explain this effect, the underlying mechanism remains the subject of debate (for recent reviews see McHugh *et al.* 1999; Proske & Morgan, 2001).

It was hypothesised by Morgan (1990) that a muscle might adapt from a period of eccentric exercise by adding sarcomeres in series to damaged and possibly non-damaged muscle fibres. This would shift the active length-tension relationship to longer muscle lengths, so that during subsequent eccentric exercise, less of the muscle's working range would involve the descending limb of the length-tension relation, the region where non-uniform lengthening of sarcomeres is thought to occur (Morgan, 1990). Experimental evidence in support of this idea was provided by Lynn & Morgan (1994). They reported that running rats downhill on a treadmill, 30 minutes a day for 5 days, led to a significant increase in the number of sarcomeres in muscle fibres of a knee extensor muscle, which had undergone eccentric exercise, compared to sedentary or uphill (concentric exercise) trained rats. Following a similar training regime, Lynn *et al.* (1998) reported that downhill trained rats suffered less damage from an acute period of eccentric contractions than uphill trained rats, as measured by changes to active mechanical properties of the muscle. Importantly, the eccentric contractions were carried out over the same working range of the muscle for both training groups, suggesting that an increased number of sarcomeres in muscle fibres after eccentric training had provided protection against damage. Further indirect evidence of such an adaptation was recently shown by Brockett *et al.* (2001). Here, it was found that a single period of eccentric exercise of human hamstring muscles led to a sustained shift of the active angle-torque relationship towards longer muscle lengths, which was accompanied by fewer signs of muscle damage from a second period of eccentric exercise performed 8 days after

the first. Interestingly, there is also some evidence to suggest that the opposite effect occurs as a result of training with concentric exercise, that is, the muscle becomes more susceptible to damage from eccentric exercise (Ploutz-Snyder *et al.* 1998; Whitehead *et al.* 1998). It was suggested that the muscle adapted to concentric exercise by reducing the number of sarcomeres in muscle fibres. Thus, during a period of eccentric exercise, the working range included more of the descending limb of the length-tension relation, so that the muscle became more extensively damaged (Whitehead *et al.* 1998).

Delayed onset muscle soreness (DOMS)

While the sensation of DOMS appears to be related to the process of muscle damage from eccentric contractions, the underlying mechanism by which this occurs remains unclear. An mentioned earlier, DOMS can be experienced as early as 8 to 10 hours after eccentric exercise, reaches peak intensity between 24 to 48 hours and has usually gone after a week (Hough, 1902; Byrnes *et al.* 1985a; Balnave & Thompson, 1993; Brockett *et al.* 2001). An interesting characteristic of DOMS is that it is only experienced when the muscle is stretched, contracted or palpated, all mechanical stimuli that are normally not painful. That is, there is typically no persistent, chronic pain in a completely relaxed muscle with DOMS (see Weerakkody *et al.* 2001).

It has been hypothesised that DOMS is associated with the inflammatory response to muscle damage (Smith, 1991). During inflammation, there is a local production of chemicals such as prostaglandins (PGE_2). These substances are known to sensitise muscle nociceptors (pain receptors). Nociceptors are free nerve endings that terminate in connective tissue adjacent to muscle fibres (Stacey, 1969), and are served by group III (small myelinated) and IV (unmyelinated) afferent fibres (Mense, 1977). Once in a sensitised state, nociceptors can respond to normally non-noxious mechanical stimuli such as stretch, contraction and palpation of the muscle, and thus signal pain. It was proposed that this process would be facilitated by muscle swelling, by increasing intramuscular pressure during a contraction and therefore providing a mechanical stimulus for the sensitised nociceptors (Smith, 1991). While this hypothesis remains a possibility, there is some experimental evidence that argues against it. In the study by Newham (1988), it was reported that the time-course of inflammation and swelling after eccentric exercise did not parallel that of DOMS. In addition, it has been shown that nonsteroidal anti-inflammatory drugs, which prevent

prostaglandin production, do not reduce DOMS, when taken before or after eccentric exercise (Croisier et al. 1996; Bourgeois et al. 1999).

An alternative hypothesis to that described above, was put forward by Weerakkody et al. (2001). They proposed that large-fibre mechanoreceptors, particularly muscle spindles, might contribute to DOMS. The experiments were carried out on subjects experiencing DOMS in the triceps surae muscle group, two days after a period of eccentric exercise. There were three pieces of evidence in support of their hypothesis. Firstly, it was shown that localised vibration (20 and 80 Hz) applied to the skin overlying a sensitive region of the muscle, increased the intensity of DOMS. This was attributed to muscle spindles, since they were known to be the only mechanoreceptors in the muscle to respond to 80 Hz vibration. Secondly, a nerve compression block, which selectively blocked large nerve fibres, led to an increase in the threshold of pain during a mechanical stimulus. Thirdly, injection of 5% sodium chloride into a sensitive area of the muscle was found not to produce more pain than an injection into an unexercised muscle. If nociceptors were in a sensitised state, as a result of the inflammation, the hypertonic saline would have been expected to produce more pain in the exercised muscle. It was concluded that while there was evidence of an involvement of large fibre mechanoreceptors in DOMS, the mechanism by which this occurred remained to be elucidated. A mechanism operating at the level of the spinal cord seemed to be the most likely possibility (Weerakkody et al. 2001).

Mechanical indicators of muscle damage

Reduction in active tension

A commonly used indicator of muscle damage after a series of eccentric contractions is a reduction in the level of tension generated during an isometric contraction. Generally, the drop in tension is greatest immediately after a period of eccentric contractions, and then slowly recovers over several days or weeks for both humans (Clarkson et al. 1992; Jones et al. 1997) and animals (McCully & Faulkner, 1985; Lowe et al. 1995). The size of the initial tension drop can vary considerably, from as little as 10% (Brockett et al. 1997) to almost 90% (Warren et al. 1999a). The size of the fall in tension depends on a number of factors relating to the eccentric contraction protocol, such as the number of contractions, the stretch amplitude, and the length range over which the contractions are carried out (Talbot & Morgan, 1998). The

level of muscle activation during the eccentric contractions, and whether or not it is synchronous activation, is also likely to be an important factor in the force drop. For most eccentric contraction protocols, part of the initial reduction in force will be due to metabolic fatigue, which usually recovers within the first 3 hours after the eccentric contractions (Faulkner et al. 1993). The remainder of the reduced tension is assumed to be due to the effects of the eccentric contractions, however the underlying mechanism remains a point of conjecture (for recent reviews see Morgan & Allen, 1999; Allen, 2001; Proske & Morgan, 2001; Warren et al. 2001).

There have been two main hypotheses for the loss of active tension in muscle after eccentric contractions, structural damage to muscle fibres and E-C coupling failure. Warren et al. (1993) proposed that impairment of the E-C coupling process was primarily responsible for the fall in active tension after eccentric contractions. They reported that following eccentric contractions of mouse soleus muscle there was a large drop in tension, more than 40%. However, the tension generated by adding caffeine was the same before and after the eccentric contractions. Since caffeine acts directly on the SR to release calcium, it was proposed that failure of the E-C coupling process at some point before calcium release from the SR was responsible for the drop in tension. A similar interpretation was reached by Balnave & Allen (1995) on single mouse fibres. They found that following a series of eccentric contractions, a moderate drop in tension was accompanied by a reduced intracellular calcium concentration during tetanic contractions. Since caffeine could restore both tension and calcium concentration to normal values, it was suggested that structural damage was minimal and that EC-coupling failure could explain the majority of the fall in tension. However, the same study showed that by imposing a more extreme eccentric contraction protocol on the muscle, in which there were more, and longer stretches, some of the tension deficit could not be recovered with caffeine. Here, it was suggested that a component of the tension drop could be explained by structural ...age to the fibre (Balnave & Allen, 1995). It has also been shown that for ...bian muscle, the reduced tension after eccentric contractions could not be ...ted to a change in calcium release (Morgan et al. 1996; Allen, 2001). Thus, ...ems to be a species difference between mammalian and amphibian muscle in ...f the underlying cause of the immediate fall in tension after eccentric ...ons (see Allen, 2001).

While E-C coupling impairment appears to contribute to the reduction in tension generating capacity of the muscle, the mechanism remains unclear. One obvious cause would be damage-induced inexcitability of some muscle fibres. However, the two studies that have examined this possibility showed conflicting results (Warren *et al.* 1993; McBride *et al.* 2000). Based on the current experimental evidence, Warren *et al.* (2001) suggested that the E-C coupling 'failure site' might be the T-tubule voltage sensors, which undergo a functional change as a result of the eccentric contractions. As mentioned earlier, there is recent evidence of structural disruption of the T-tubule system following repeated eccentric contractions, consistent with mechanical damage from non-uniform lengthening of sarcomeres (Takekura *et al.* 2001; Yeung *et al.* 2002a). If ruptured T-tubules sealed over or remained disconnected, this could lead to a localised loss of conduction, a reduced calcium release from the SR, and possibly a drop in tension (Yeung *et al.* 2002a). In addition, a rise in resting calcium concentration from damaged T-tubules could also affect calcium release from the SR (Yeung *et al.* 2002a). Since disrupted T-tubules have been identified extensively throughout damaged fibres, and were evident for several days after eccentric exercise (Takekura *et al.* 2001), it is possible that T-tubule damage might also contribute to the prolonged reduction in tension. This would be consistent with the findings of Ingalls *et al.* (1998), who suggested that E-C coupling failure could account for more than half of the tension deficit up to 5 days after eccentric contractions. Disruption of contractile filaments and the cytoskeleton have also been proposed to contribute to the drop in tension. If the damage involved many adjacent myofibrils, then tension transmission along the fibre could be affected. Also, disruption of the cytoskeleton, in particular desmin, could effect lateral transmission of tension across the fibre (Morgan & Allen, 1999).

Shift in optimum length

Katz (1939) first observed that stretching an active muscle sometimes led to a shift to longer lengths of the optimum length for active tension. He reported that such shifts were evident only when the active stretches ended at lengths beyond the initial optimum length, and proposed that they were due to the 'partial transformation of active contractile into passive elastic tissue'. Similar shifts in the length-tension relationship after repeated eccentric contractions have now been shown in a number of studies, carried out on single frog fibres (Morgan *et al.* 1996), bundles of rat

muscle fibres (Yeung *et al.* 2002b), whole amphibian muscle (Wood *et al.* 1993; Talbot & Morgan, 1996) and human muscle (Saxton & Donnelly, 1996; Jones *et al.* 1997; Whitehead *et al.* 1998; Brockett *et al.* 2001). It has been proposed that after eccentric contractions, some disrupted sarcomeres will be unable to generate active tension, and so represent passive elements in series with the still functioning sarcomeres. This would increase the series compliance of the muscle and increase the rest length of disrupted sarcomeres, which would consequently shift the optimum length towards longer muscle lengths (see Morgan & Allen, 1999). In this way, the shift in optimum length would provide a measure of the average number of disrupted sarcomeres in myofibrils after a series of eccentric contractions. Experimental support for this idea was provided by a study in which the size of the shift in optimum length increased, following eccentric contractions carried out further down the descending limb of the length-tension relationship (Talbot & Morgan, 1998), in accord with the predictions of the Morgan (1990) model. This study also showed that the shift in optimum length correlated with the drop in active tension after eccentric contractions (Talbot & Morgan, 1998).

Since a shift in optimum length is evident immediately after a series of eccentric contractions, it has been considered to be a useful indicator of the extent of sarcomere disruption and muscle damage, and is independent of muscle fatigue (Proske & Morgan, 2001). Importantly, the shift can also account for some of the immediate reduction in active tension, whenever tension is compared at the same length before after eccentric contractions. In some cases this overestimation of the drop in tension can be significant, with the fall at the initial optimum length being more than twice that measured at the post-eccentric contractions optimum length (see Yeung *et al.* 2002b; Fig. 3). A reversal of the shift in optimum length has been shown to occur by about 5 hours after eccentric contractions in toad sartorius muscle (Jones *et al.* 1997) and within 1 to 2 days after eccentric exercise for human triceps surae (Jones *et al.* 1997; Whitehead *et al.* 1998). It was proposed that a reversal of the shift was due to the reinterdigitation of overextended sarcomeres in muscle fibres, a process that occurred over a period of several hours after the eccentric contractions (Jones *et al.* 1997). This idea was supported by the finding of a reduced number of disrupted sarcomeres in toad muscle fibres 5 hours after eccentric contractions (Jones *et al.* 1997). A reversal of the shift, however, could also be

explained for some fibres, by small areas of disrupted sarcomeres progressing into larger regions of damage, leading to the point where the fibre was no longer able to contract and so did not contribute to the length-tension relationship (Morgan & Allen, 1999).

While it has been proposed that the shift in optimum length is due to an increase in series compliance of the muscle, it could also be argued that the shift is due to an impairment of E-C coupling. That is, if the level of activation had been reduced because calcium release from the SR was less, a shift in optimum length would occur because of the increased calcium sensitivity at long lengths (Endo, 1973). However, there is experimental evidence that argues against this as the only mechanism. It has been shown that the length-tension curves before and after eccentric contractions sometimes cross, so that at longer lengths, a higher tension is produced after the eccentric contractions (Morgan et al. 1996; Brockett et al. 2001; Brockett et al. 2002; Yeung et al. 2002b). This effect is not consistent with a lower level of activation. The shift in optimum length might also contribute to a phenomenon known as 'low-frequency fatigue', in which, after eccentric contractions, there is a proportionally greater drop in tension at low compared to high stimulation frequencies (Davies & White, 1981; Newham et al. 1983a; Newham et al. 1987; Jones et al. 1989; Yeung et al. 2002a; Yeung et al. 2002b). While it has been proposed that 'low-frequency fatigue' might reflect a reduced calcium release as a result of E-C coupling failure, it could also be partly due to an increased series compliance, which would tend to slow the rate of tension rise to a greater extent at low frequencies (Allen, 2001).

Passive mechanical properties

While extensive research has been carried out on changes to the active contractile function of muscle after eccentric contractions, by comparison, changes to passive mechanical properties have been little studied. A common symptom experienced after unaccustomed eccentric exercise is the feeling of muscle stiffness, which accompanies DOMS. Studies carried out on human elbow flexor muscles, have proposed that an increased muscle stiffness leads to a reduced range of motion (Howell et al. 1985), and a more flexed resting elbow angle (Clarkson & Tremblay, 1988; Cleak & Eston, 1992; Brockett et al. 1997; Chleboun et al. 1998). It has also been shown that passive tension and stiffness of the resting muscle increases after eccentric exercise (Jones et al. 1987; Howell et al. 1993; Chleboun et al. 1998).

Jones *et al.* (1987) reported that following a period of eccentric exercise, the external force required to extend the elbow increased immediately after eccentric exercise, and reached a maximum between 24 and 72 hours post-exercise. Using the same muscle group, Howell *et al.* (1993) and more recently Chleboun *et al.* (1998) measured static passive torque over a range of elbow angles up to full elbow extension, and calculated passive stiffness from the slope of a straight line fitted to torque values at short to intermediate muscle lengths. In both studies, passive stiffness increased immediately after a period of eccentric exercise and remained fairly constant over the next 3 to 5 days, before returning to control values. Passive torque of human triceps surae has also been shown to increase after eccentric exercise (Jones *et al.* 1997; Whitehead *et al.* 1998).

There have been various mechanisms put forward for the rise in passive stiffness after eccentric exercise. Firstly, it is unlikely that the increased stiffness is due to neural activity, since measurements carried out on the passive muscle are not accompanied by any electromyographic (EMG) activity (Howell *et al.* 1985; Howell *et al.* 1993; Chleboun *et al.* 1998). This implies that the increased stiffness is the result of changes to intrinsic passive properties of the muscle as a result of damage from the eccentric contractions. Jones *et al.* (1987) proposed that the increased stiffness was due to damage and shortening of parallel, connective tissue structures as a result of the eccentric contractions. It has also been suggested that damage-induced swelling within the muscle compartment might exert strain on perimysial connective tissue elements, leading to a restricted range of motion (Howell *et al.* 1985) and an increased passive stiffness at long muscle lengths (Howell *et al.* 1993). However, Chleboun *et al.* (1998) measured passive stiffness at longer lengths and found that there was no significant change as a result of the eccentric exercise. In addition, they reported that the increased passive stiffness at intermediate lengths, measured immediately after eccentric exercise, could not be attributed to muscle swelling, since this did not become significant until 48 hours post-exercise (Chleboun *et al.* 1998).

It was postulated by Howell *et al.* (1993) that the initial increase in passive stiffness at intermediate muscle lengths might be the result of a low-level of activation of the contractile machinery, triggered by an increased sarcoplasmic calcium concentration as a result of damage to internal muscle fibre membranes. In order to explain the

progressive increase in passive tension with length, the authors suggested that the level of activation became greater with stretch, possibly due to an increased calcium release via stretch-activated calcium channels (Howell *et al.* 1993). While this hypothesis remains a possibility, based on the fact that a small rise in resting calcium concentration has been shown after eccentric contractions (see above), there is at present, however, no direct evidence that the intracellular calcium concentration increases with stretch of a damaged muscle. Furthermore, this proposal is inconsistent with the fact that passive stiffness does not increase at longer muscle lengths (Chleboun *et al.* 1998).

An alternate hypothesis, one that is able to account for an immediate rise in passive stiffness, is that during eccentric contractions, an uneven distribution of the length change by sarcomeres along muscle fibres (Morgan, 1990), leads to strain and tearing of membranous structures (T-tubules and SR), causing a localised rise in calcium concentration and the development of contracture in parts of some muscle fibres. There is experimental evidence in support of such regions of contracture after a series of eccentric contractions, as described earlier. It seems plausible that the tension produced by fibres undergoing a contracture would show up as a rise in whole muscle passive tension in the absence of any neural activation.

The work presented in Chapters 3, 4 and 5 of this thesis tested this hypothesis more formally, by measuring changes to the passive mechanical properties of both human and animal muscle after a period of eccentric contractions. It was hoped that investigating the mechanism underlying changes to passive properties of muscle would provide new insight into the process of muscle damage from eccentric contractions.

The history-dependence of passive tension in cat medial gastrocnemius muscle

Introduction

The experiments described in this chapter aimed to explore the thixotropic properties of resting, whole mammalian muscle, and to test the hypothesis that such behaviour was consistent with the presence of stable cross-bridges in the resting muscle. This remains an issue of importance since alternative interpretations of the history-dependent properties of passive muscle have been recently proposed, as described in Chapter 1.

It was hypothesised that stable cross-bridges would manifest themselves in two ways, as a component of the resting tension, and as the initial steep rise in tension during a slow passive stretch (Hill, 1968). This hypothesis was examined in a number of ways, by looking at both the history and length-dependent properties of the passive muscle. The important new approach taken here was to use different forms of muscle conditioning, in an attempt to differentiate between the passive mechanical properties of cross-bridges and other passive structures within the muscle.

Methods

The experiments were carried out on a total of 11 cats (*Felis domesticus*) of both sexes weighing between 2.5 and 6 kg. All experiments were undertaken with approval from the Monash University Committee for Ethics in Animal Experimentation.

Anaesthesia

General anaesthesia was induced by an intraperitoneal injection of 40 mg kg^{-1} sodium pentobarbitone (Nembutal®, Rhone Merieux, Australia), in isotonic saline, and was maintained throughout the course of the experiment with additional doses (6 mg) delivered into the cephalic vein. The presence of a withdrawal reflex, and a reduction in the end-tidal carbon dioxide concentration below 5%, indicated that a supplementary dose of anaesthetic was required.

Surgical procedures

After the animal was anaesthetised, a glass cannula was inserted into the trachea for ventilation, and the cephalic vein of the right forelimb was cannulated, for the administration of additional anaesthetic. The animal was then placed in the prone position on a metal frame and secured with a head clamp. The hips were fixed in place with steel pins screwed into the iliac crest bilaterally. A rectal probe was used to measure core body temperature, which was maintained at ~37°C by means of a feedback-regulated heating blanket. The expired carbon dioxide concentration was monitored using an infrared capnograph (Normocap, Datex, Finland). In some experiments, it was necessary to use a pump to ventilate the animal via the tracheal cannula.

An incision was made along the midline of the dorsal aspect of the trunk, from the sacrum to the third lumbar vertebra. The dorsi communis, longissimus dorsi medialis and multifidus spinae muscles on both sides of the vertebral column were dissected free and removed. The ligamentum flavum, located between the L7 vertebra and the sacrum was carefully cut and removed. A laminectomy of the dorsal aspect of vertebrae L4 to L7 and the sacrum was then performed using bone ronguers, exposing the spinal cord in this region. The spinous process of L3 vertebra was clamped and fixed to the metal frame. The dura was opened, and the ventral and dorsal spinal roots of L6 to S2 were exposed and then cut at their point of entry into the spinal cord. The exposed tissue was packed with sterile gauze soaked in Ringer, and the skin was clamped with Michel clips (Martin Brand, Germany).

The experiments were carried out on either the medial gastrocnemius (MG) (n=8) or soleus (n=3) muscles of the left hindlimb. These muscles, together with the lateral gastrocnemius (LG), form the triceps surae muscle group, the principle extensors of the ankle. An incision was made along the skin from the popliteal fossa to the calcaneum. The small saphenous vein was ligated and the distal tendons of the peroneal muscles were cut close to the calcaneum. This exposed a small section of the lateral surface of the fibula, on which a mark was made with the cautery. The distal tendon of the muscle chosen for the experiment was located, and a thin, nylon thread was tied to it. The hindlimb was then adjusted to the approximate position it would adopt during the experiment, and the ankle was maximally dorsiflexed. At

this point, while all of the ankle extensor muscles were still attached, the distance between the markers on the fibula and tendon was noted, and used later to determine the maximum physiological length of the muscle (L_{max}).

When the experiments were carried out on MG, it was necessary to first separate it from LG. Then, the distal tendons of LG, plantaris and soleus were located and cut, leaving only the tendon of MG attached to the calcaneum. A similar procedure was carried out for experiments using soleus, however, in this case, the MG tendon was cut and the soleus tendon was preserved. After this, LG, plantaris and the non-experimental muscle (soleus or MG) were separated from adjacent connective tissue, and any blood vessels supplying them were tied off. Each muscle was then ligated as close as possible to its proximal end, and removed. This process ensured that only the muscle chosen for the experiment remained, physically isolated from any other musculature, with its nerve, blood supply, and both proximal and distal tendons intact.

After removing the superficial, popliteal fat, the nerve to either MG or soleus was located and carefully separated from the tibial nerve over a length of about 20 mm, so that it could be easily placed on the stimulating electrodes. The remaining portion of the tibial nerve was then ligated and cut. An extensive dennervation of other hindlimb nerves, including the common peroneal and sural nerves, and those innervating the hip, was also carried out. The hip dennervation involved dissecting the gluteus maximus, caudofemoralis and pyriformis muscles, sectioning their nerves, and reflecting them to expose the sciatic nerve. The nerves to gluteus inferior, gluteus superior, and the muscular branch of the sciatic nerve were ligated and cut.

During both the laminectomy and hindlimb dissection procedures, exposed tissues were kept moist with Ringer solution. After the surgical procedures were complete, exposed spinal and hindlimb tissues were covered with mineral paraffin oil aerated with carbogen (95% O_2, 5% CO_2), in baths fashioned from skin flaps. Throughout the experiment, the baths were maintained to within 1 to $2°$ C of core body temperature by radiant heat.

Experimental equipment and data acquisition

The calcaneum was cut and the piece attached to the MG or soleus tendon had a 2 mm diameter hole drilled through it. A threaded rod was passed through the hole and was clamped to the calcaneum by a pair of nuts and washers. The rod was attached to a transducer, which measured the tension generated by the muscle. It consisted of two silicon strain gauges, which formed one half of a Wheatstone bridge circuit. Output signals from the transducer were further amplified at a gain of 10 or 100 X for passive measurements, and low-pass filtered, usually at 1 kHz. A 50 Hz notch filter was also used.

The tension transducer was connected, via a metal shaft, to a custom designed electromagnetic servomotor (Department of Physiology, Monash University), with a position feedback from an LVDT (linear variable differential transformer) displacement transducer. This provided an output voltage, which could be calibrated to measure the change in length of the muscle. Before the commencement of the experimental procedure, the servomotor stretched the muscle to L_{max}, as determined by the distance between the tendon and bone markers measured beforehand, and this length was then designated as zero.

The signals from both tension and length transducers were processed by a commercial analogue to digital converter (MacLab/8s, AD Instruments, Australia) and recorded using the program 'Chart' (AD Instruments, Australia) running on a Macintosh G4 computer (Apple, U.S.A.). Data was then analysed using the software Igor Pro (Wavemetrics, Ore, U.S.A.).

Experimental procedure

Active length-tension relationship

The active-length tension relationship of the whole muscle was measured to determine the length at which maximum isometric active tension occurred, the optimum length (L_{opt}), which was used as a reference length for all passive tension measurements. With the muscle held at a given length, the nerve of the experimental muscle was stimulated with bipolar, platinum wire electrodes at a supra-maximal voltage, which was verified by increasing the stimulus current until tension no longer increased. MG was stimulated at 80 pulses per second (pps) for 0.5 s while for soleus, a rate of 50 pps for 1 s was used.

Muscle conditioning procedures

In order to investigate the thixotropic properties of the passive muscle, two conditioning procedures were used, referred to as 'hold-test' and 'hold-long', which were based on previously used methods (see Morgan *et al.* 1984; Proske & Morgan, 1999). For hold-test conditioning, the muscle was moved to the test length and stimulated for 0.5 s at 15 pps. For hold-long conditioning, the muscle was stretched by 3 mm at 10 mm s^{-1}, then stimulated as for hold-test, and held for 4 s before returning to the test length. It has been shown that holding the muscle at a longer length for 3 s before returning to the test length is required to maximise the effects of hold-long conditioning on intrafusal fibres (Morgan *et al.* 1984). Following both conditioning procedures, a test stretch of 3 mm amplitude at 1 mm s^{-1} was given 25 s after the stimulation period. At the end of each test stretch, the muscle was shortened to a length where the tendon was visibly slack, so that tension was close to zero. These measurements were made at 2 mm intervals over muscle lengths ranging from L_{opt}-10 mm to L_{opt}+6 mm.

Passive tension measurements

After both conditioning procedures, the tension recorded before the test stretch, when the muscle was held at the test length, was referred to as the 'resting tension' (Figure 2.1 A). This was calculated from the difference between the mean tension, measured over the 1 s period before onset of the test stretch, and zero tension, determined by shortening the muscle until the tendon was visibly slack. During the test stretch, there were two components; an initial steep rise in tension, referred to as the 'short-range component', followed by a more gradual change in tension. The peak of the short-range component was called the 'yield point', and the tension difference between the resting tension and yield point was termed the 'short-range tension' (see Figure 2.1). The 'short-range stiffness' was calculated as the slope of the short-range component as a function of muscle length. At muscle lengths longer than L_{opt}, the rise in passive tension beyond the yield point became much steeper. This component of the tension response to stretch was assumed to be due to parallel elastic structures, so it was called the 'elastic tension' (Figure 2.1 B). Since the aim was to determine the nature of the short-range tension, it was, therefore, necessary to account for any possible contribution from the elastic tension. To measure the elastic tension, a line was fitted to the slope of tension rise beyond the yield point, and this was then

Figure 2.1 *Method of measuring short-range tension.*

(A) Tension response to a test stretch at an intermediate muscle length (L_{opt}-2 mm). The level of resting tension (RT) before the stretch (dashed line) was taken as that above zero tension (dotted line), where the muscle was shortened until the tendon fell slack. The short-range tension (SRT) is measured as the increase in tension above the RT, up to the yield point. (B) At long muscle lengths (L_{opt}+6 mm), an additional component of tension during the stretch was attributed to elastic structures such as titin filaments. This elastic tension (ET) component was calculated by fitting a line to the second, less steep tension rise. This line was then shifted vertically so that it intersected the tension trace at the onset of the stretch. The ET component was then subtracted from the original record, to give the short-range component (lower trace).

A $L_{opt}-2$ mm

SRT

RT

0.1 s

B $L_{opt}+6$ mm

SRT

ET

SRT

0.2 N

50 ms
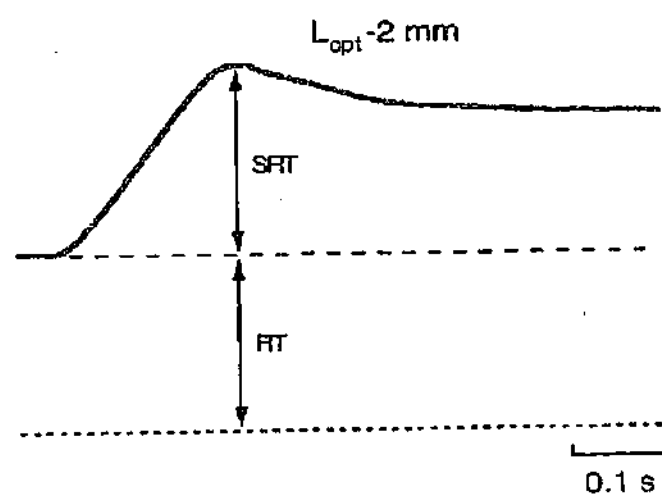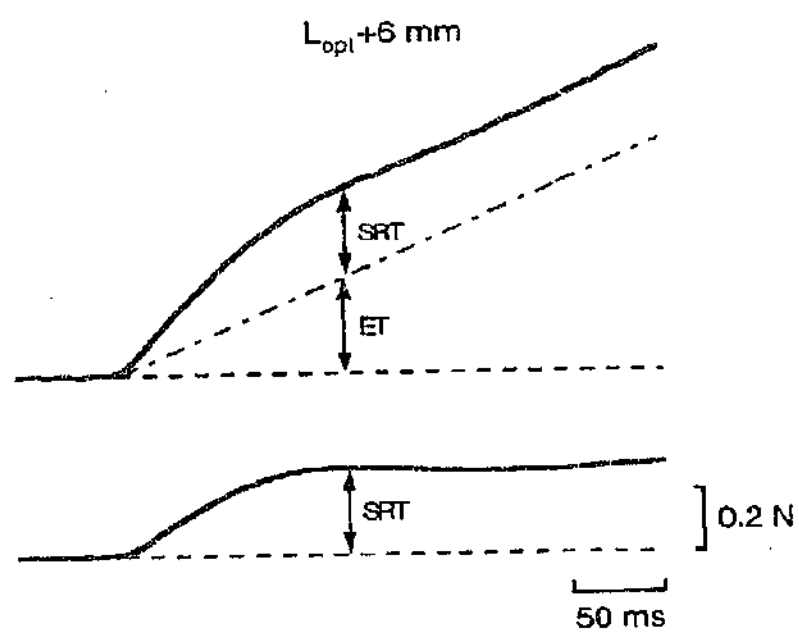
displaced vertically so that its starting point coincided with the tension trace at the start of the stretch. Tension values along the line were then subtracted electronically from the original record, and the remaining tension was attributed to short-range properties of the passive muscle (see Figure 2.1). A similar method was employed by Haugen & Sten-Knudsen (1981).

It has been shown previously that short-range tension takes time to re-develop following a stretch-shortening movement (Campbell & Lakie, 1998). In order to obtain a measure of the rate of development of short-range tension, test stretches were applied at various times after conditioning. For this, the muscle was conditioned using the hold-test procedure, that is, an isometric contraction, 0.5 s in duration at 15 pps. A second method of conditioning was also used, which involved a series of rapid stretch-shortening movements of 5 mm amplitude at 50 mm s^{-1} (see Figure 2.13). This was similar to the conditioning procedure used by Morgan et $al.$ (1984). A test stretch was carried out at intervals between 1 and 60 s after both forms of conditioning. The test length was L$_{opt}$.

In an early experiment, hold-test conditioning was carried out using a stimulation rate of 80 pps for MG. At the time, it was noted that the short-range tension measured after such conditioning was considerably smaller than after conditioning using a stimulation rate of 15 pps. Thus, in two experiments on MG, the effect of grading the size of the conditioning contraction on the measured short-range tension was investigated. In order to achieve smooth isometric tension profiles over a wide range of tensions at sub-maximal stimulation rates, the ventral roots with motor axons to MG (L7 to S1) were divided into five approximately equal portions, and these were stimulated sequentially (distributed stimulation) with supra-maximal stimuli over a range of frequencies from 7 to 80 pps (see Rack & Westbury, 1969; Wise et $al.$ 2001). The procedure for these experiments began with hold-long conditioning, as described previously, followed by distributed stimulation 5 s later. The duration of the stimulation was 1 s. The muscle was then brought back to the test length and held there for a further 50 s before the test stretch was applied. The test length for these experiments was L$_{opt}$.

Data analysis

For all parameters measured, the mean plus or minus the standard error of the mean ($\pm$ S.E.M.) was calculated. The significance level for all experiments was set at $P < 0.05$. A three-factor ANOVA with interactions was used to test for significant differences between the two types of conditioning. The factors were conditioning, length and animal. Where the interactions of conditioning and length were significant, a least significant difference (LSD) *post hoc* test was used to identify individual lengths at which conditioning effects were significant. Relationships between some parameters were determined by linear regression analysis. The statistical program used was Data : ·· '' (Ithaca, NY, USA).

Results

In three preliminary experiments, the soleus muscle was used. In line with previous studies (Rack & Westbury, 1969; Walmsley & Proske, 1981), it was found that soleus has a very broad active length-tension curve and low passive tension over most of the physiological range of muscle lengths (Figure 2.2). Since one of the main aims of these experiments was to examine passive properties over a wide range of muscle lengths, including the descending limb of the active length-tension relation, it was preferable to use a muscle with higher resting tension and narrower active length-tension relation. Thus, an additional eight experiments were carried out on MG, as it satisfied both of these criteria. The results presented here, unless otherwise stated, are taken from the experiments performed on MG. Here, it should be pointed out that the general features of the passive properties in MG, as described below, were also found in soleus.

Resting tension

One of the main aims of these experiments was to examine whether a component of the resting tension, particularly at short to intermediate muscle lengths, could be attributed to stable cross-bridges. It was found that resting tension was both length and history-dependent, that is, dependent on the preceding history of activation and length changes (Figure 2.3). This figure shows examples of the history-dependence of resting tension at short, intermediate and long muscle lengths. At short lengths (L_{opt}-6 mm), resting tension after hold-long conditioning was close to zero, while there was a small but positive resting tension after hold-test. At intermediate lengths

Figure 2.2 *Length-tension relationships for MG and Soleus.*

An example of active (A) and resting (B) length-tension relationships for MG (blue) and soleus (red). Muscle length is relative to the optimum length (L_{opt}) for both muscles.

A

B

Length relative to L_opt (mm)
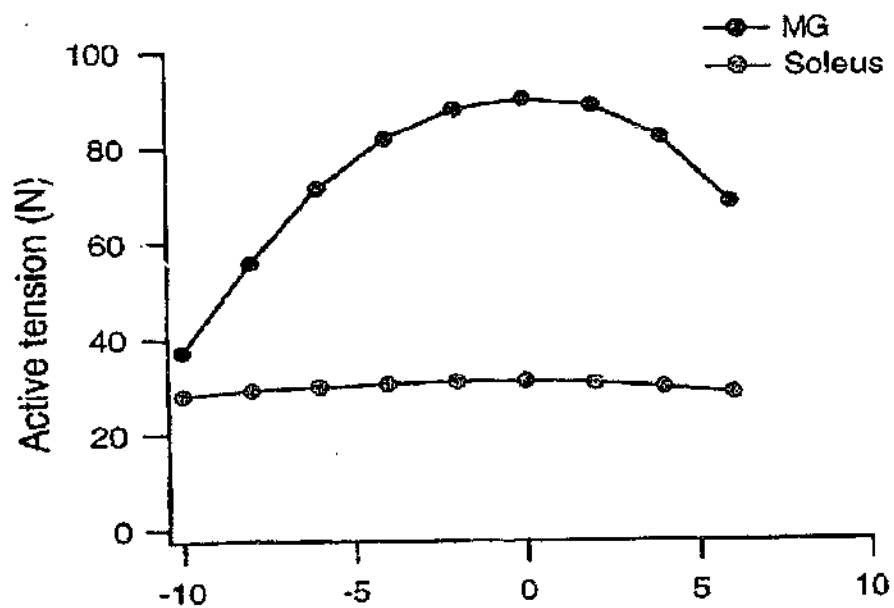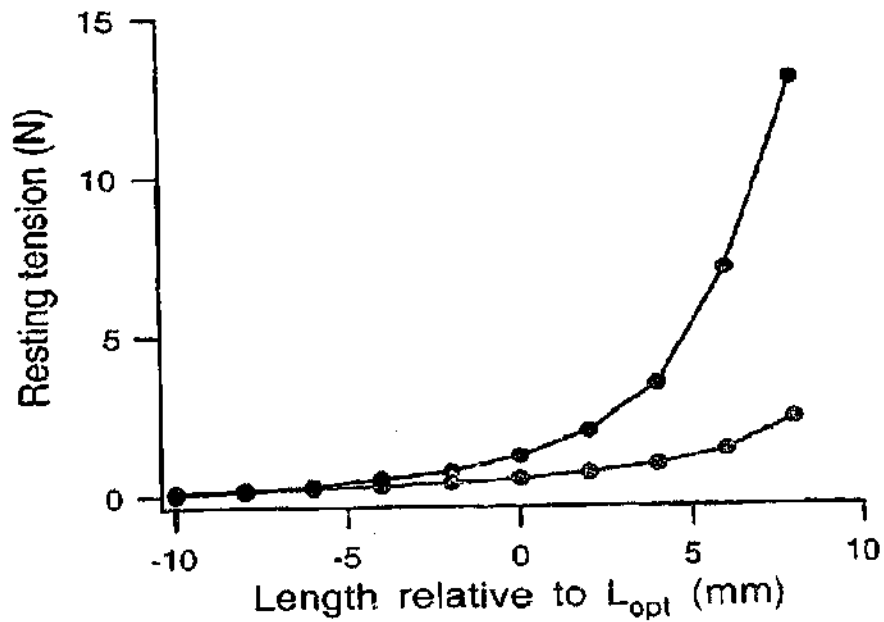
Figure 2.3 *Tension responses after muscle conditioning.*

The resting tension, and tension response to a test stretch, as measured after hold-test (blue) and hold-long (red) conditioning, has been shown at short (L_{opt}-6 mm), intermediate (L_{opt}) and long (L_{opt}+4 mm) muscle lengths. Zero tension, measured at a short length where the tendon lay visibly slack, is shown in each case by the dotted line. The lowest traces represent the length changes and the black bars indicate the period of stimulation at 15 pps.

L_{opt}-6 mm

0.5
N
0

L_{opt}

1
N
0

L_{opt}+4 mm

2
N
0

2 mm

5 s
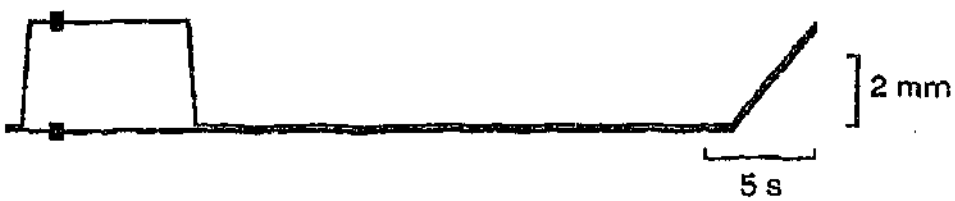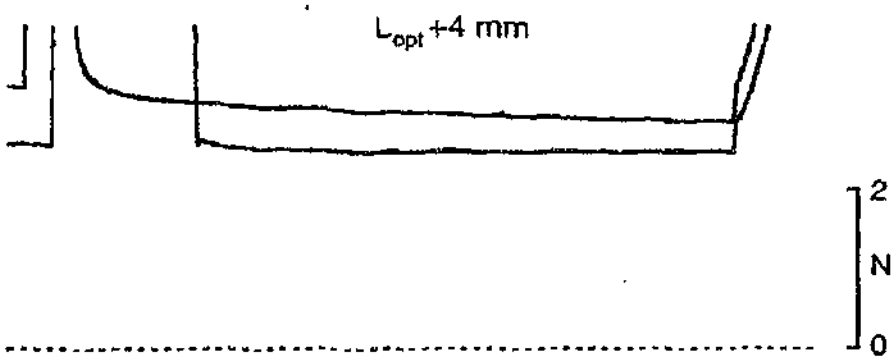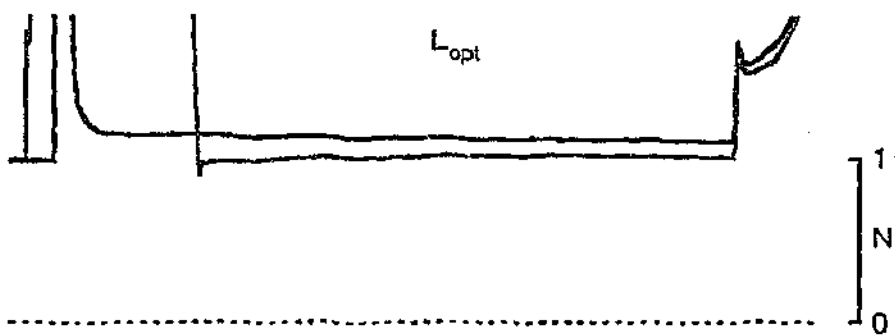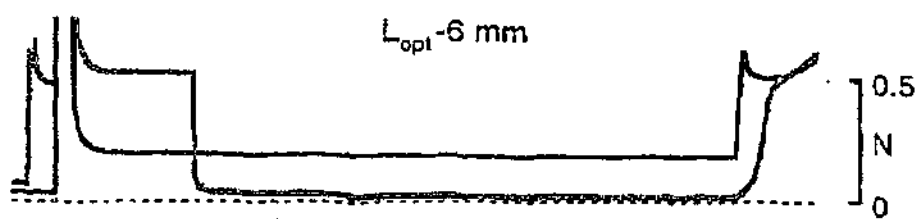
(L_{opt}), the resting tension after both forms of conditioning had increased, that measured after hold-test being a little higher. At long lengths (L_{opt}+4 mm), the resting tension increased further, as did the history-dependent component. The mean resting tension from 5 experiments, as measured after both forms of conditioning, is plotted against muscle length in Figure 2.4 A. Analysis showed that for lengths L_{opt}-6 mm or longer, there was a significant effect of conditioning on the short-range tension (P < 0.05, three-factor ANOVA). There was also a significant interaction between conditioning and length (P < 0.01, three-factor ANOVA) and at some, but not all lengths, hold-test and hold-long values were significantly different (P < 0.05, LSD *post-hoc* test).

In order to estimate the history-dependent component of resting tension at each length, the difference in resting tension after the two forms of conditioning was expressed as a percentage of the resting tension measured after hold-test (Figure 2.4 B), since it was proposed that the resting tension measured after hold-long was predominantly due to passive structures other than cross-bridges (see Discussion). At short muscle lengths, although the differences in resting tension were small in absolute terms, the majority of the resting tension was history-dependent, with a mean value of 96.3% ($\pm$ 3.7) at L_{opt}-10 mm. As muscle length increased, the history-dependent resting tension progressively decreased to a minimum of 8.8% ($\pm$ 1.0) at L_{opt}+2 mm, before increasing again with length to 30.3% ($\pm$ 2.5) at L_{opt}+6 mm.

An interesting observation, illustrated in Figure 2.5, was that at short muscle lengths, the tension traces after the two conditioning procedures converged at some point beyond the yield point during the test stretch, despite a history-dependent difference in resting tension. However, at lengths longer than L_{opt}, the traces no longer converged, at least over the 3 mm test stretch used here, so that any history-dependent resting tension was maintained throughout the stretch. It appeared that this effect at longer lengths was not permanent, since reproducible results were found during several hold-test, hold-long sequences.

Short-range component

The short-range component of the tension response to the test stretch was both history and length-dependent. At short muscle lengths, where the resting tension was close to zero, there was often no discernible short-range component. Instead, the

Figure 2.4 *History and length-dependence of resting tension.*

(A) The mean (± S.E.M.) resting tension as measured after hold-test (blue) or hold-long (red) conditioning as a function of muscle length. Asterisks indicate lengths where values after the two forms of conditioning were significantly different (P < 0.05, LSD *post hoc* test). (B) The history-dependent component of resting tension, at each length, was calculated as the difference in resting tension after the two forms of conditioning, expressed as a percentage of the resting tension measured after hold-test. Here, the mean (± S.E.M.) history-dependent resting tension is plotted against muscle length.
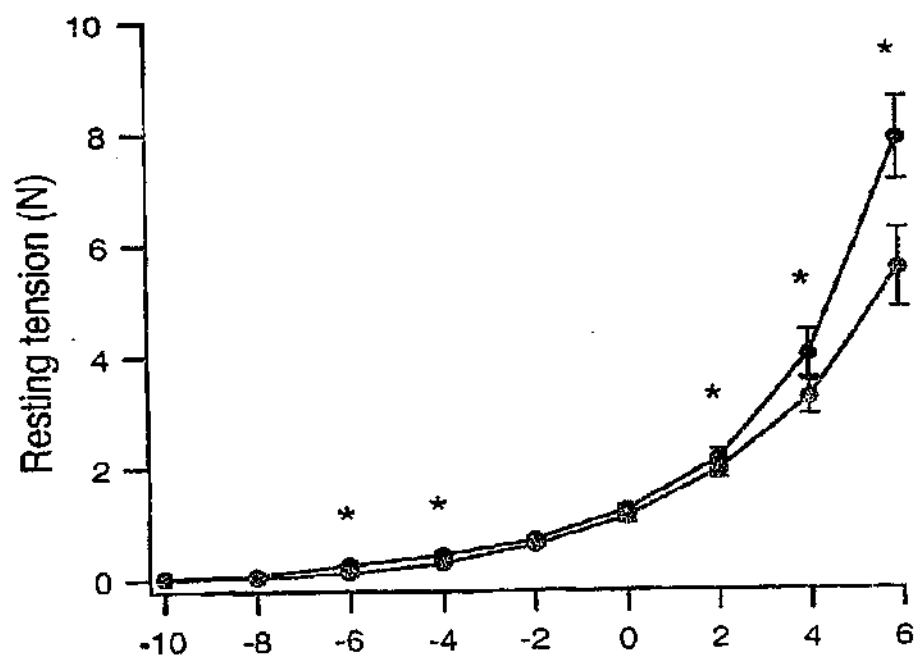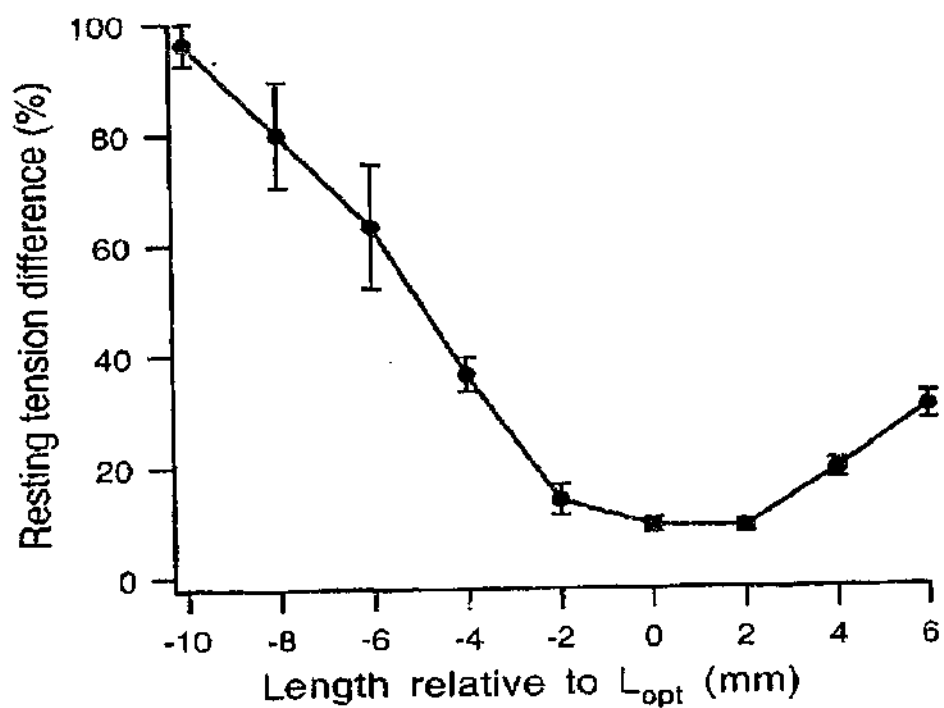
Figure 2.5 *Tension changes during a test stretch after the two forms of conditioning.*

Tension responses to a test stretch at four different lengths (as indicated), after hold-test (blue) and hold-long (red) conditioning. The bottom trace indicates the length change. The dotted line represents zero tension, measured at a short length where the tendon lay visibly slack.

$L_{opt}+4$ mm

$L_{opt}+2$ mm

L_{opt}

$L_{opt}-4$ mm

1

N

0

1 mm

0.5 s
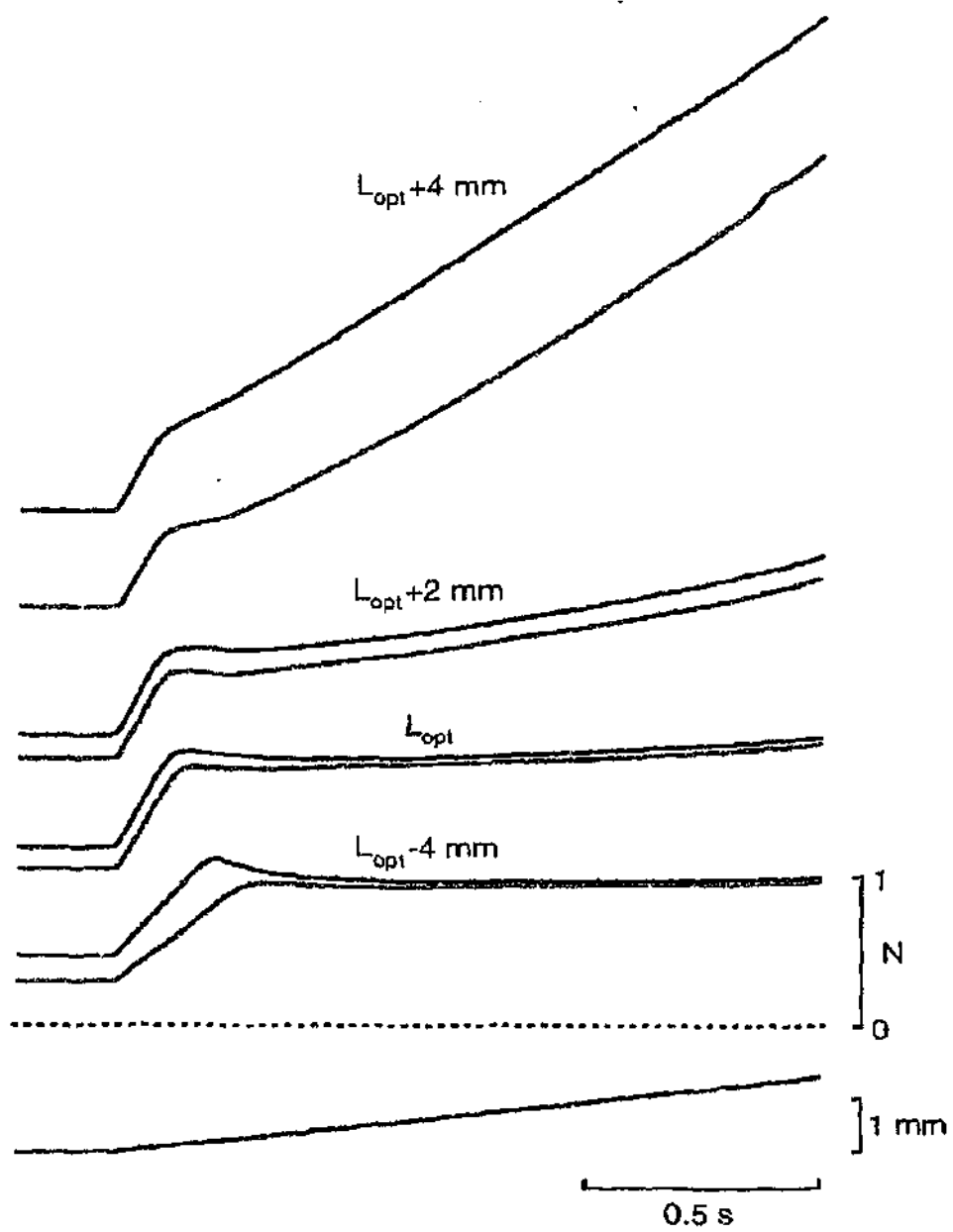
ension profile was rather rounded (see Figure 2.3, top panel), and the yield point was sometimes not reached during the 3 mm test stretch. This was most evident after hold-long conditioning at lengths less than L_{opt}-6 mm, and was attributed to the presence of slack as a result of the conditioning procedure. At muscle lengths between L_{opt}-6 mm and L_{opt}+6 mm, the short-range tension could be measured after both forms of conditioning, with mean values increasing with length to a peak of 0.68 N ($\pm$ 0.06) at L_{opt}-2 mm after hold test and 0.74 N ($\pm$ 0.08) at L_{opt} after hold long (Figure 2.6). At longer lengths, short-range tension decreased, when measured as described in the Methods (see Figure 2.1 B), and at lengths greater than L_{opt}+6 mm, the slope of the short-range component was almost indistinguishable from that of the secondary slope beyond the yield point. This suggested that passive, elastic structures were now dominating the tension response to stretch. Over the range of muscle lengths L_{opt}-6 mm to L_{opt}+6 mm, short-range tension measured after hold-long was consistently higher than following hold-test, and this effect was significant (P < 0.05, three-factor ANOVA).

The slope of the tension rise during a stretch, the short-range stiffness, also showed a length dependence (Figure 2.6), with mean values peaking at L_{opt}+2 mm for both hold-test (6.3 N mm^{-1} $\pm$ 0.42) and hold-long (6.25 N mm^{-1} $\pm$ 0.37), again, provided they were measured after subtraction of the elastic tension component (see Figure 2.1 B). At short to intermediate lengths, short-range stiffness was greater after hold-test than hold-long (see Figure 2.6), and this interaction between conditioning and length was significant (P < 0.01, three-factor ANOVA).

The fact that short-range tension peaked at L_{opt}, and showed a length-dependence similar to that of active isometric tension (see Figure 2.2), the possibility was considered that this might provide evidence in support of a cross-bridge origin of the short-range component. To further explore this point, short-range tension and active tension, measured at different lengths for both forms of conditioning, were expressed as a percentage of their maximum values for each experiment. Regression analysis gave a significant linear relationship between active tension and short-range tension, with a slope of 1.76 (r^2 = 0.63; P < 0.001) for values between L_{opt} -2 mm and L_{opt} +4 mm (Figure 2.7). It meant that over this length range a unit increase in active tension led to a 1.76 times increase in short-range tension. When values at shorter muscle lengths were included in the analysis, the r^2 value was reduced.

Figure 2.6 *Length-dependence of the short-range component after muscle conditioning.*

(A) Raw records with resting tensions aligned of the tension responses to a test stretch at three different muscle lengths, after hold-test (blue) or hold-long (red) conditioning. (B) The mean ($\pm$ S.E.M.) short-range tension (upper panel) and short-range stiffness (lower panel) plotted against muscle length, as measured after hold-test (blue) and hold-long (red). Asterisks indicate lengths where values after the two forms of conditioning were significantly different ($P < 0.05$, LSD *post hoc* test).

A

L_{opt}-4 mm L_{opt} L_{opt}+4 mm

] 0.2 N

0.5 s

B
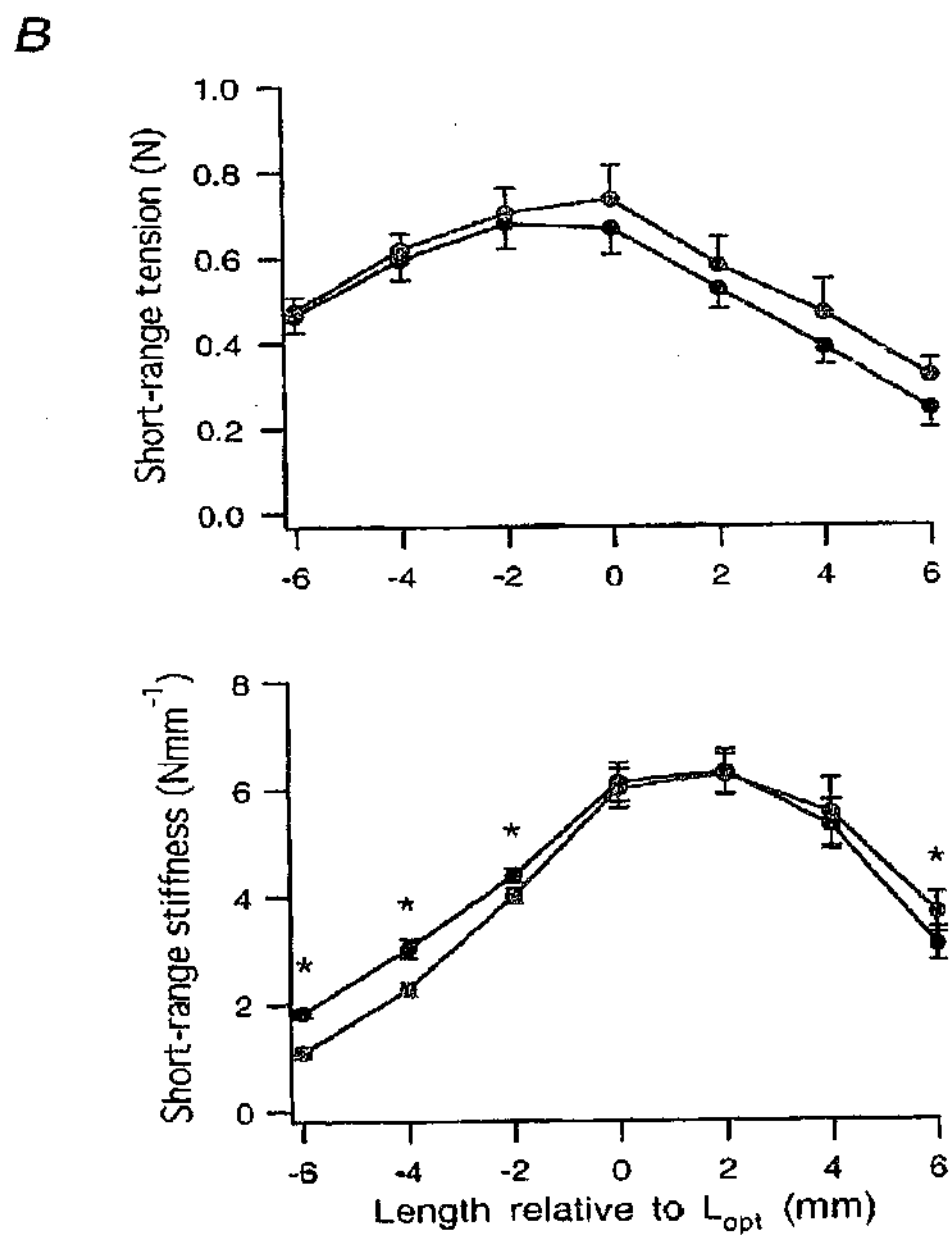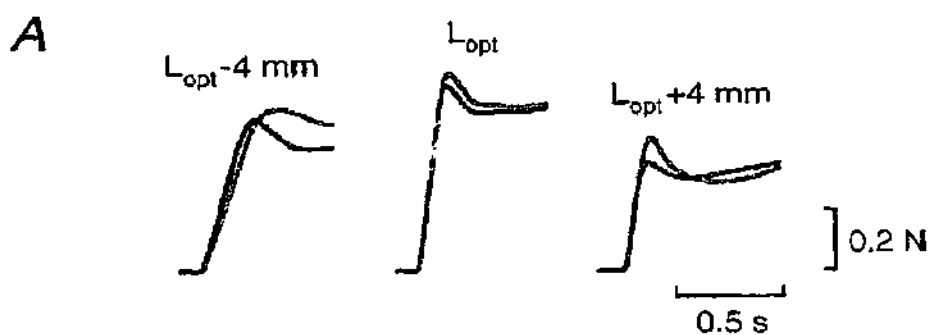
Figure 2.7 *Relationship between short-range tension and active tension.*
Active tension measured during maximal isometric contractions plotted against
short-range tension at the corresponding muscle length, for five experiments.
Values for both parameters are expressed as a percentage of the maximum.
Hold-test (blue) and hold-long (red) values over the length range L_{opt}-2 mm to
L_{opt}+4 mm are shown. A regression line has also been fitted.
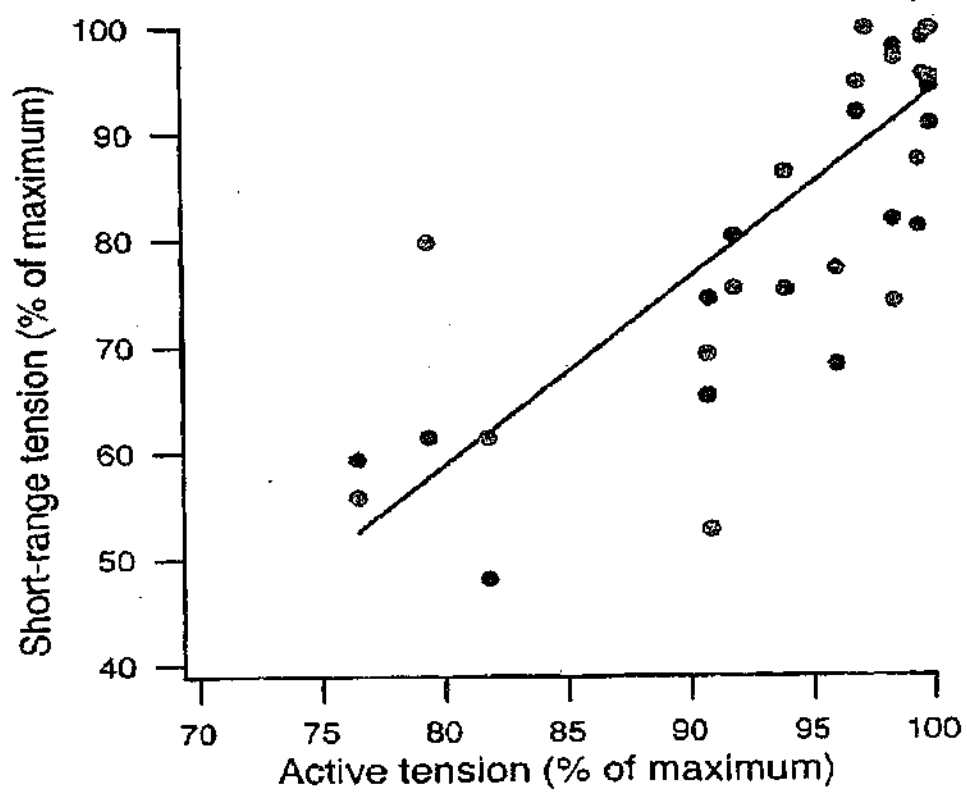
While most of the passive tension measurements for MG were similar to those found for soleus, one notable difference was the length-dependence of the short-range tension, which, for soleus, was found to increase at lengths longer than L_{opt}, up to $L_{opt}+8$ mm (Figure 2.8). This finding prompted a re-evaluation of the relationship between the short-range tension and active tension for MG. The possibility was considered that the higher resting tension for MG at long lengths could lead to strain of stable cross-bridges, causing them to detach earlier during the test stretch, and thereby reducing the size of the short-range tension (Figure 2.9; see also Discussion). This point was examined in two ways (Figure 2.10). Firstly, the history-dependent differences in short-range tension and resting tension, expressed as a percentage of the hold-test value, were plotted against one another. Regression analysis showed that, for muscle lengths from L_{opt} to $L_{opt}+6$ mm, there was a significant linear relationship between these variables, with a slope of -1.2%, ($r^2 = 0.56$, $P < 0.001$). Secondly, it was decided to examine if the level of resting tension, independent of the form of conditioning, was related to the amplitude of the short-range tension. In this case, values were expressed as a percentage of the maximum isometric tension for each experiment, in order to normalise for any differences in tension across experiments. Here, there was also a significant relationship, with a slope of -0.06% ($r^2 = 0.62$, $P < 0.001$).

The history-dependence of the length change required to reach the yield point during the test stretch, was another way in which the thixotropic properties of the passive muscle were examined (Figure 2.11 A). Mean values of the length to the yield point as a function of muscle length are plotted in Figure 2.11 B. At $L_{opt}-10$ mm, there was no measurable yield point after hold-long conditioning while after hold-test, it was not well defined and could only be measured in three experiments. At $L_{opt}-8$ mm, values from only two experiments could be used after hold-long conditioning. At $L_{opt}-6$ mm, measurements were made in all 5 experiments for both forms of conditioning. Here, the length change to the yield point was 0.29 mm (± 0.02) (hold-test) and 0.78 mm (± 0.15) (hold-long). As muscle length increased, the length to the yield point became progressively less, reaching a minimum value of 0.11 mm (± 0.01) at $L_{opt}+2$ mm after hold-test conditioning, and then remaining fairly constant at longer lengths. There was a significant effect of conditioning on the length change to the yield point, for muscle lengths including and less than $L_{opt}-4$ mm ($P < 0.05$, three-

Figure 2.8 *Length-dependence of the short-range tension for soleus.*

(A) Sample records from one experiment carried out on soleus, at three different muscle lengths, showing the short-range tension as measured after hold-test (blue) and hold-long (red) conditioning. (B) Short-range tension plotted against muscle length after the two forms of conditioning from the same experiment as shown in (A).
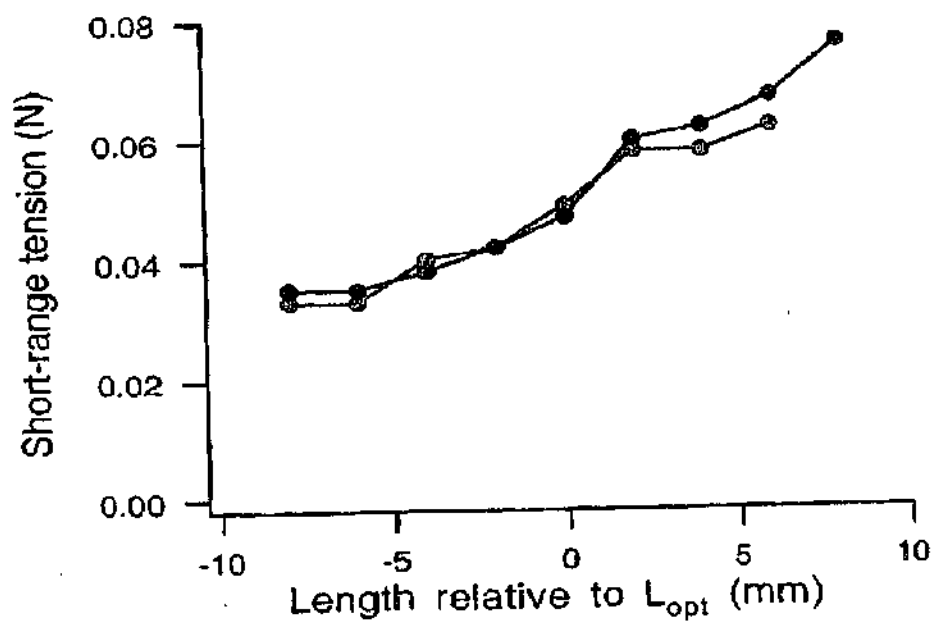
A

L_{opt}-8 mm

L_{opt}-2 mm

L_{opt}+4 mm

0.02 N

0.2 s

B

Short-range tension (N)

0.08

0.06

0.04

0.02

0.00

-10   -5   0   5   10

Length relative to L_{opt} (mm)
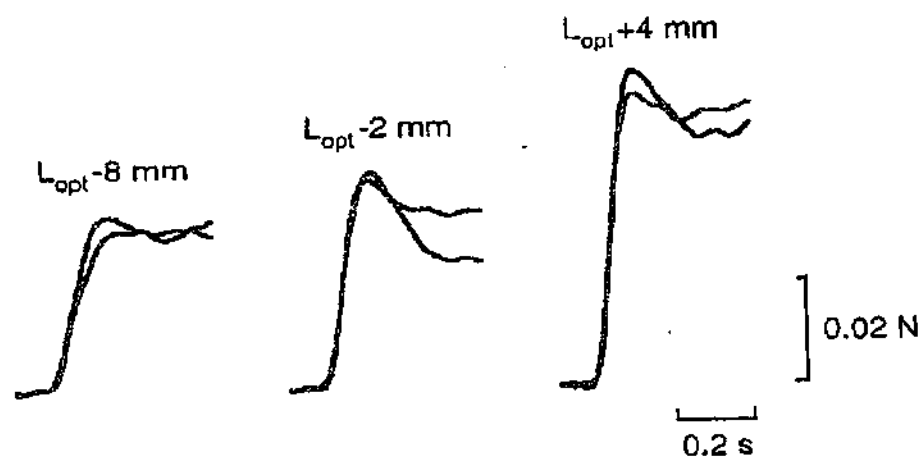
Figure 2.9   *The proposed history and length-dependent effects of resting tension on short-range tension at long lengths.*

A schematic representation of two sarcomeres (upper and lower panels) in a resting muscle at a long muscle length, showing a titin filament and an attached cross-bridge. The sarcomere in the upper panel has undergone hold-long conditioning (red). Therefore, it has a lower level of resting tension, produced by titin, than the sarcomere in the lower panel, which has been conditioned with a hold-test (blue). This is represented by the more compressed central, tension-bearing segment of the titin filament for the sarcomere in the upper panel compared to that in the lower panel. It was hypothesised that the higher resting tension in sarcomeres after hold-test would put more strain on attached cross-bridges, so that during a test stretch, the length change required for these cross-bridges to detach (ΔL) would be less than following hold-long. This would lead to a smaller short-range tension after hold-test than after hold-long. It was also suggested that ΔL would become progressively less as muscle length became longer and the level of resting tension increased. These ideas were based on the results presented in Figure 2.10.

Hold-Long

Z-line · Actin · ΔL · Cross-bridge · Titin · Myosin

Hold-Test

ΔL
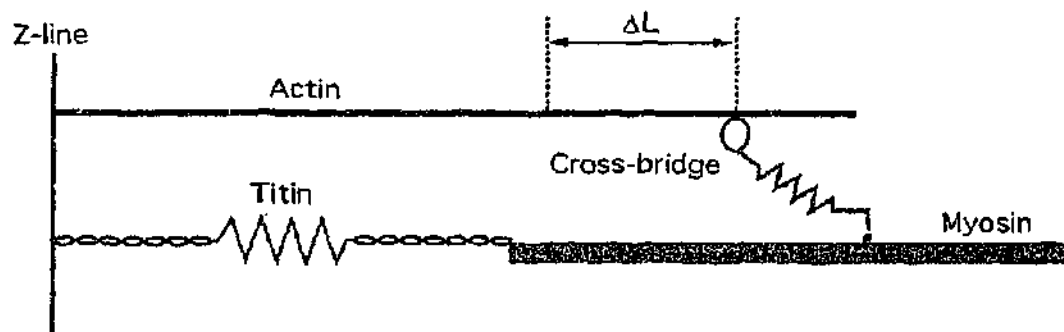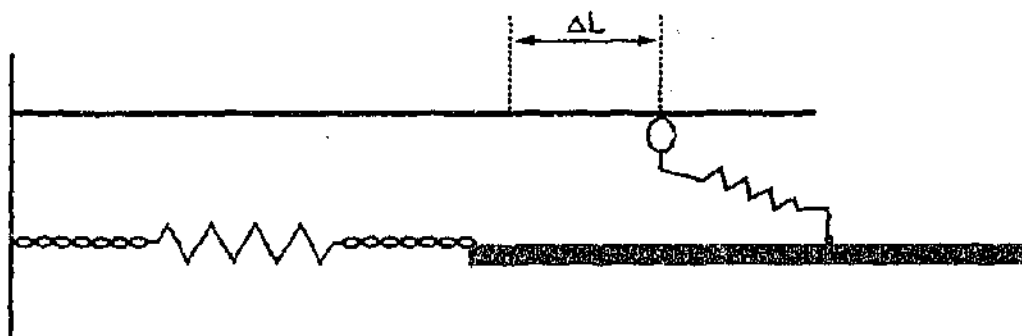
Figure 2.10 *Relationship between resting tension and short-range tension at long muscle lengths for MG.*

(A) The relationship between the history-dependent difference (see text) in resting tension and short-range tension is shown for values measured over the length range L_{opt} to $L_{opt}+6$ mm. A regression line has been fitted. The different symbols correspond to values at different lengths; circles (L_{opt}), squares ($L_{opt}+2$ mm), triangles ($L_{opt}+4$ mm) and diamonds ($L_{opt}+6$ mm). (B) The relationship between resting tension and short-range tension, after hold-test (blue) and hold-long (red) conditioning, as measured over the same length range as shown in (A). For both parameters, values from each experiment are expressed as a percentage of the maximum isometric tension generated by the muscle. A single regression line has been fitted to values after both forms of conditioning. The different symbols represent the same lengths as indicated for (A).

A
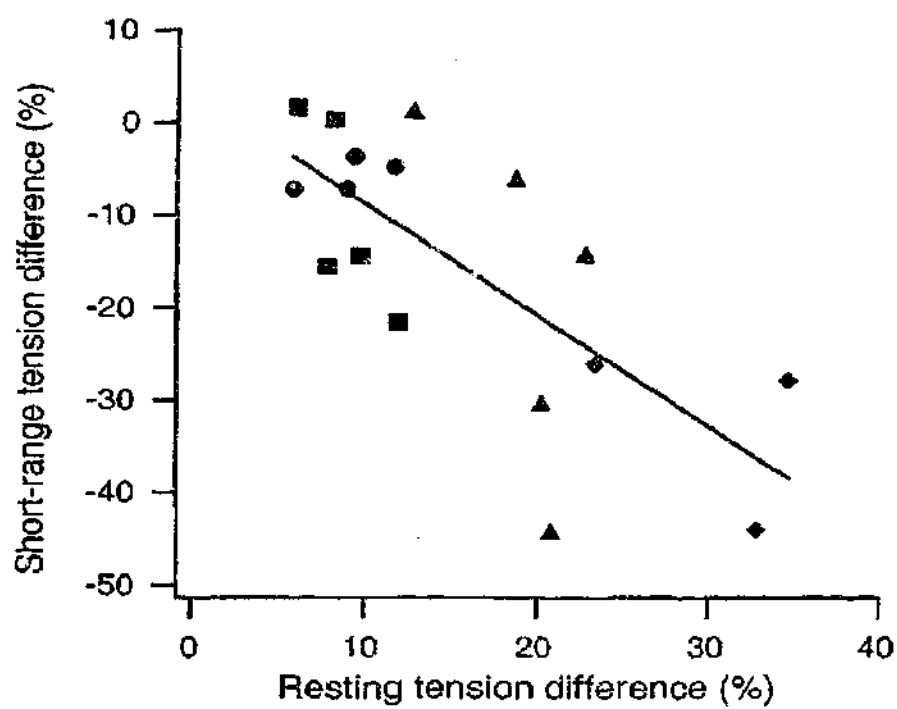
B
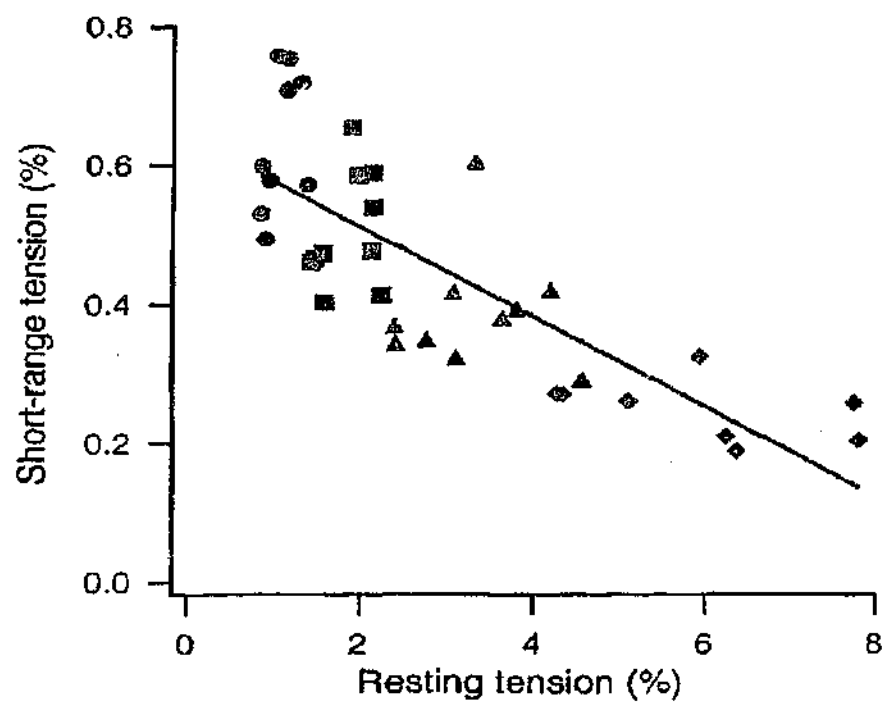
Figure 2.11 *The length change required to reach the yield point.*

(A) Sample records from three different muscle lengths showing the length change required to reach the yield point of the short-range component (arrows) during the test-stretch. Lowest trace represents the length change. (B) The mean (± S.E.M.) length change to the yield point after hold-test (blue) and hold-long (red) conditioning. After hold-long, there was no measurable yield point at L_{opt}-10 mm, while at L_{opt}-8 mm, measurements from only two experiments could be used. After hold-test, only three muscles gave measurable values at L_{opt}-10 mm. Asterisks indicate lengths where values after the two forms of conditioning were significantly different ($P < 0.05$, LSD *post hoc* test).

A

L$_{opt}$-4 mm

L$_{opt}$-2 mm

L$_{opt}$

0.5 N

1 mm

0.2 s

B

Length change to yield point (mm)

Length relative to L$_{opt}$ (mm)
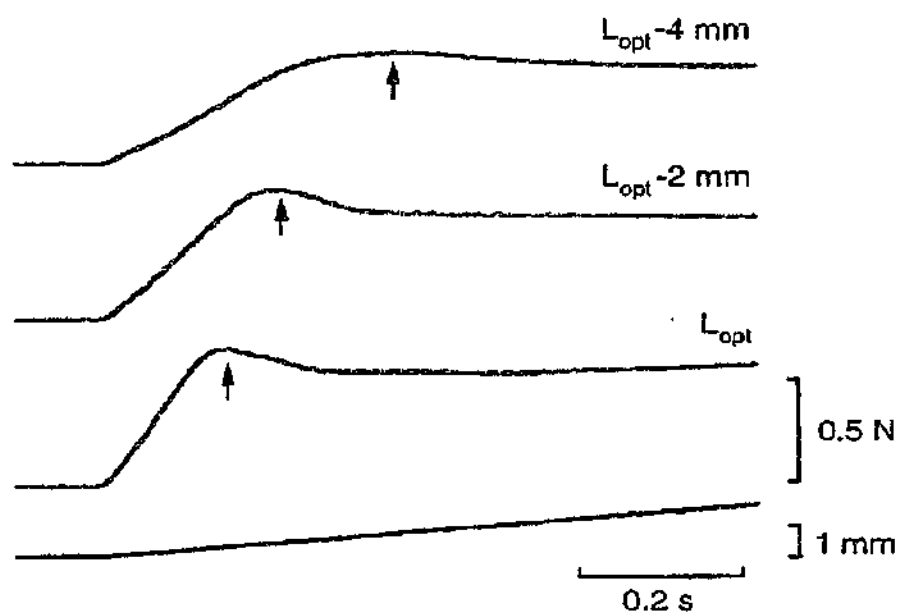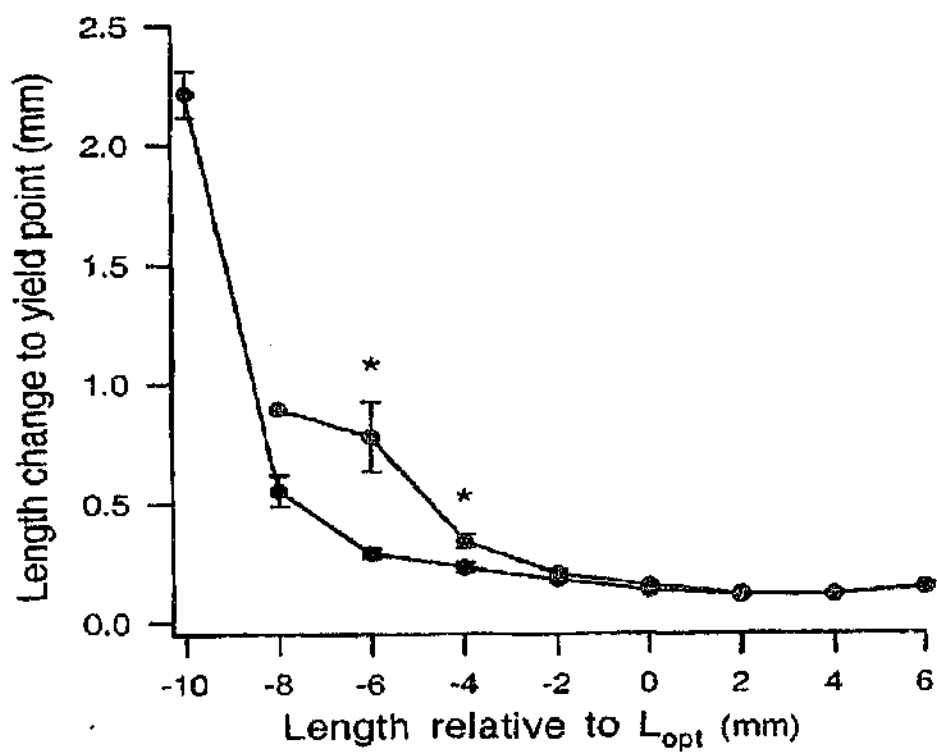
factor ANOVA), but no significant difference at longer lengths. In order to compare these findings with previous studies, a length change of 0.11 mm represents a fibre length change of 0.55%, assuming an average muscle fibre length of about 20 mm for MG (Walmsley & Proske, 1981).

The relationship between the size of the tension generated during the hold-test conditioning contraction and the resultant short-range tension was examined in two experiments. In one of these experiments, however, isometric tension exceeded the voltage limit of the servomotor at a stimulation rate of 30 pps, which limited the number of measurements that could be made. Therefore, attention was focused on the other experiment, where isometric tension was graded over a wider range, from 18 N at a stimulation rate of 7 pps to 129 N at 80 pps. That is, an increase of more than 700%. Over this range, the short-range tension decreased by 27%, from 1.06 N to 0.77 N (Figure 2.12). Regression analysis showed a strong relationship between active tension and short-range tension ($r^2 = 0.81$, $P < 0.001$).

Time course of short-range tension re-development

An example of the tension response to a test stretch carried out 5 s after conditioning with rapid stretch-shortening movements is shown in Figure 2.13 A. As the time after conditioning increased from 1 s up to 60 s, so too did the size of the short-range tension following both movement and isometric contraction conditioning, with values consistently higher after movement conditioning (Figure 2.13 B). When the time after conditioning was 60 s, the mean short-range tension following movement conditioning was 0.7 N (± 0.02), compared with 0.65 N (± 0.04) after contraction conditioning at 15 pps, and 0.33 N following contraction conditioning at 80 pps for one experiment (Figure 2.13 C). In order to measure the time-constant of short-range tension recovery, an exponential function was fitted to the plot of short-range tension against time after conditioning, for values from each experiment. The mean time constants were 6.6 s (± 0.5) (movement conditioning), 9.9 s (± 0.6) (15 pps) and 28.8 s (80 pps).

Figure 2.12 *The effect of grading isometric tension during conditioning on short-range tension.*

(A) Sample records from an experiment of isometric tension (upper traces) measured using distributed stimulation with rates ranging from 7 pps to 70 pps. The lower traces are the measured short-range tension, with resting tension aligned, following conditioning at the corresponding rate. (B) Data from the same experiment as in (A) showing the relationship between active tension and short-range tension. A linear regression line has been fitted to the data.

A

7 pps 12 pps 25 pps 70 pps

50 N

1 s

0.5 N

0.5 s

B

Short-range tension (N)

Active tension (N)
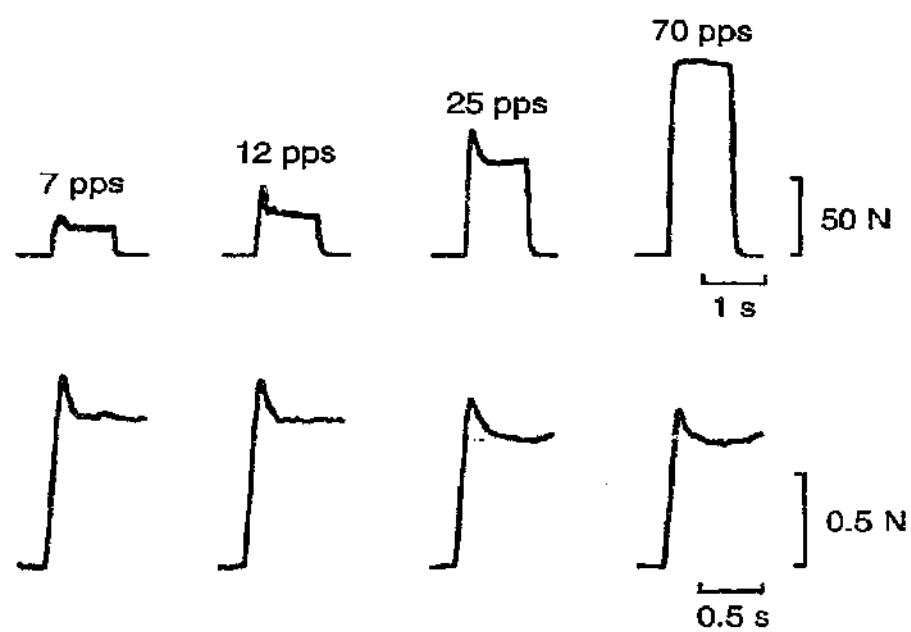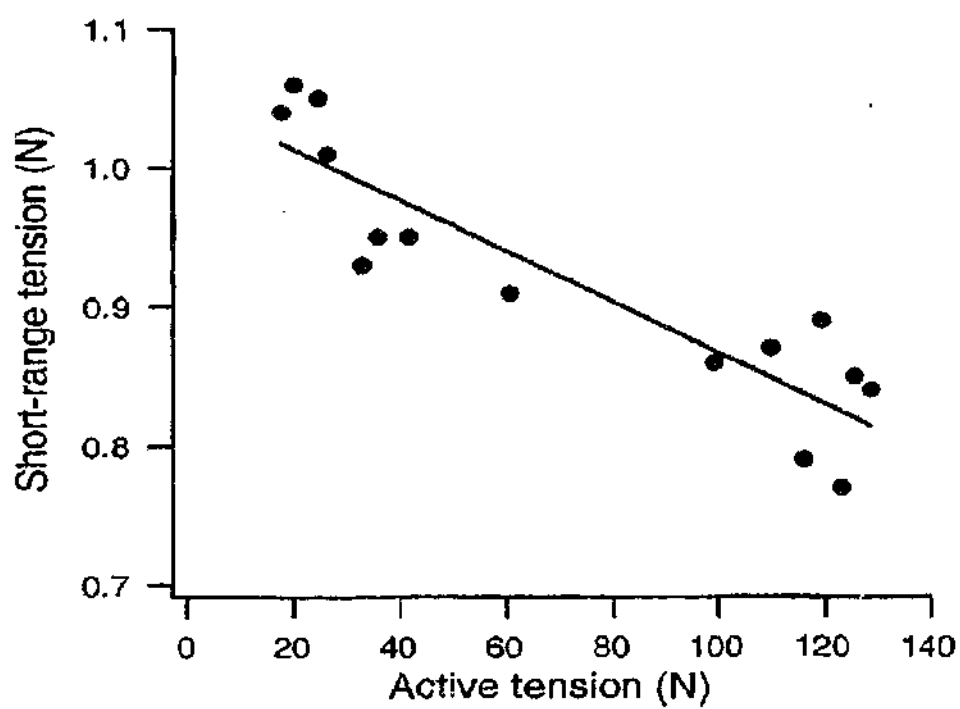
Figure 2.13 *The time-course of re-development of short-range tension after conditioning.*

(A) A record of the tension response to a test stretch carried out 5 s after conditioning with rapid, stretch-shortening movements (see text). Tension (upper trace), length change (lower trace). The test length was L_{opt}. (B) Sample records of the short-range tension recorded at various times after movement (red) or hold-test (blue) conditioning. (C) The mean ($\pm$ S.E.M.) short-range tension measured after conditioning with movement (red) or hold-test (blue filled symbols). Values from one experiment where the hold-test stimulation rate was 80 pps (blue open symbols) is also shown.

A
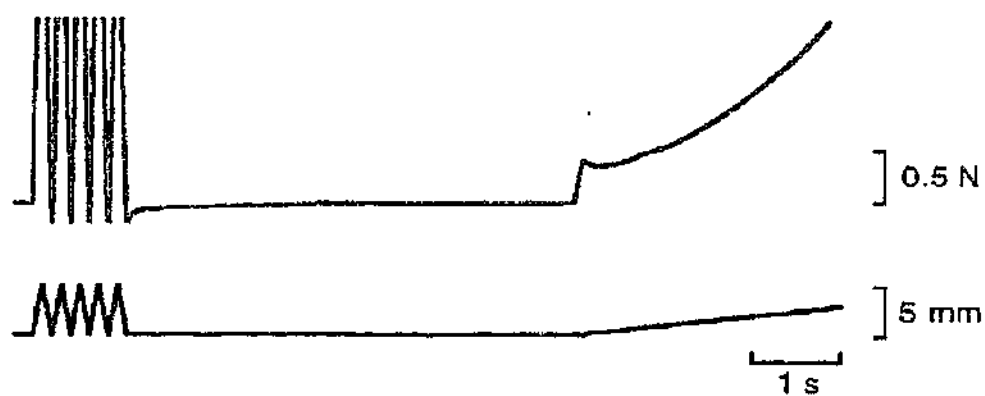
B
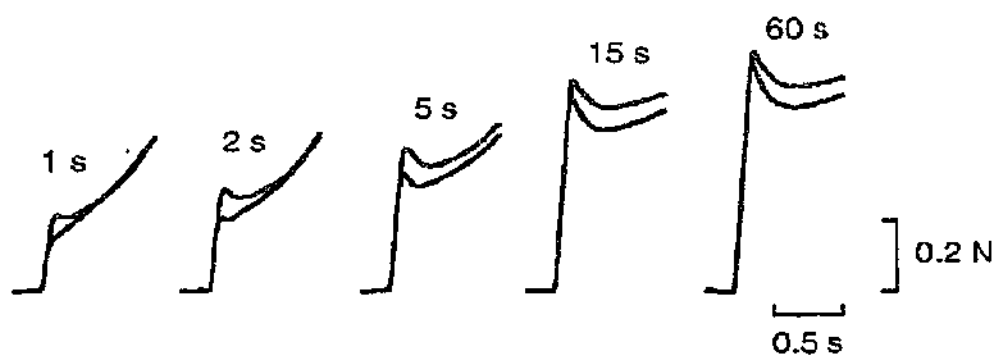
C
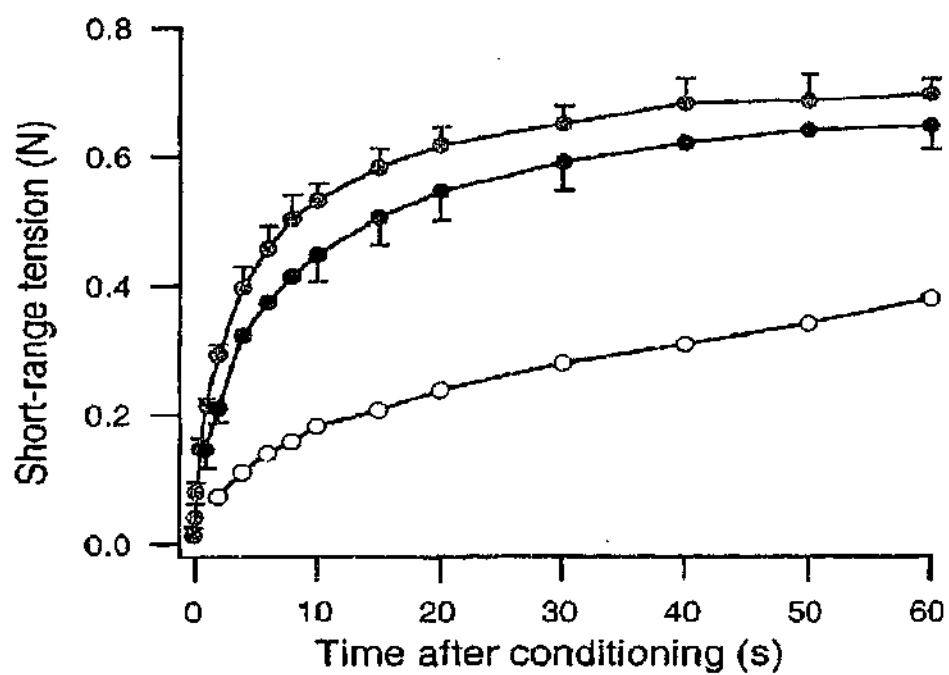
Discussion

The central aim of these experiments was to determine if an intact, whole mammalian muscle displayed thixotropic properties that could be best explained in terms of stable cross-bridges in resting muscle. It was hypothesised that stable cross-bridges would contribute to the resting tension and the short-range tension during a slow passive stretch, both of which would be dependent on muscle history and length.

Resting tension

An important piece of evidence in support of the contribution of stable cross-bridges to resting tension was the finding that thixotropic properties were evident at short muscle lengths, where the resting tension was close to zero, so that the resting tension generated by passive, elastic structures such as titin was likely to be negligible. This was highlighted by the large history-dependent difference in resting tension, of 80% or more, found at short lengths (L_{opt}-8 mm and L_{opt}-10 mm) (see Figure 2.4 B). Here, it was hypothesised that during hold-long conditioning, stable cross-bridges would form at the longer length and stiffen the muscle so that upon shortening back to the test length, sarcomeres in muscle fibres would not follow the length change but retain their longer length, or at least something intermediate between the two lengths, causing the muscle-tendon unit to fall slack. Since cat MG has a tendon that is about five times longer than its muscle fibres (Walmsley & Proske, 1981), and likely to be more compliant than the muscle at these short lengths, it seemed plausible that it would absorb most of the imposed length change during muscle shortening and fall slack. Under these conditions, tension was close to zero, which meant that attached cross-bridges, after hold-long conditioning, produced negligible resting tension. With hold-test conditioning, in which the muscle was isometrically contracted at the test length, it was envisaged that slack would be removed by the active cycling of cross-bridges, so that when the muscle had relaxed after the contraction, cross-bridges would attach and be under some strain, as they generated resting tension. Thus, it was suggested that at short muscle lengths, a significant component of the resting tension could be attributed to stable cross-bridges.

Hill (1968) first proposed that a component of the resting tension, the FRT, was attributable to stable cross-bridges. He found that for a frog sartorius muscle weighing 100 mg, the FRT was about 150 mg at a sarcomere length of 2 µm. Since the mean weight of cat MG has been shown to be 9.8 g (Spector *et al.* 1980), an equivalent, weight adjusted value of FRT for this muscle would be about 15 g or 0.15 N. This value is remarkably close to the history-dependent resting tension (0.1 N to 0.15 N) found in the current series of experiments at lengths shorter than optimum length. While such a comparison must take into account certain assumptions, it does, nevertheless, suggest a general similarity between amphibian and mammalian muscle in terms of the contribution of stable cross-bridges to resting tension.

As muscle length increased from short to intermediate lengths, and tendon compliance became progressively less, it was proposed that a greater number of cross-bridges would detach during the shortening phase of hold-long conditioning, and therefore, less slack would be produced. This was highlighted by a gradual reduction of the history-dependent component of resting tension, down to a minimum value of 8% at $L_{opt}+2$ mm. In other words, at these intermediate muscle lengths, the majority of the resting tension, more than 90% at L_{opt} and $L_{opt}+2$ mm, was likely to have been produced by non-cross-bridge, passive structures such as titin filaments. This suggestion seemed reasonable, based on the fact that titin filaments have been proposed to start producing resting tension at a sarcomere length of 2.6 µm (Wang *et al.* 1993), which is just short of the optimum length for mammalian muscle.

An interesting finding was that at longer muscle lengths, the history-dependent resting tension increased again, up to a value of 30% at $L_{opt}+6$ mm. It was observed that for lengths up to L_{opt}, any history-dependent resting tension did not persist throughout the test-stretch, that is, the tension traces converged beyond the yield point. This was attributed to cross-bridges, since it was expected that their contribution to resting tension would not persist during the stretch, as they became detached. However, at longer lengths, beyond L_{opt}, there was a lack of convergence of the tension traces after the two forms of conditioning, with the difference in resting tension remaining throughout the stretch. (see Figure 2.5). Since titin filaments were likely to be the major source of resting tension at these longer lengths, it was postulated that the increased history-dependence of resting tension at these

lengths might be attributable to the mechanical properties of titin. As described in Chapter 1, there is some recent experimental evidence to suggest that titin displays long-range thixotropic properties, characterised by a shift of the extension-force relationship to longer lengths, during stretch-shortening cycles that produce forces within normal physiological limits (Kellermayer *et al.* 1998; Kellermayer *et al.* 2001). This type of behaviour would be consistent with a lower level of resting tension after hold-long conditioning, where the muscle was stretched and shortened back to its original length, compared to that measured after hold-test. The current results also suggested that any structural reorganisation of titin filaments was reversible with subsequent muscle conditioning, since the history-dependent component of resting tension at long lengths remained about the same throughout several consecutive trials. Again, this is in line with the reported mechanical properties of titin (Kellermayer *et al.* 2001).

Short-range component

Thixotropy

The thixotropic properties of muscle and their possible explanation in terms of stable cross-bridges during a slow, passive stretch, was examined in a number of different ways. Firstly, it was found that over most of the physiological length range, a clear short-range component was observed. That is, tension increased linearly with stretch to a length close to the yield point, indicative of strain of an elastic component. The slow stretch speed used in these experiments also ensured that the initial steep rise in tension had a negligible viscous component (see Chapter 1). With further stretch beyond the yield point, tension usually fell slightly and reached a plateau, before increasing again with further stretch, as parallel, passive structures were lengthened. The underling mechanism for the tension plateau beyond the yield point remains unclear, however two possibilities are the sarcomere non-uniform lengthening hypothesis (Proske & Morgan, 1999) and the movement enhancement hypothesis (Campbell & Lakie, 1998) (see Chapter 1, page 15).

As with resting tension, the short-range component was also history-dependent. This was particularly evident at short muscle lengths where, after hold-long conditioning, the tension response was often rather rounded, indicative of slack in the tendon being taken up during the stretch. At muscle lengths from L_{opt}-6 mm to L_{opt}+6 mm, the short-range tension was found to be consistently higher after hold-long, while the

short-range stiffness, except at $L_{opt}+6$ mm, was lower after this form of conditioning. These findings can be explained in terms of the proposed mechanical properties of cross-bridges. It was hypothesised that the size of the short-range tension would depend on both the number of attached cross-bridges before the onset of the stretch, and the average tension produced by these cross-bridges during stretch up to the yield point, which, in turn would depend on whether the cross-bridges were strained before the stretch. It was envisaged that there would be little difference in the number of attached cross-bridges before the test-stretch, from the two forms of conditioning, since this would largely depend on the cross-bridge attachment rate in the resting muscle, which was presumably the same in both cases. However, as proposed earlier, particularly at short muscle lengths, very little resting tension would be produced by cross-bridges after hold-long, while after hold-test, the strained cross-bridges would generate some resting tension. Thus, following hold-long conditioning, cross-bridges would not be detached during a test stretch until the slack in the tendon had been removed and the tension level was high enough. This resulted in a more rounded, and less steep rise in tension. After hold-test, the strained cross-bridges would detach earlier during the stretch, thereby producing a lower short-range tension, but a more steep tension rise and so a greater short-range stiffness (see Figure 2.3, top panel).

As muscle length was increased up to L_{opt}, the history-dependence of both short-range tension and stiffness became progressively less, as the slack produced during hold-long became less. However, at longer lengths, a history-dependence of short-range tension again emerged. A possible explanation for this finding was that the rising level of resting tension at these lengths, from passive structures such as titin, placed extra strain on cross-bridges, so that they detached earlier during the stretch, thereby generating less tension and leading to a reduction in the size of the short-range tension (see Figure 2.9). Such an effect would have been more pronounced following hold-test, because the level of resting tension was greater after this form of conditioning. This idea was supported by the finding that as the history-dependent component of resting tension increased at longer lengths, there was a close to proportional decrease in the history-dependent short-range tension (see Figure 2.10 A). Furthermore, over the same length range, there was also a significant relationship between the measured short-range tension and resting tension, when

both parameters were expressed relative to the peak, active isometric tension for each experiment (see Figure 2.10 B). Together, these findings suggest that at long muscle lengths, as the level of resting tension increases, either by conditioning or increasing length, there is a proportional decrease in the size of the short-range tension

In the original experiments by Hill (1968), it was shown that frog sartorius muscle had to be stretched by about 0.2% of its fibre length in order to reach the yield tension. However, in more recent experiments on mammalian muscle fibres, an 'apparent break point' was not reached until the muscle had been stretched by 1 to 2% of fibre length and so this was taken to be inconsistent with Hill's hypothesis (Mutungi & Ranatunga, 1996a). This finding, though, was made on passive muscle with an unknown previous mechanical history. As shown in the current experiments, the length change required to reach the yield point during a slow stretch was strongly history and length-dependent, with values ranging between approximately 10% of fibre length at short muscle lengths, down to 0.55% at intermediate and long lengths. Importantly, in recent experiments, Campbell & Lakie (1998) reported that the elastic limit of the SREC was reached after stretching single frog fibres 0.4% of their initial length, a value twice that reported by Hill (1968). Thus, based on this finding, it means that the minimum value of 0.55% found in the current series of experiments is not so different from that reported for frog muscle fibres.

It is important, here, to consider the role of the tendon during a passive stretch. It was envisaged that at short muscle lengths, high tendon compliance might contribute to delaying the length to the yield point (see Figure 2.11). It seemed unlikely, though, to have had a significant effect at intermediate and long lengths, since values remained fairly constant, and were independent of muscle history, despite a 5 to 10-fold increase in resting tension between L_{opt}-2 mm and L_{opt}+6 mm. Therefore, this implied that provided the tendon was not affected by conditioning, the changes in tension must be attributable to the muscle fibres. However, as pointed out by Herbert & Crosbie (1997), despite the fact that tendon is intrinsically stiffer than muscle, a small strain of the tendon during stretch can translate into a larger absolute length change, if the tendon is considerably longer than the muscle fibres. As mentioned previously, cat MG has a tendon that is about 5 times longer than its muscle fibres (Walmsley & Proske, 1981), so it is possible that tendon stretch provides some contribution to the change in length up to the yield point, which

remains more or less constant at long lengths. This would mean that the component of the length change to the yield point attributable to stretch of muscle fibres was somewhat less than 0.55%, so that the actual value was now closer to that reported by Hill (1968).

Length-dependence of short-range tension

The finding of a relationship between short-range tension and active tension for MG, over a range of muscle lengths, was considered as evidence in support of the hypothesis that the magnitude of the short-range tension was related to the number of attached cross-bridges that had formed in muscle fibres before the onset of the test stretch.

Previous studies, though, have shown that such a correspondence between the length-dependence of the short-range tension and active tension is not present for amphibian muscle fibres. For example, Haugen & Sten-Knudsen (1981) reported that short-range stiffness and the 'stationary tension plateau', the equivalent of the short-range tension of the present study, peaked at a sarcomere length of 3 μm, about half way down the descending limb of the active length-tension curve for frog muscle fibres. Similarly, the elastic modulus of the SREC has been shown to reach maximum values at sarcomere lengths between 2.4 and 3 μm (Hill, 1968; Moss & Halpern, 1977). As described in Chapter 1, it has been suggested that a reduced myofilament spacing and increased calcium sensitivity at long lengths, might provide possible explanations for the fact that the short-range tension peaks on the descending limb for frog muscle (Proske & Morgan, 1999).

An alternative idea to explain the length-dependence of short-range tension for MG, was prompted by the finding that the short-range tension for soleus continued to increase at lengths longer than the optimum, up to $L_{opt}+8$ mm. Since the level of resting tension begins to increase more steeply at a shorter length for MG than soleus, the possibility was considered that for MG, at lengths beyond L_{opt}, the higher level of resting tension in muscle fibres might act to reduce the size of the short-range tension, as strained cross-bridges detached earlier during the stretch, as suggested earlier. Interestingly, the results of Haugen & Sten-Knudsen (1981) also lend support to this proposal, as they showed that the level of resting tension was negligible at lengths less than 2.6 to 2.8 μm, but then increased more steeply at

longer lengths. In other words, the length at which the 'stationary tension plateau' peaked in their study also corresponded with the length at which resting tension began to rise more steeply. Therefore, it seemed possible that the length-dependence of the short-range tension was affected not only by the number of attached cross-bridges but also by their degree of strain, which, in turn, would depend on the level of resting tension generated by the muscle. This point deserves further investigation in future experiments.

Time-course of short-range tension re-development

The re-development of the short-range tension as measured at different times after muscle conditioning was used as an estimate of the attachment rate of stable cross-bridges in resting muscle. Previous experiments have examined cross-bridge attachment rates using pairs of triangular stretches, separated by different time intervals (Hufschmidt & Schwaller, 1987; Campbell & Lakie, 1998). Here, two conditioning procedures were u..ed. The first method involved hold-test conditioning, where it was proposed that during the contraction there would be active cycling of cross-bridges, and that stable cross-bridge formation would commence at the end of the contraction. However, a potential complication with this method was that as tension fell during the relaxation phase of the contraction, it was envisaged that recoil of the tendon would be accompanied by non-uniform lengthening of sarcomeres (Huxley & Simmons, 1970). This interfilamentary movement might detach cross-bridges in some sarcomeres, thereby reducing the number of attached cross-bridges at the onset of the test stretch. Thus, a second method was employed, which involved a series of rapid stretch-shortening movements. The aim here, was to achieve a defined muscle history, but without the potentially complicating factors associated with the isometric contraction conditioning.

It was found that the mean time constant of short-range tension recovery was 6.6 s with movement conditioning and about 10 s after a conditioning contraction at 15 pps. These values compare reasonably well with time constants of about 4 s for human ankle extensor muscles (Hufschmidt & Schwaller, 1987) and 12.5 s for single frog fibres (Campbell & Lakie, 1998). However, following a conditioning contraction at 80 pps, it was found that the time constant was much longer, close to 30 s. It was also noted that the measured short-range tension was less after conditioning with isometric contractions than rapid movements, particularly when

the higher stimulation rate was used. It was proposed that as the level of tension generated by an isometric contraction increased, and the relaxation phase became more prolonged, less stable cross-bridges would form, for the afore mentioned reasons (above), and the size of the short-range tension would become less. This idea was supported by the findings from two experiments, in which a 700% increase in the level of isometric tension, using distributed stimulation, led to about a 30% reduction in short-range tension (see Figure 2.12). Therefore, these findings suggested that the rate of cross-bridge attachment in resting muscle, and the resultant amplitude of the short-range tension, can be affected by the type of muscle conditioning, particularly as the strength of an isometric contraction is varied.

To summarise, this chapter has provided experimental evidence in support of the mechanical properties of stable cross-bridges in resting muscle. This was shown by a history-dependent component of resting tension at short lengths, and an initial steep rise in tension (short-range component) during a slow stretch, which was measured over a wide range of muscle lengths. It was also observed that at intermediate and long muscle lengths, the contribution to resting tension from non-cross-bridge sources, such as titin filaments, became increasingly more prominent, and furthermore, these structures also appeared to display thixotropic behaviour at long lengths. The functional significance of these findings will be discussed later (see Chapter 6).

The effects of eccentric and concentric exercise on passive properties of human triceps surae

Introduction

The work carried out in this chapter aimed to investigate the immediate and longer time-course of changes to passive mechanical properties of human ankle extensor muscles following an acute bout of eccentric exercise. As described in Chapter 1, it was hypothesised that eccentric contractions would cause localised regions of damage to muscle fibre membranes, triggering a calcium-activated contracture that would raise the level of passive tension generated by the muscle, particularly during the early post-exercise period. This process would occur in the absence of any neural input to the muscle fibre, so there was expected to be no accompanying electromyographic (EMG) activity. These experiments also examined whether muscle swelling provided a contribution to the rise in passive tension a few days after eccentric exercise, as recently proposed by Chleboun *et al.* (1998).

It is known that concentric exercise does not cause structural damage to muscle or lead to DOMS (Newham *et al.* 1983a; Newham *et al.* 1983b). Thus, a second group of subjects in the present study were subjected to a period of concentric exercise, in order to test the hypothesis that the rise in passive tension after eccentric exercise was attributable to muscle damage and not to any effects of the exercise *per se*. Another important aim of this chapter was to elucidate whether an increase in passive tension after eccentric exercise could be related to other indicators of muscle damage, particularly changes to the active length-tension relationship. The long-term goal of this analysis was to determine if changes to passive properties of muscle could be used as reliable indicators of muscle damage after eccentric exercise.

Methods

Subjects

The experiments were carried out on 13 subjects, 10 male (mean age, 21 years) and 3 female (mean age, 20 years). All subjects were free of any musculoskeletal abnormalities and at the time of the experiment were not participating in regular

exercise programmes. Subjects gave their written consent to participate in the study, which had been approved by the Monash University Human Ethics Committee and conformed to the Helsinki Declaration on Human Experimentation.

Exercise

Subjects were assigned to either an eccentric (n=7) or concentric (n=6) exercise group. The exercise was carried out on the ankle extensor muscles, formed principally by the triceps surae muscle group (TS). TS comprises soleus, and the medial and lateral gastrocnemius muscles, which are also knee flexors. All three muscles are innervated by the tibial nerve, one of two terminal branches of the sciatic nerve. For both exercise groups, the TS of one leg performed exercise while the TS of the other leg was unexercised and acted as a control. Both forms of exercise were carried out on a treadmill (Heartmaster, Tetley Technologies), inclined at approximately 13° and moving at a speed of 2.2 km h^{-1}.

Eccentric exercise

Subjects were required to step backwards with the experimental leg, using a toe to heel action to ensure that TS was actively contracting while being stretched (Figure 3.1). Subjects were asked to step back with the leg fully extended, in order to increase the length range over which the gastrocnemii were being actively stretched. After each step, the control leg was placed flat on the treadmill, alongside the experimental leg, to ensure that its TS was not contracted or stretched. The subject was then carried up the treadmill in preparation for the next step backwards. Subjects performed the exercise for 1 hr, at a stepping rate of 30-35 steps min^{-1}. A weight belt of 5-10 kg was carried in order to increase recruitment of TS during the eccentric contractions.
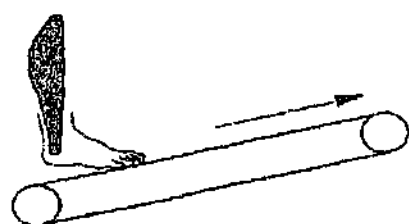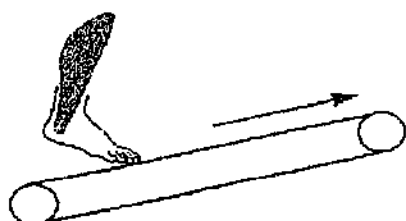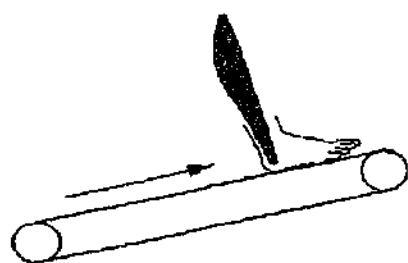
Concentric exercise

The concentric exercise started at the bottom of the treadmill (see Figure 3.1). Subjects were asked to plantarflex the foot of the experimental leg and to lift themselves up onto their toes, thereby performing a concentric contraction of TS. The control leg then stepped forwards and was placed on the treadmill using a flat foot, and the experimental leg was brought alongside it. The treadmill then carried the subject down to the bottom of the treadmill, ready for the next contraction. Subjects carried out a contraction every 2 to 3 s, a rate of 20 to 30 steps min^{-1}. Since

Figure 3.1 *Method of eccentric and concentric exercise.*

A schematic representation of the eccentric and concentric exercise protocols carried out on TS. Note that the treadmill was moving in opposite directions for the two forms of exercise (green arrows). Each contraction cycle has been represented by four phases (numbered). Eccentric exercise required the subject to step backwards with the experimental leg, using a toe-heel action while the muscle was active (eccentric contraction; phases 2 and 3). The subject was then carried forwards (arrow) to the top of the treadmill (phases 4 to 1), in preparation for the next step back. Concentric exercise began with subject at the bottom of the treadmill. The foot of the experimental leg was plantarflexed as the subject performed a concentric contraction of TS (phases 2 and 3). The control leg then stepped forward to the top of the treadmill, remaining essentially relaxed (not shown), and the experimental leg was placed beside it (phase 4). The treadmill then carried the subject backwards to the bottom of the treadmill (phases 4 to 1) for the next contraction. For both exercise protocols, the phases when the muscle was active (red muscle) and passive (blue muscle) are indicated.

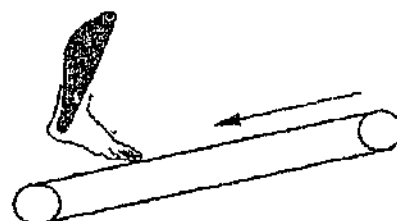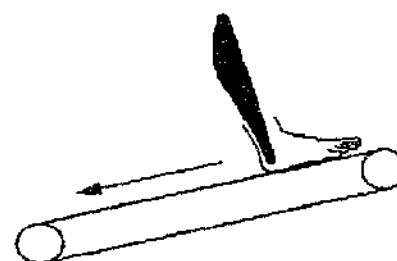Eccentric   Concentric

1
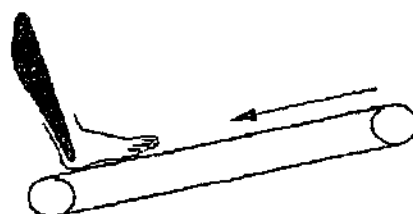
2

3

4

the concentric exercise was more exhausting than the eccentric exercise, subjects were unable to perform concentric exercise continuously without periods of rest. Thus, a 1 min rest period was given after each 5 min of exercise. In total, 8 periods of 5 min of exercise were carried out. Subjects also carried a 5 to 10 kg weight belt during this exercise.

Measurements

Angle-torque relationships

The passive and active length-tension properties of TS were measured as the relationship between ankle angle and torque about the ankle joint, which is the product of muscle force and moment arm, the perpendicular distance between the achilles tendon and the joint axis of rotation. It is known that the achilles tendon moment arm decreases as the ankle joint is rotated from a plantarflexed to more dorsiflexed foot position, in which muscle length is increased (Rugg et al. 1990; Maganaris et al. 1998). Thus, the tension produced by TS will be somewhat underestimated by measuring torque at longer lengths. However, for the current experiments, moment arm was not taken into account, since the main aim was to compare changes to angle-torque relationships after the eccentric exercise with those before the exercise, and it seemed unlikely that moment arm would be affected by the exercise.

Measurements of both passive and active angle-torque relationships were carried out on the apparatus shown in Figure 3.2. It consisted of a chair that was attached to a steel frame, which, in turn, supported two wooden footplates via a rotatable axle. The subject was seated on the chair and both feet were firmly strapped to the footplates with Velcro straps. For each subject, the horizontal position of the chair was adjusted so that when the footplates were locked into a set position, the angle subtended between the shin and footplate was approximately 90°. The knee angle, defined as the included angle between the thigh and shin, was held constant at approximately 140° by means of a brace placed over the lower thigh and clamped to the steel frame. The angle of the footplate, and thus the ankle angle, could be altered in 5° increments by rotating the axle with a handle, which could then be locked into place with a metal pin. The axle was aligned with the axis of rotation of the ankle joint. Torque about the ankle joint was measured by two strain gauges glued to the axle. The gauges were aligned at 45° to the axis, along the planes of maximum

principle stress during twisting, as the foot pushed on the footplate. Output from the strain gauges was sent to a MacLab/8s (ADInstruments, Australia) where it was amplified and converted from an analogue to digital signal, before being recorded in 'Chart' (ADInstruments, Australia) running on a Macintosh 6100 computer (Apple, U.S.A).

To contract TS, the tibial nerve was stimulated using bipolar electrodes. The cathode was a 1.5 cm diameter sphere wrapped in gauze, and moistened with saline solution (0.9%). The cathode was mounted in a metal frame, enabling it to be placed behind the knee in the popliteal fossa, and fastened to the subject's lower thigh with Velcro straps. The frame also allowed for the position of the electrode to be altered if required. The anode was a thin, rectangular brass plate (6 x 4 cm), which was covered in electrode conducting gel and taped to the anterior surface of the leg, just inferior to the patella. Nerve stimulation was triggered by the MacLab, while the timing of each impulse was set by a sequencer (Pulsemaster A300, World Precision Instruments, Florida, USA). This, in turn, was connected to a stimulator (Digitimer DS7, Hertfordshire, England) which had a maximum pulse width of 0.2 ms, designed for experimentation on human subjects.

At the start of each testing session, the stimulus current required to provide a maximal contraction of the TS of both legs was determined. This involved delivering single pulses (0.2 ms width) to the tibial nerve with progressively increasing current strengths. The twitch torque produced by each stimulus was recorded. When torque no longer increased, the current strength was considered to be supramaximal. For most subjects, this occurred when the stimulus current was between 30 and 60 mA. A warm-up procedure was then carried out, in which subjects voluntarily contracted TS by pushing down on the footplates for 3 s, every 15 s, until 10 contractions had been completed. This served to reduce potentiation effects from the preceding electrical stimulation.

Active and passive angle-torque relationships of both legs were measured simultaneously. Subjects were requested to remain completely relaxed during this procedure, particularly during the measurement of passive torque. In order to ensure that subjects were relaxed, the electromyographic (EMG) activity of TS was recorded for two subjects, before and after eccentric exercise. Two adhesive Ag-

Figure 3.2 *Equipment for measuring angle-torque curves.*

A schematic representation of the apparatus used for the measurement of the passive and active angle-torque relationships of TS (red). The subject was seated in a chair with each foot strapped to a footplate. A brace was secured over the lower thigh to prevent movement of the knee joint. Electrical stimulation of the tibial nerve was used to activate the muscle. The cathode (-) was placed in the popliteal fossa, while the anode (+) was positioned just below the patella. Active and passive torque was measured with strain gauges attached to the axle, which was coincident with ankle joint. Ankle angle could be adjusted by moving a handle attached to the axle and set into position with a locking pin.

knee brace

handle

footplate

chair

locking pin
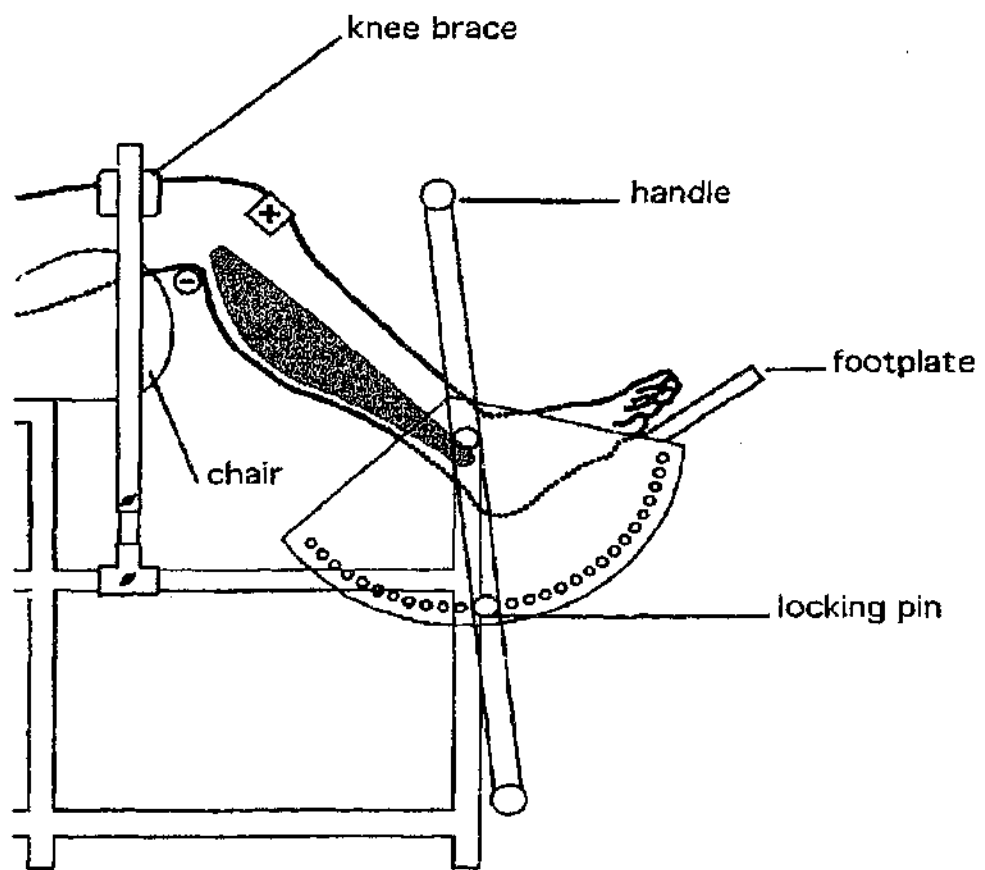
AgCl recording electrodes (3M 'Red Dot') were placed on the skin overlying TS, approximately 2 cm apart, while an earth electrode was positioned on the anterior surface of the leg, overlying the tibia. The outline of the recording electrodes was marked on the skin, so that they could be re-applied in nearly the same position from one measurement session to the next. Output signals from the electrodes were sent directly to the MacLab, before being recorded and stored in 'Chart'.

Angle-torque measurements were performed over an ankle angle range of 40°, starting at 90° and rotating the ankle in 5° increments up to 50° (Figure 3.3). Here, a decrease in the ankle angle represented an increase in muscle length, as the foot became more dorsiflexed. At each angle, a 10 s period was given for the muscle to relax to allow for the measurement of passive torque. The tibial nerve was then stimulated with double pulses (0.2 ms pulse width, 20 ms apart) and the resultant active torque was recorded. Tetanic stimulation was not used as previous experience had shown that it was too uncomfortable for subjects. A second set of measurements was made, to establish repeatability of active angle-torque measurements. In the intervening period of 1 to 2 min between the first and second set of measurements, the ankle angle was returned to 90° and subjects were asked to perform a voluntary contraction of TS, in order to remove any slack due to muscle thixotropy.

Active angle-torque curves were plotted for each measurement session and Gaussian curves were fitted to values above 75% of the peak torque, to locate the optimum angle for peak torque generation (Figure 3.3 B). Passive torque at each angle was taken as the mean torque recorded over the final 0.5 s before nerve stimulation. Values were then plotted relative to the torque measured at 90°. Passive angle-torque curves were constructed by fitting a single exponential curve to the data points, as it was found that this function provided a good approximation of the passive angle-torque relationship (see Figure 3.3 B). Since there were differences in the location of the optimum angle for active torque between subjects, it was decided to compare changes in passive torque after exercise with those beforehand, at the pre-exercise optimum angle for each subject.
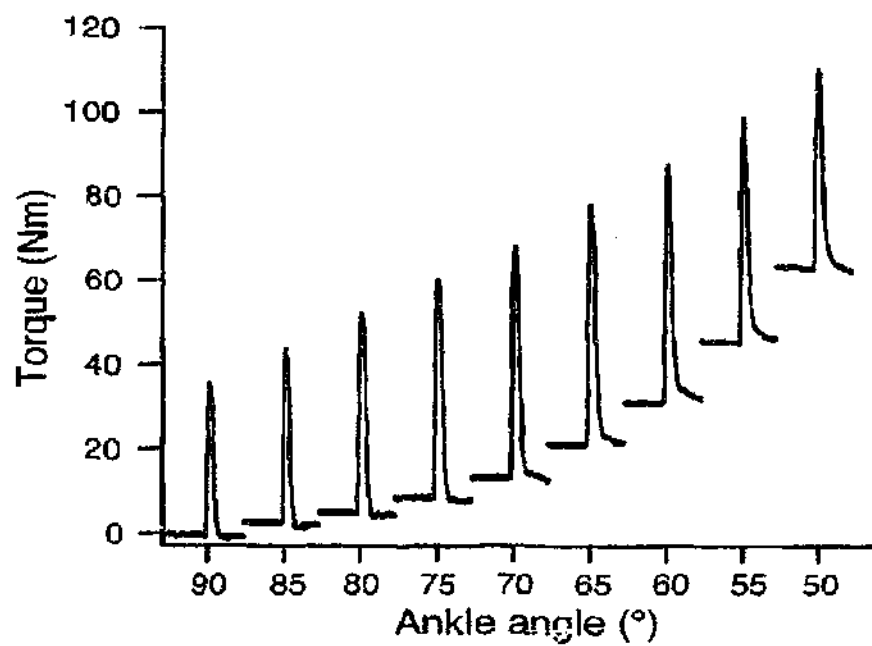
Leg volume

The volume of the leg below the level of the knee was measured in order to determine the degree of swelling that occurred after the exercise. Subjects placed

Figure 3.3 *Active and passive angle-torque measurements.*

(A) An example for one subject of the torque recorded at each ankle angle before exercise. At each angle, passive torque is shown over the final 0.5 s before nerve stimulation, and each trace has been offset relative to the value at 90° (zero torque). Active torque was generated by double pulse stimulation of the tibial nerve. (B) Active (open circles) and passive (filled circles) angle-torque relationships from the data shown in (A). A Gaussian curve (dotted line) was fitted to values above 75% of the maximum active torque. The optimum angle for peak active torque is indicated (arrow). An exponential curve (solid line) was fitted to passive angle-torque measurements.

A

B
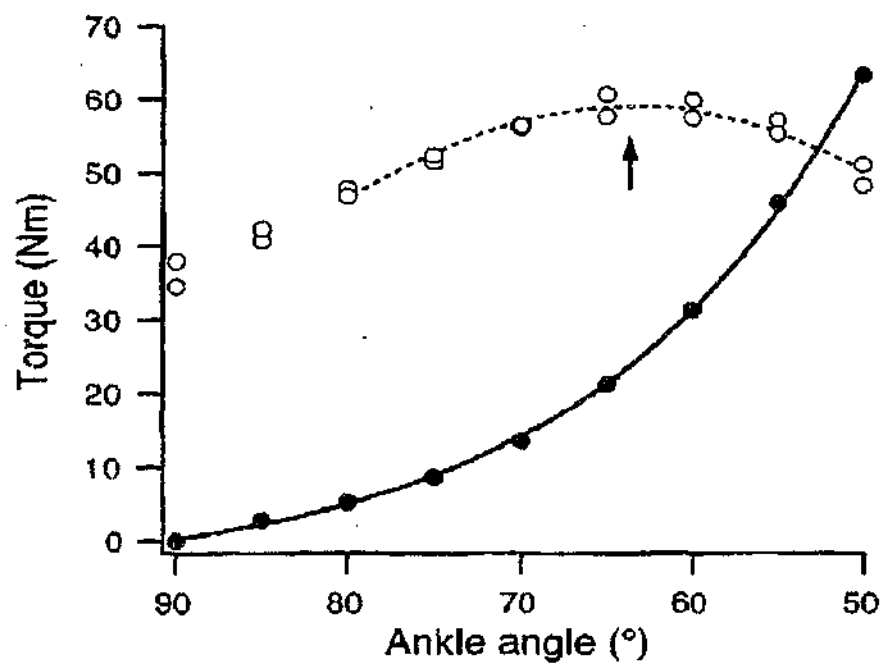
each leg, in turn, in a calibrated Perspex container, which was filled with warm water to the level of the popliteal fossa. The leg was then withdrawn, and the amount of water displaced was used to calculate the leg volume. It was proposed that any change in leg volume after eccentric exercise would be attributed to swelling of TS, which would show up as an increase in total leg volume.

Pre and post-exercise measurements

Angle-torque and leg volume measurements were made three to four times before exercise, immediately and 2 hours afterwards, and on each of the following 4 days post-exercise. In addition, after exercise, subjects were asked to rate their perceived level of muscle soreness during slow walking and stretch of the muscle on a scale from 0 to 10, where 0 represented no pain, and 10 represented intolerable, intense pain.

Data analysis

All parameters were calculated as the mean $\pm$ S.E.M., with the level of significance set at $P < 0.05$. A three-factor ANOVA with interactions was used to test the significance between legs following eccentric or concentric exercise. The factors were time (after the exercise), leg (exercised or control) and subject. Where the ANOVA was significant, an LSD post hoc test was used to determine significant differences between legs at different times. A linear regression analysis was used to test the significance of relationships between some variables. The statistical program used was Data Desk (Ithaca, NY, USA).

Results

The main aim of these experiments was to investigate the effects of an acute period of eccentric or concentric exercise on changes to the passive mechanical properties of human TS. However, it was first necessary to establish the extent of the damage to the muscle, after each form of exercise, by examining changes to the active angle-torque relationship. Examples of active angle-torque curves measured before and immediately after either eccentric or concentric exercise are displayed in Figure 3.4. Here, it can be seen that after eccentric exercise, there was both a shift of the optimum angle (arrow) of $5°$ in the direction of longer muscle lengths, and a reduction in peak active torque of 14 Nm, or 40%. In comparison, after concentric

Figure 3.4 *Active angle-torque curves before and after exercise.*

Active angle-torque curves as measured before (blue) and immediately after (red) a period of either (A) eccentric or (B) concentric exercise of TS. For each curve, the optimum angle for peak torque generation is indicated (arrow).

A

Active torque (Nm)

B

Active torque (Nm)

Ankle angle (°)
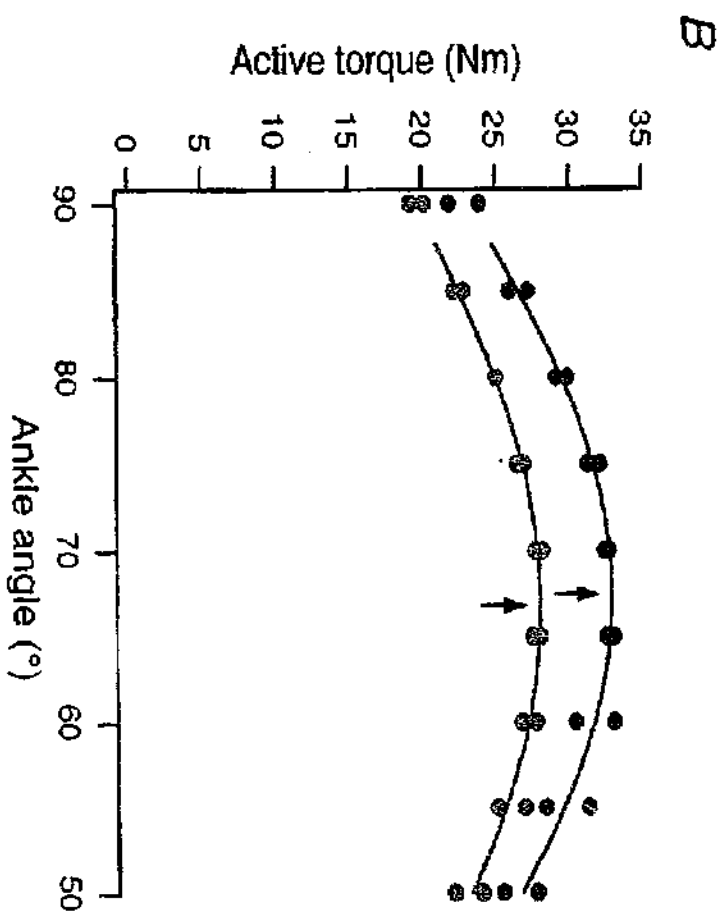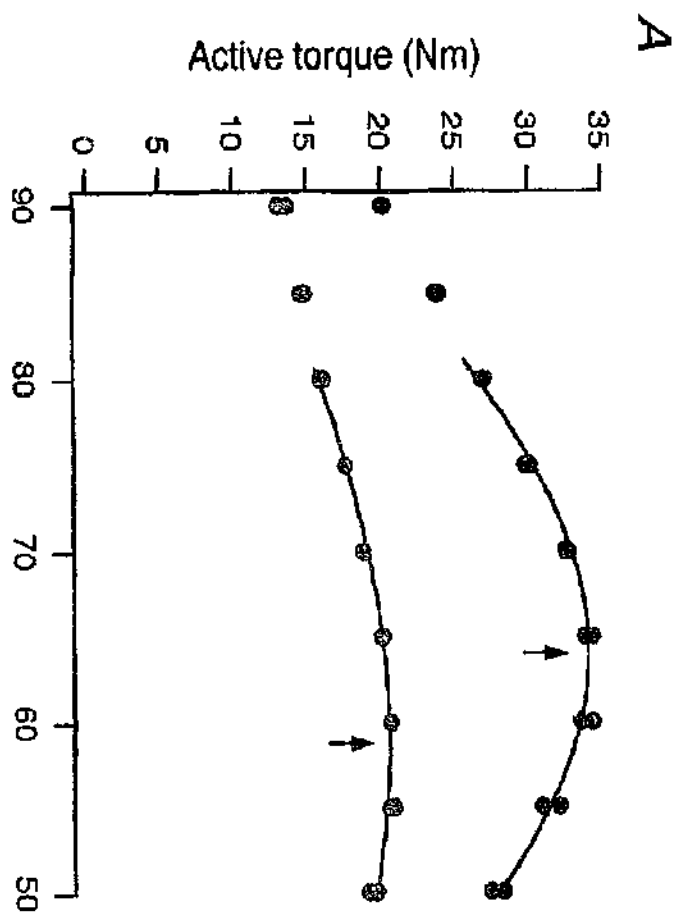
exercise, there was a negligible shift in optimum angle (0.6°), while peak torque dropped by 5 Nm, or 15%.

The pooled data for the shift in optimum angle for both exercise groups, as measured up to 96 hours post-exercise is shown in Figure 3.5. The mean shift in optimum angle for the eccentrically exercised TS was 6.0° (± 2.3) immediately after exercise and reached a peak of 7.6° (± 2.9) at 2 hours post-exercise. Over this period, there was a significant difference between values for the eccentrically exercised and unexercised muscles (P < 0.05, three-factor ANOVA). By 24 hours post-exercise, the shift in optimum angle measured after eccentric exercise was no longer significantly different to that of the control leg. After concentric exercise, there was no significant shift in optimum angle, at any time point, for the exercised TS compared with that of the control muscle.
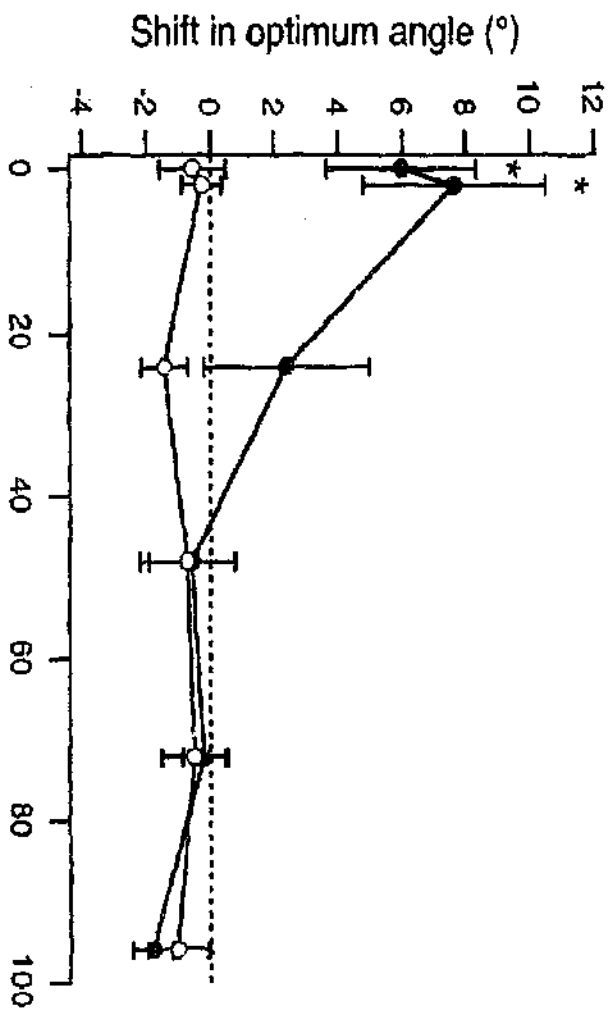
Changes in peak active torque after exercise are shown in Figure 3.6. Immediately after eccentric exercise, torque had fallen by 36.8% (± 3.9) compared with pre-exercise values, and then gradually recovered with time to be 4.6% (± 7.5) lower by 96 hours post-exercise. At all times after the exercise, there was a significant difference between values for exercised and unexercised muscles (P < 0.05, three-factor ANOVA). After concentric exercise, there was an immediate drop in peak torque of 13.1% (± 2.4) for the exercised leg, which was shown to be significantly different to that of the unexercised leg (P < 0.05, three-factor ANOVA). This drop in torque was most likely due to muscle fatigue, since values between legs were no longer significantly different by 2 hours post-exercise.

The changes to the active angle-torque curve supported the hypothesis that eccentric exercise led to muscle damage while concentric exercise did not. This point was further highlighted by the fact that only eccentric exercise resulted in delayed onset muscle soreness (DOMS). Subjects first reported muscle pain and tenderness in TS 24 hours after eccentric exercise, which was felt during contraction, stretch and palpation of the muscle. Typically, the soreness seemed to arise more in the gastrocnemii than soleus, although there was some variability in the distribution of the soreness (see Weerakkody et al. 2001). When values were pooled, muscle soreness during slow walking and stretching increased to a peak of 5 (± 0.72) out of

Figure 3.5 *Shift in optimum angle after exercise.*

The mean (± S.E.M.) shift in optimum angle plotted against time after (A) eccentric or (B) concentric exercise for the experimental (filled symbols) and control (open symbols) muscles. For this and subsequent figures, values at the time '0 hr' represent measurements made immediately after the exercise. Values are expressed relative to those measured before the exercise (dotted line). Positive values indicate a shift to smaller ankle angles, that is, in the direction of longer muscle lengths. Asterisks indicate times when there was a significant difference between the exercised and control legs ($P < 0.05$, LSD *post hoc* test).
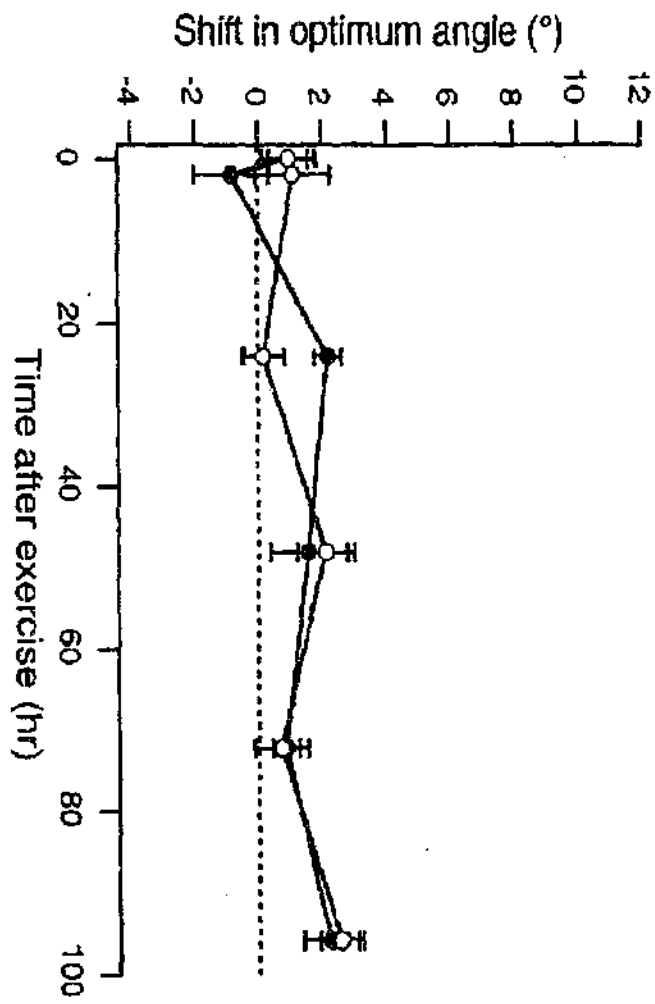
A

Shift in optimum angle (°)

B

Shift in optimum angle (°)

Time after exercise (hr)

Figure 3.6 *Peak active torque change after exercise.*

The mean (± S.E.M) change in peak torque relative to pre-exercise values after (A) eccentric or (B) concentric exercise for muscles of the experimental leg (filled circles) and control leg (open circles). Negative values represent a decrease in active torque relative to those measured before the exercise (dotted line). Asterisks indicate times when there was a significant difference between the exercised and control legs ($P < 0.05$, LSD *post hoc* test).
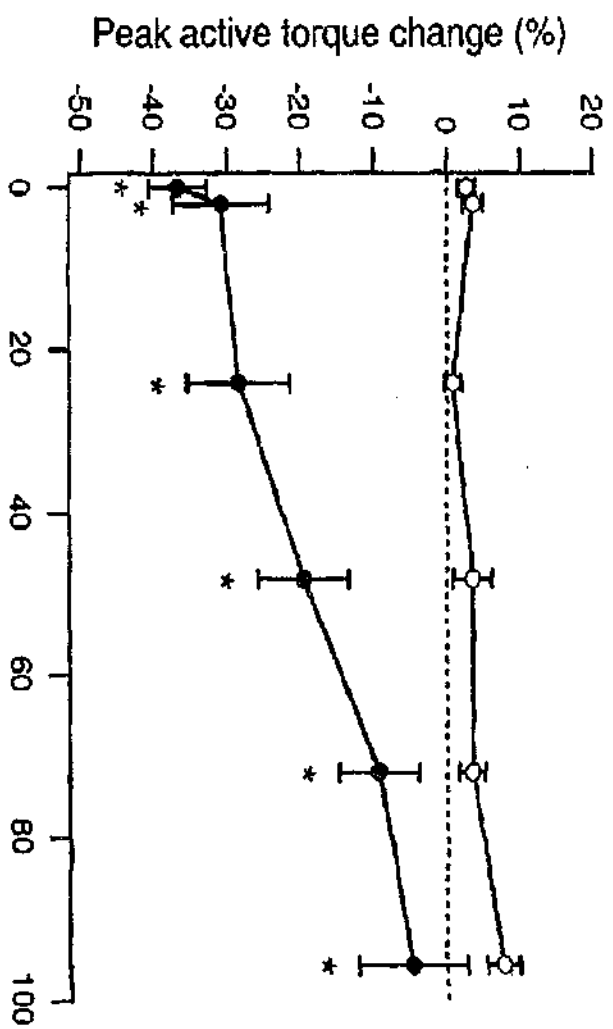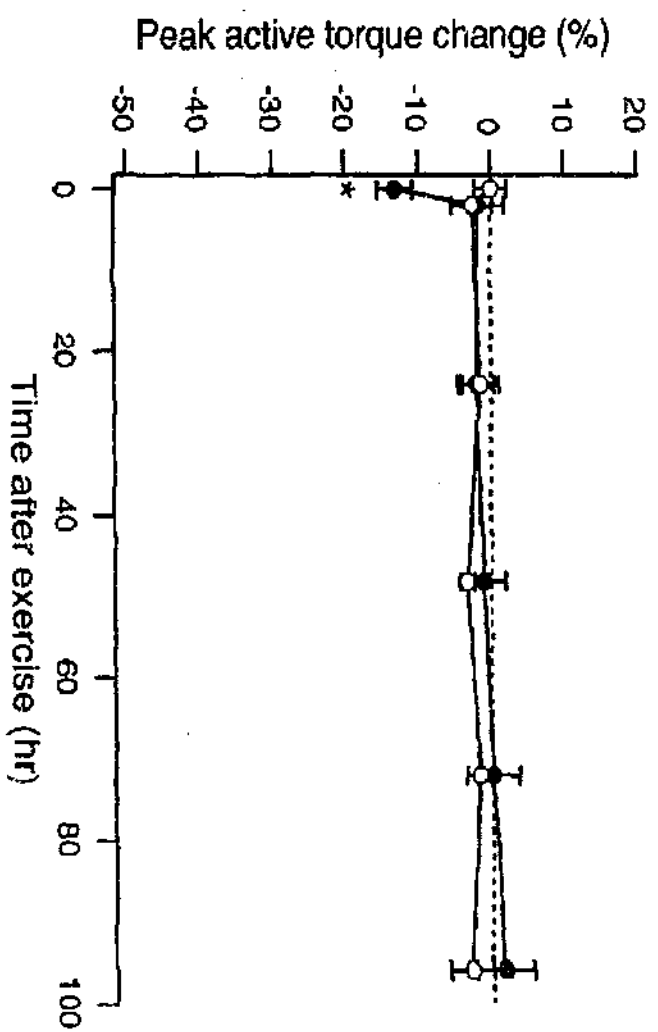
10, at 48 hours post-exercise. Values then fell to 3.7 (± 0.72) by 96 hours after the exercise.

These findings suggested that muscle damage had occurred after eccentric exercise and therefore injury contractures in muscle fibres might be present. This was investigated by measuring changes to passive mechanical properties after exercise. An example of passive angle-torque curves for two subjects, as measured before, and immediately after eccentric or concentric exercise, is shown in Figure 3.7. Here, it can be seen that after eccentric exercise, passive torque had increased at all angles measured. At the pre-exercise optimum angle for active torque (arrow), the increase was 4.7 Nm, or 55%. In comparison, after concentric exercise there was an increase of 8.9%, which was not a consistent finding across subjects (see below). The pooled data for changes in passive torque is shown in Figure 3.8. Immediately after eccentric exercise, passive torque was 40.8% (± 13 above the pre-exercise value. Over the next 24 hours, there was a further, small increase in passive torque, before it fell to 30% (± 9) at 48 hours post-exercise, and remained fairly constant thereafter. At all times after exercise, passive torque for the eccentrically exercised TS was significantly higher than that of the unexercised muscle (P < 0.05, three-factor ANOVA). After concentric exercise, passive torque of the exercised leg did not really differ from pre-exercise values and, over time, there was found to be no significant difference between exercised and unexercised muscles.

An interesting result, which was consistent with findings to be shown later in this thesis (see Chapters 4 and 5), was that the increase in passive torque immediately after eccentric exercise, when expressed as a percentage of pre-exercise values, varied with ankle angle. This was particularly evident for one subject, who had more extensive muscle damage than the other subjects, as shown by a large immediate shift in optimum angle (12°) and peak active torque drop (55%). In Figure 3.9, the percent increase in passive torque, as measured immediately after eccentric exercise, has been plotted for this subject. Here, it can be seen that values increased to a maximum of 140% at an angle of 60° and then decreased, but remained above control values, as muscle length increased further. For this subject, a similar angle-dependent relationship was found at all times after the eccentric contractions, despite

Figure 3.7 *Passive angle-torque curves before and after exercise.*

Passive angle-torque curves as measured before (blue) and immediately after (red) a period of either (A) eccentric or (B) concentric exercise of TS. Values of passive torque before and after exercise were compared at the pre-exercise optimum angle for active torque generation (arrows).
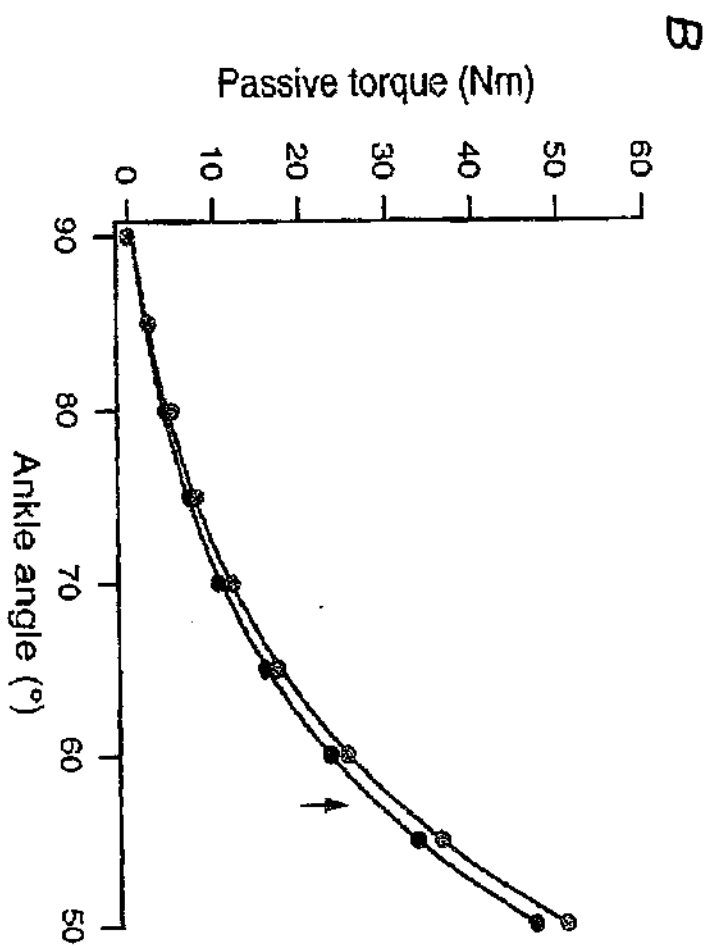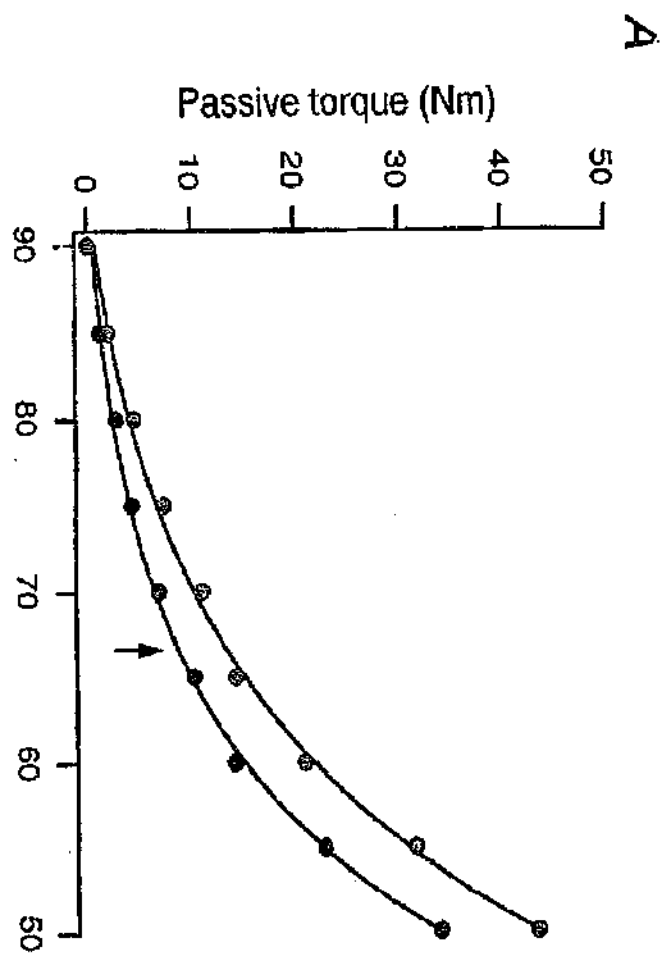
A

Passive torque (Nm)

Ankle angle (°)

B

Passive torque (Nm)

Ankle angle (°)

Figure 3.8 *Changes in passive torque after exercise.*

The mean ($\pm$ S.E.M) change in passive torque relative to pre-exercise values after (**A**) eccentric or (**B**) concentric exercise for muscles of the experimental (filled circles) and control (open circles) legs. Positive values indicate an increase in passive torque after exercise relative to those measured before the exercise (dotted line). For eccentric exercise values, there was a significant difference between the exercised and control legs at all times, as shown by the asterisks ($P < 0.05$, LSD *post hoc* test).
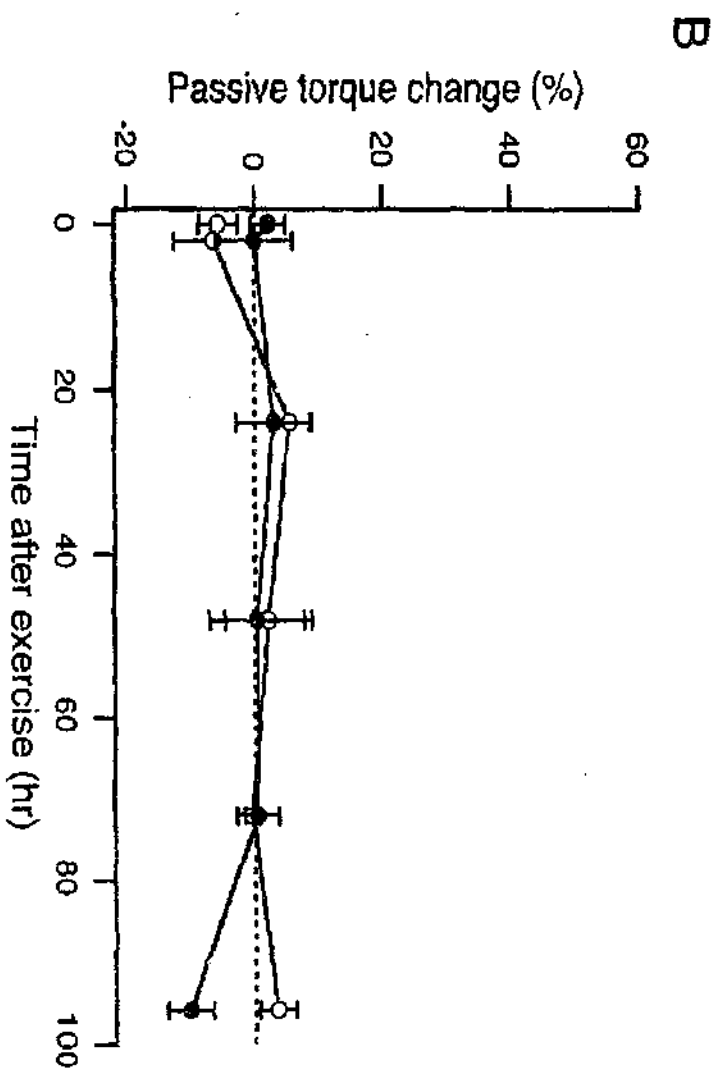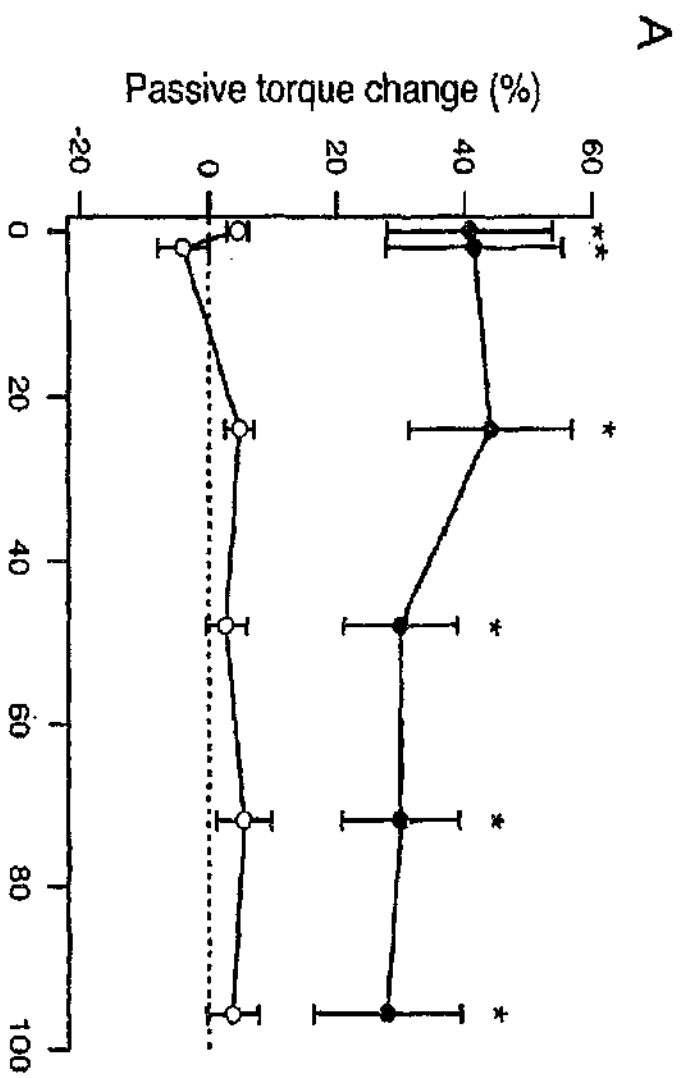
Figure 3.9 *Increase in passive torque at different angles.*

Data from one subject showing passive angle-torque curves as measured before (blue) and immediately after (red) a period of eccentric exercise. The percent increase in passive torque after eccentric exercise, expressed as a percent increase above pre-exercise values, has also been plotted against ankle angle (black, open circles, dotted line).
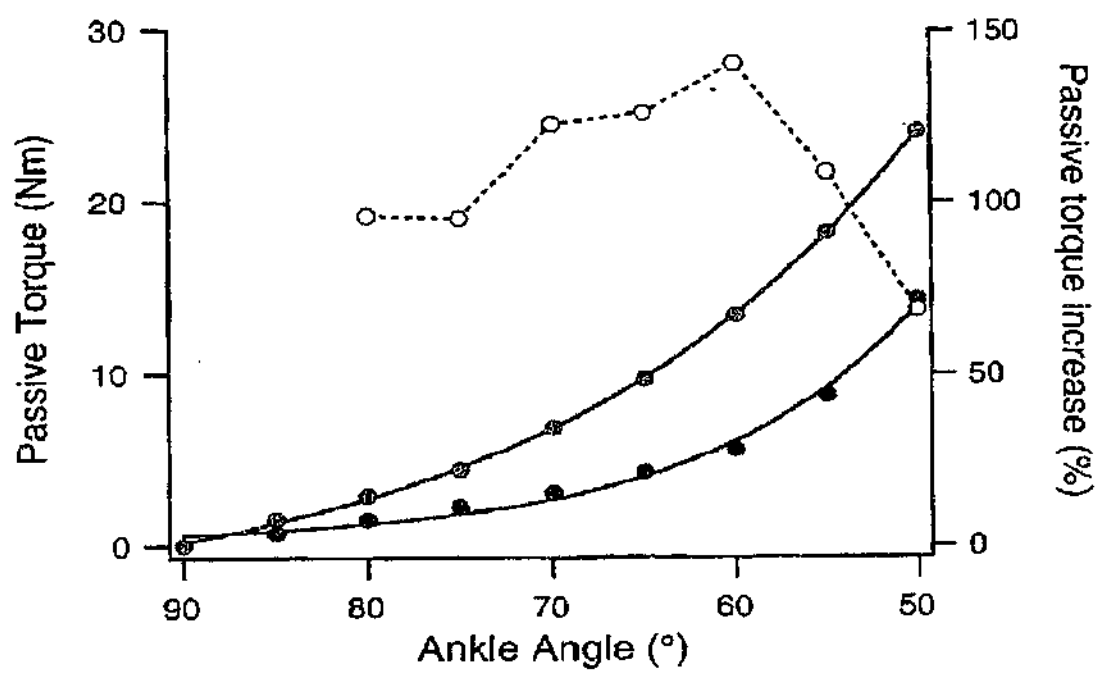
the fact that the maximum increase in passive torque had dropped to about 100% from 48 hours post-exercise onwards.

Another measure used to quantify the increase in passive torque after eccentric exercise was the amount of work done on the muscle when it passively stretched over the full range of angles used in these experiments. Unfortunately, passive shortening movements were not performed here, so measurement of the work absorbed by the passive muscle could not be calculated. The work done on the muscle was measured from the area under the exponential curve, fitted to the passive angle-torque data (Figure 3.10 A). An advantage of using this method to quantify the increase in passive torque was that it included values over the entire passive angle-torque relationship, thereby accounting for any variation in the angle-dependent changes in passive torque for different subjects. After eccentric exercise, the percent increase in work done on the passive muscle (Figure 3.10 B) was quantitatively similar to the increase in passive torque measured at the optimum angle, which was confirmed by the strong linear relationship between the two variables, including values at all times after eccentric exercise ($r^2 = 0.92$, $P < 0.001$).

The volume of the leg, below the knee, was measured before and after exercise, and an increase was attributed to swelling of TS. As shown in Figure 3.11, there was no swelling of eccentrically exercised TS until 24 hours after the exercise, and a peak value of 2.04% ($\pm$ 0.43) was reached at 48 hours post-exercise. Over the period from 24 to 96 hours post exercise, there was significant swelling of the leg that carried out eccentric exercise compared to the control leg ($P < 0.05$, three-factor ANOVA). Concentric exercise resulted in no significant swelling compared to the control leg, at any time post-exercise.

One of the aims of this experiment was to elucidate whether swelling could contribute to the rise in passive torque after eccentric exercise. Since there was no measurable swelling over the first two hours post-exercise, it seemed unlikely that it could be responsible for the immediate post-exercise rise in passive torque. However, its possible contribution at later time points needed to be examined. Thus, a regression analysis was performed to examine the relationship between the increase in passive torque and swelling over the period when swelling was significant, 24 to 96 hours post-exercise (Figure 3.12). There was found to be a close to significant

Figure 3.10 *Work done on the passive muscle before and after eccentric exercise.*

(A) Passive angle-torque curves from a subject as recorded before (blue) and immediately after (red) eccentric exercise. The work done on the passive muscle was calculated as the area underneath the exponential curve. This is shown before exercise (blue shaded area), and after exercise (red plus blue shaded areas). (B) The mean ($\pm$ S.E.M) change in work done on the passive muscle after eccentric exercise (filled circles), relative to pre-exercise values. Values for the muscle of the control leg are also shown (open circles). Positive values indicate an increase in the amount of work done on the muscle after exercise relative to that measured before the exercise (dotted line).
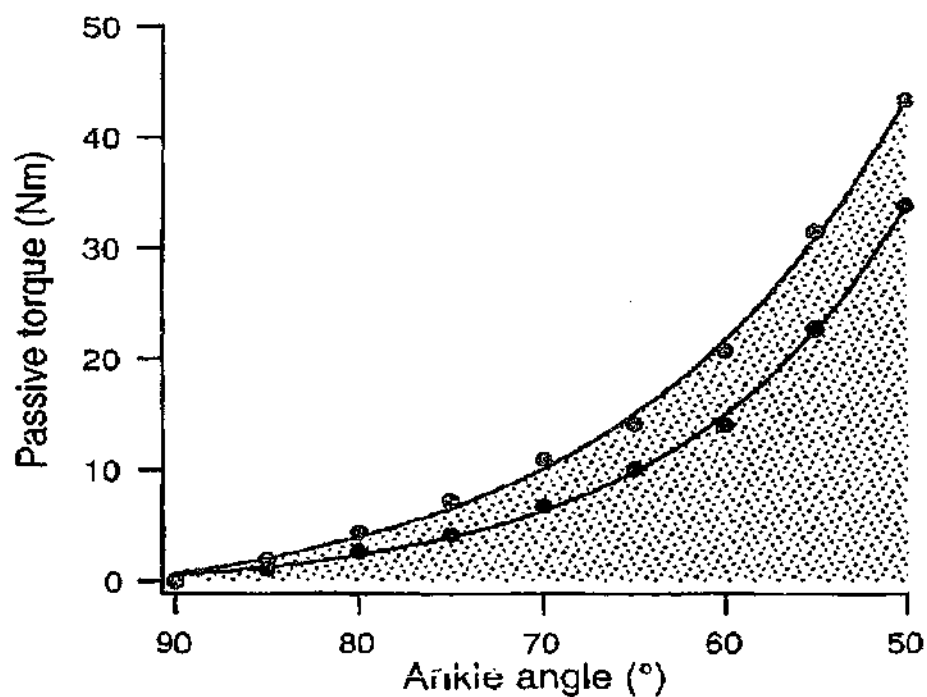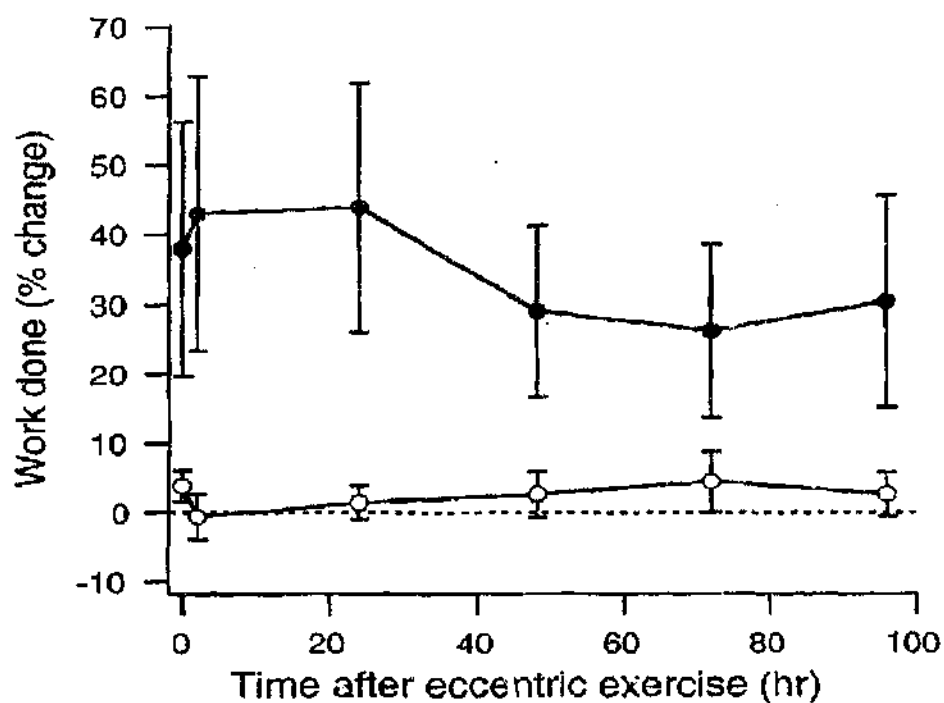
A

B

Figure 3.11 *Swelling after exercise.*

The mean ($\pm$ S.E.M) swelling after (A) eccentric or (B) concentric exercise. Swelling was taken as an increase in leg volume after exercise above that measured before exercise. Values for both the experimental leg (filled symbols) and control leg (open symbols) are shown. Values are expressed relative to those measured before the exercise (dotted line). Asterisks indicate times when there was a significant difference between the exercised and control legs ($P <$ 0.05, LSD *post hoc* test).
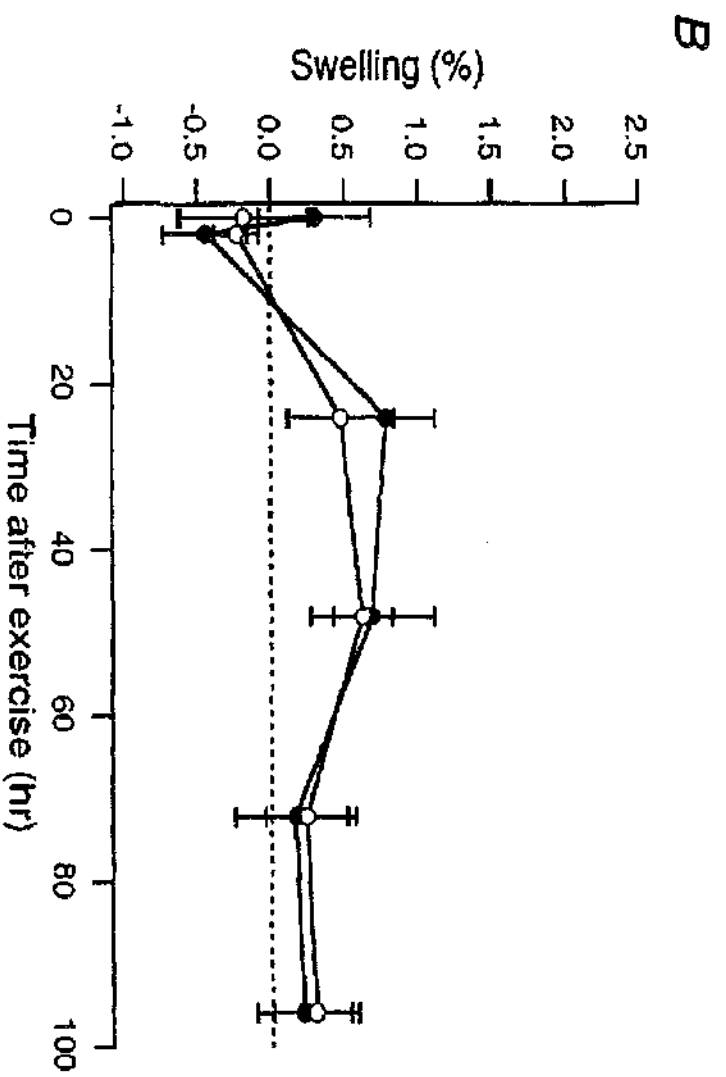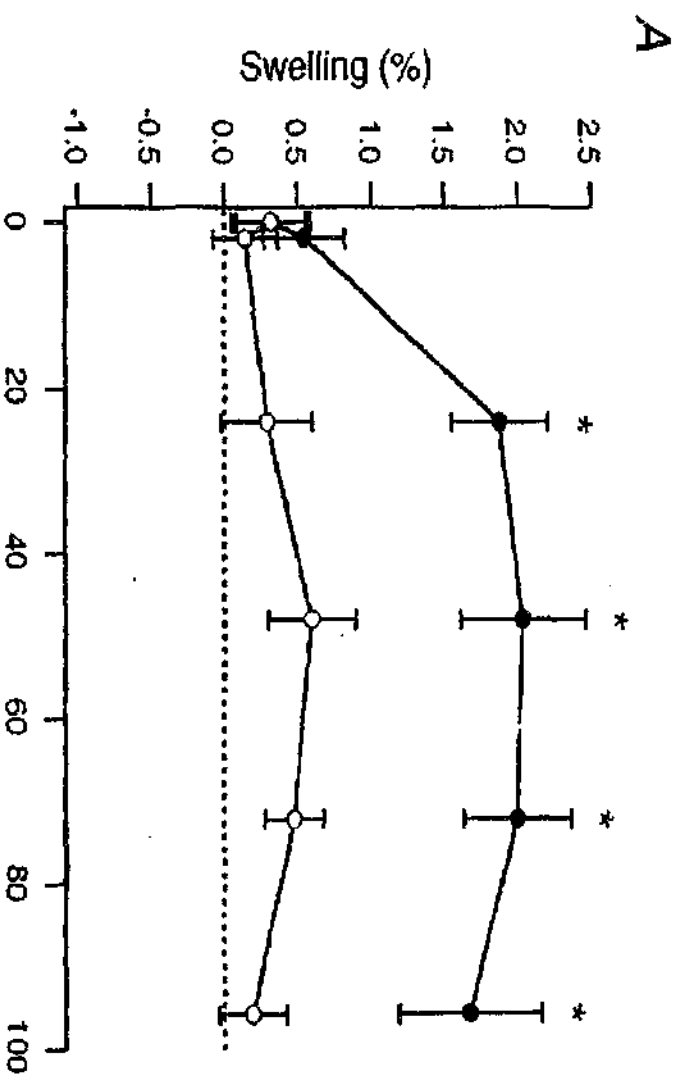
Figure 3.12 *The relationship between passive torque and swelling.*
Plot showing the relationship between the increase in passive torque and swelling after eccentric exercise. Since swelling of the eccentrically exercised leg was not significantly different from the control leg over the first two hours post-exercise (open symbols), regression analysis was performed on values from 24 to 96 hours post-exercise (filled symbols, regression line).
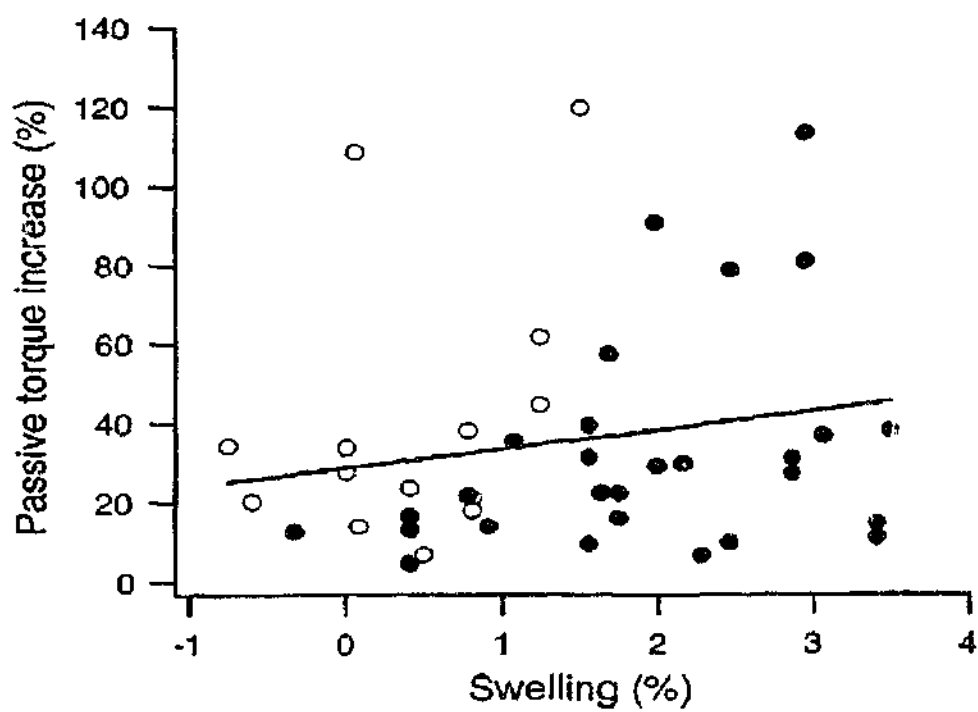
slope of the regression line (P = 0.07) but only a weak relationship (r^2 = 0.12) between these parameters.

It was hypothesised that the rise in passive torque after eccentric exercise was attributable to injury contractures in muscle fibres, which would occur in the absence of any neural activation. In order to test this proposal, the surface EMG activity of TS of two subjects was recorded during passive torque measurements before, and at all times after eccentric exercise. An example from one subject of the passive torque and corresponding EMG activity, as measured at three ankle angles before, and 2 and 48 hours post-exercise, is shown in Figure 3.13 A. Note that, despite an increase in passive torque after the eccentric exercise, there was no change in the amplitude of the EMG activity at any angle compared to that measured beforehand. During measurements of passive torque, EMG activity of TS was quantified by taking the root mean square (RMS) of the raw EMG signal over the final 0.5 s before nerve stimulation. RMS is a commonly used indicator of the magnitude of a raw EMG signal Herzog *et al.* 1999. For each measurement session after eccentric exercise, the change in RMS-EMG from pre-exercise values, at each angle, was calculated and these values were then pooled. This is shown for each of the two subjects (Figure 3.13 B). One subject had essentially no change in EMG after the eccentric exercise, while the other subject showed more variability, but the changes were still small (less than 1 μV at most time points). Analysis showed no significant difference between the exercised and unexercised legs, for changes in EMG after the eccentric exercise at all angles (P = 0.62, four-factor ANOVA).

In order to investigate whether the rise in passive torque after eccentric exercise was related to other indicators of muscle damage, the relationships between the increase in passive torque and both the shift in optimum length and drop in active torque were examined (Figure 3.14). The shift in optimum angle correlated well with the drop in peak active torque up to 2 hours post-exercise (r^2 = 0.60, P < 0.01) but was not as strongly related to the increase in passive torque over the same period (r^2 = 0.29, P < 0.05). The increase in passive torque after eccentric exercise, however, was strongly correlated with the drop in peak active torque over the first 2 hours post exercise (r^2 = 0.82, P < 0.001). These parameters were also a significantly related between 24 and 96 hours post-exercise (r^2 = 0.66, P < 0.001) but here, the slope of the regression line was less steep (-1.2 compared with -3.1 over the first 2 hrs post-exercise).

Figure 3.13 *EMG activity during passive torque measurements.*

(A) Data from one subject showing the raw EMG (bottom traces) recorded during the measurement of passive torque, over a period of 0.5 s before nerve stimulation. Records are shown at three ankle angles before (blue), 2 hrs (red) and 48 hrs (green) post-eccentric exercise. Torque (upper traces) has been offset relative to the passive torque measured at 90°. The twitch torque, as measured following double pulse stimulation of the tibial nerve, is also shown in each record. (B) The mean (± S.E.M) change in RMS-EMG activity after eccentric exercise for two subjects (different symbols). The dotted line represents pre-exercise values.

A

90°   70°   50°

]20 Nm

]20 µV

0.5 s
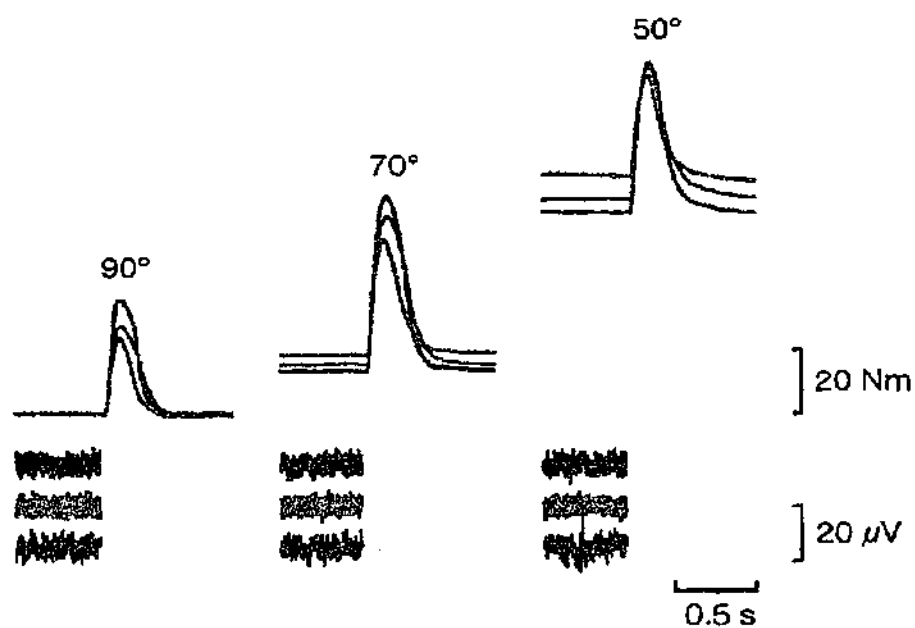
B
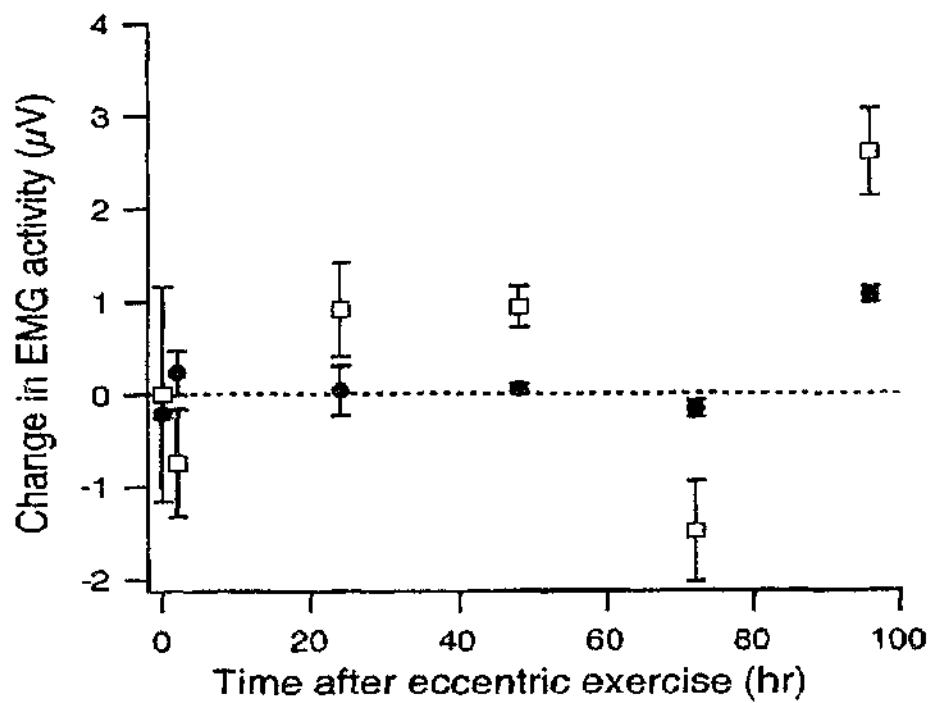
Figure 3.14 *Relationships between active and passive angle-torque measurements.*

The relationships between **(A)** change in peak active torque and shift in optimum angle, **(B)** change in passive torque and change in peak active torque, and **(C)** change in passive torque and shift in optimum angle. All values are plotted relative to those measured before eccentric exercise. For all 3 plots, a significant relationship was found over the first two hours post-exercise (filled symbols), while for (B), a strong relationship was also found over the period from 24 to 96 hours post-exercise (open symbols).
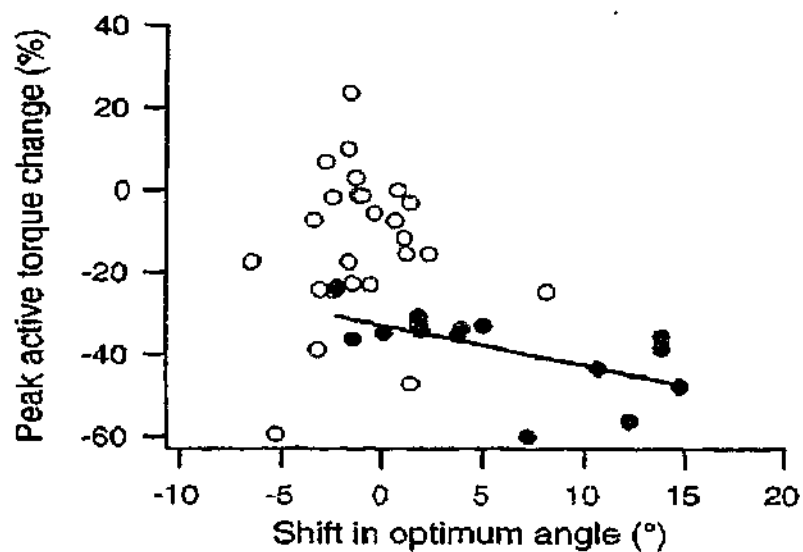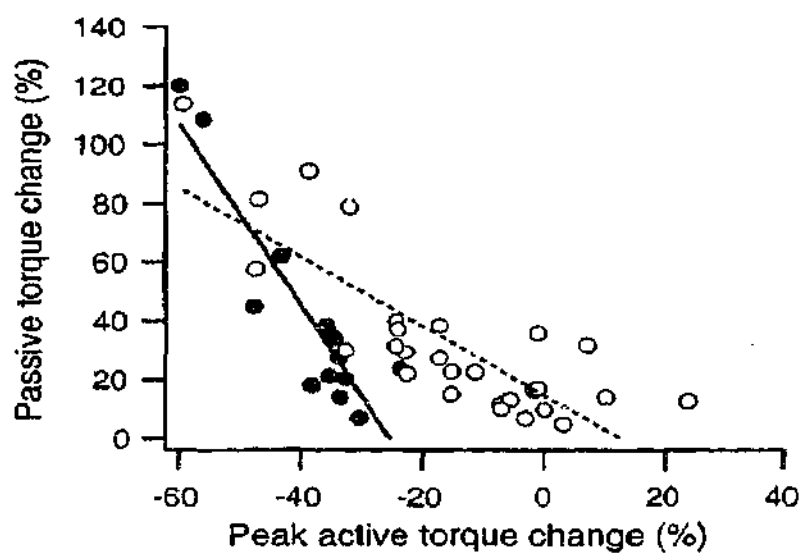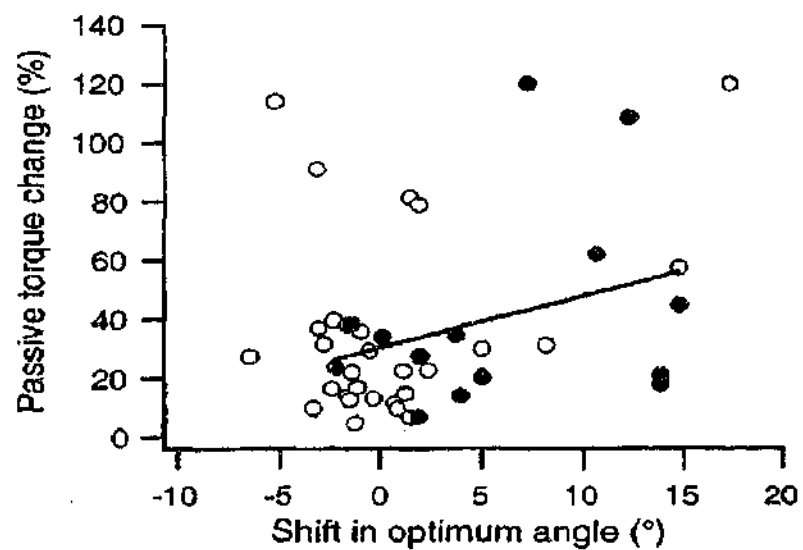
Discussion

The main purpose of this. chapter was to investigate the time-course of changes to the passive mechanical properties of human ankle extensor muscles after a period of eccentric exercise. Passive torque was measured before and after eccentric exercise at each subject's pre-exercise optimum angle. It was found that passive torque increased immediately after eccentric exercise with values increasing slightly more over the next 24 hours, before falling but remaining above control values 4 days after the exercise. A similar trend was found for the time-course of the increase in work done by the muscle during passive angle-torque measurements. Previous work carried out on human TS has also shown an increase in passive torque following eccentric exercise (Jones *et al.* 1997; Whitehead *et al.* 1998), however values in these studies were not significant until 24 hours post-exercise. This difference was attributed to the fact that in these earlier experiments, passive torque measurements were carried out from dorsiflexion to plantarflexion, that is, from long to short muscle lengths. Passive torque values measured in this way were likely to be lower, particularly during the early stages after eccentric exercise, as a result of muscle thixotropy (see Chapter 4).

Importantly, the increase in passive torque after eccentric exercise was accompanied by other mechanical indicators of muscle damage, measured from the active angle-torque relationship. A shift to longer lengths of the optimum angle and a reduction in peak active torque were found immediately after eccentric exercise. The optimum angle returned to its pre-exercise value 24 hours after exercise, while peak torque had not fully recovered by 96 hours post-exercise. These findings were consistent with those from previous studies carried out on TS (Jones *et al.* 1997; Whitehead *et al.* 1998). It was proposed that the recovery of the shift in optimum angle reflected the reinterdigitation of overextended sarcomeres in damaged fibres, while the slower recovery of peak torque corresponded to the repair of damaged fibres (Jones *et al.* 1997). Other commonly used indicators of muscle damage, swelling and DOMS (Smith, 1991), were also evident after the eccentric exercise, with both parameters becoming significant from 24 hours post-eccentric exercise onwards. In contrast, following concentric exercise, there was no change to the passive angle-torque relationship, nor were there any other measurable signs of muscle damage. The only change was a 13% drop in peak active torque immediately post-exercise, which

recovered rapidly, within two hours, and so was most likely due to muscle fatigue (Faulkner *et al.* 1993). Thus, these results strongly suggested that the rise in passive torque was attributable to muscle damage from eccentric contractions and not to any other effects of the exercise.

It was hypothesised that the rise in passive torque, particularly during the period immediately after the exercise, was due to the tension produced by injury-contractures in damaged muscle fibres. This proposal was supported by the fact that after eccentric exercise, there was no change in the EMG activity of TS during passive angle-torque measurements, despite an increase in passive torque of more than 40%. This ruled out reflex activation of the muscle as the cause of the rise in passive torque. Previous studies have also reported no increase in EMG activity of the relaxed elbow flexors after eccentric exercise, using both surface (Howell *et al.* 1993; Chleboun *et al.* 1998) and intramuscular (Howell *et al.* 1985) recording electrodes. The fact that there was no measurable swelling of TS until 24 hours post-exercise implied that the immediate rise in passive torque was also not likely to be due to swelling-induced strain of connective tissue, which could potentially lead to an increase in passive tension, based on the biomechanical model of Purslow (1989). A similar conclusion was reached by Chleboun *et al.* (1998), where passive stiffness of the elbow flexors increased by 60% immediately post-exercise and remained relatively constant over the subsequent 5 days, while swelling did not become significant until 48 hours post-exercise. In the present study, the fact that swelling showed a delayed onset, also argues against possible swelling-induced changes to the moment arm or the mechanical properties of joint structures, as being responsible for the immediate rise in passive torque.

It was postulated by Howell *et al.* (1993), and more recently by Chleboun *et al.* (1998), that low-level calcium activation could be responsible for the increased passive stiffness after eccentric exercise. They suggested that this effect became greater as muscle length increased and that stretch activated calcium channels in the surface membrane may be involved. However, in their studies, it was found that while passive stiffness increased at short to intermediate lengths, there was little to no change in stiffness at longer lengths, suggesting that the increase in passive torque had become less at these lengths. The results of the present series of experiments also showed that the percent increase in passive torque was dependent on muscle

length, with values increasing to a maximum and then falling at longer lengths. This relationship was most prominent for one subject, who showed signs of having more extensive muscle damage than the other subjects who had undergone eccentric exercise. For this subject, as the muscle was stretched by dorsiflexing the foot, passive torque increased to reach a peak of 140% at an ankle angle of 60° (see Figure 3.9), which corresponded to the pre-exercise optimum angle for peak torque. The proposed mechanism underlying the length-dependent rise in passive tension after eccentric contractions will be discussed later, in Chapters 4 and 5.

While swelling was unlikely to be the cause of the increase in passive torque over the first few hours post-exercise, the possibility was considered that it might provide a contribution at later times, as suggested by Chleboun *et al.* (1998). However, it was found that during the period where swelling became significant, 24 to 96 hours post-exercise, there was no additional increase in passive torque. In fact, values dropped by about 15% from 48 hours post-exercise onwards. Over this period, the increase in passive torque was not related to swelling. It was also found that the degree of swelling, measured as an increase in leg volume, was quite small, about 2%. Even if it is assumed that TS conservatively occupies one third of the total leg volume, this gives a value of 6% swelling for TS. Comparatively, this value is still considerably lower than the 26% swelling of the elbow flexors reported by Chleboun *et al.* (1998). However, the degree of muscle damage, and hence swelling, was likely to have been greater in their study because subjects were required to maximally activate the muscle during eccentric contractions, whereas the eccentric exercise carried out on TS was submaximal. Here, it must be pointed out that the method of measurement of swelling in the present series of experiments was likely to have been less accurate than the ultrasound technique employed by Chleboun *et al.* (1998). Thus, it is possible that swelling does provide some contribution to the rise in passive torque from 24 hours post-exercise onwards, although further experiments are necessary in order to investigate this idea.

While contracture was proposed to be the cause of the immediate rise in passive torque, it remains unclear, based on the current data, how long such a contracture tension might be sustained after eccentric exercise. One possible scenario is that during the damage process, there may be an intermediate stage whereby the contracture ceases to generate active tension but remains as a clot with high stiffness,

because the resting calcium concentration of damaged muscle fibres returns to normal or there is insufficient ATP to maintain the ongoing contraction (see Armstrong *et al.* 1991). It was envisaged that the presence of such a stiff segment of a fibre would tend to reduce its functional length, thereby shifting the passive length-tension relationship towards shorter lengths and raising the level of passive tension at a given muscle length. Under these conditions, it seems likely that the level of passive tension would be less than that produced by fibres undergoing an active contracture. This might provide a possible explanation for the drop in passive torque from 24 to 48 hours post-exercise. However, this idea is speculative and certainly requires further investigation.

The relationships between the rise in passive torque and changes to the active angle-torque curve after eccentric exercise were considered to be important in terms of gaining new insights into the damage process. As expected, there was a significant correlation between the shift in optimum angle and the reduction in active torque immediately post-exercise. This is in line with the results of previous studies on human TS (Jones *et al.* 1997) and toad sartorius (Talbot & Morgan, 1998). The results of the current series of experiments also showed a strong correlation between the rise in passive torque and both the shift in optimum length and drop in active tension. The relationship between the increase in passive torque and the drop in active torque was considered to be particularly important as it provided some support for the proposal that damage to muscle fibre membranes leads to the development of contracture, as well as a reduction or loss of active tension generation. There is some experimental support for this proposal (Warren *et al.* 1995). They reported a relationship between the reduction in active tension and the frequency of damaged muscle fibres with swollen regions, a few hours after eccentric exercise. These swollen regions were found to have a high calcium concentration, so there is a possibly that they represented a region of contracture (Warren *et al.* 1995). Therefore, these relationships have provided new evidence that a rise in passive tension can be used alongside changes to active mechanical properties, as a reliable indicator of the damage to human muscle after eccentric exercise.

C h a p t e r   F o u r

The effects of eccentric contractions on passive mechanical properties and Golgi tendon organ responses of cat medial gastrocnemius

Introduction

In the previous chapter, passive tension of human muscle was found to increase immediately after eccentric exercise and remain at a higher level for several days. In terms of gaining insight into the process of muscle damage, the immediate rise in passive tension was considered to be of particular importance, based on the proposition that it was a direct consequence of the initial mechanical disruption to the muscle from eccentric contractions. The central aim of the work undertaken in this chapter was to further explore the hypothesis that the increase in passive tension immediately after a series of eccentric contractions was attributable to regions of injury contracture in some muscle fibres. To test the hypothesis, a series of experiments was carried out on cat medial gastrocnemius (MG), in which passive tension and work absorption were measured during passive stretch-shortening movements, before and immediately after a series of eccentric contractions. It was proposed that accompanying a rise in passive tension would be an increase in work absorbed by the passive muscle, reflecting the energy absorbed by actively cycling cross-bridges within regions of contracture.

In a second series of experiments, also on cat MG, another approach was used to test the contracture hypothesis. It involved recording the responses of Golgi tendon organs, the muscle's tension sensors, during passive movements before and after eccentric contractions. Tendon organs are located at the myotendinous junction, where they lie 'in series' with muscle fibres and signal the tension produced by the muscle during both active contractions and passive movements (see Proske, 1981). Here, it was hypothesised that following eccentric contractions, a rise in whole muscle passive tension, due to regions of contracture in some muscle fibres, would lead to a corresponding increase in tendon organ responses during a passive stretch.

Methods

The work described in this chapter was taken from two series of experiments, carried out on a total of 16 cats of both sexes weighing between 3 and 7.9 kg. The first study (n=5) investigated changes in passive mechanical properties after a series of eccentric contractions. In the second study (n=11), recordings from Golgi tendon organ afferent fibres were made during passive tension measurements before and after eccentric contractions.

For both studies, animals were anaesthetised and surgical procedures were carried out in the same way as described in Chapter 2. In the second study, though, during the laminectomy, both the ventral and dorsal roots of L7 to S1 were exposed and cut as they entered the spinal cord, so that recordings from tendon organ afferents could be made. All experiments were performed on the medial gastrocnemius muscle (MG).

Study 1: Experimental procedure

Active length-tension measurements

The active length-tension relationship for MG was measured over a range of muscle lengths in the same way as described in Chapter 2. However, in 2 of the 5 experiments, active tension generated by the whole muscle exceeded the limits of the servomotor, so that length-tension measurements on part of the muscle were carried out.

The measured optimum length (L_{opt}) before the eccentric contractions was used as the reference length for active and passive tension measurements both before and after eccentric contractions. For active length-tension measurements, the optimum length, as calculated from a Gaussian curve fitted to tension values above 75% of the maximum tension, was used measure the shift in optimum length after eccentric contractions. In general, the fitted optimum length before the eccentric contractions was very close to L_{opt} (see Figures 4.2 & 4.3). Peak active tension, before and after the eccentric contractions, was also calculated from the fitted curve.

Passive length-tension measurements

The passive length-tension relationship was determined in two ways (Figure 4.1). The first method (static passive tension) involved shortening the muscle to L_{opt}-10 mm. At this length, the passive muscle lay quite slack and tension was close to zero.

Figure 4.1 *Static and dynamic passive length-tension measurements.*

(A) An example of static (top) and dynamic (bottom) passive length-tension measurements taken before a period of eccentric contractions. In each panel, the upper traces are tension, and the lower traces indicate muscle length. Static tension measurements were made at 2 mm intervals, over the muscle length range from L_{opt}-10 mm to L_{opt}+10 mm. For dynamic tension measurements, the muscle was slowly stretched and then shortened over the same length range as for static measurements. (B) Passive length-tension curves for static (filled circles) and dynamic (line) measurements shown in (A). The dynamic length-tension curve was taken from the lengthening phase of the movement, shown as a continuous trace of tension against length.

A

Static

10
N
0

10 mm

50 s

Dynamic

10
N
0

10 mm

10 s

B

Passive tension (N)

Length relative to L$_{opt}$ (mm)
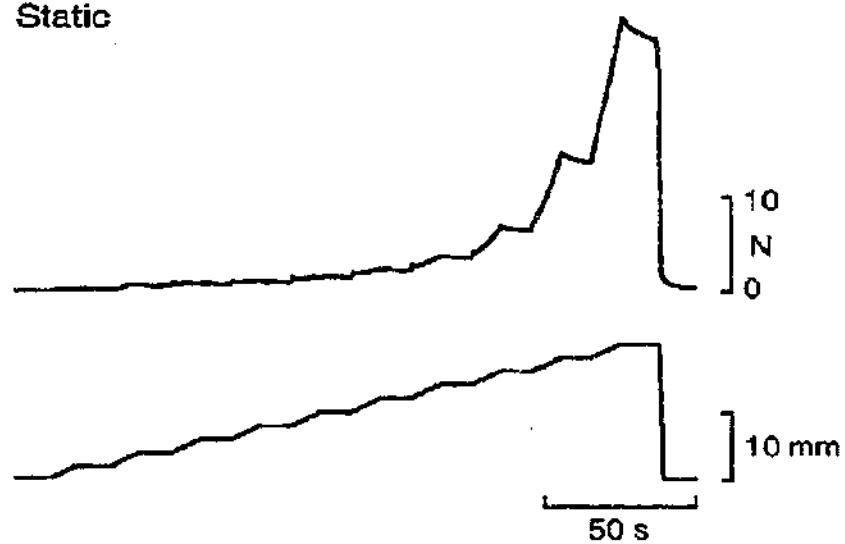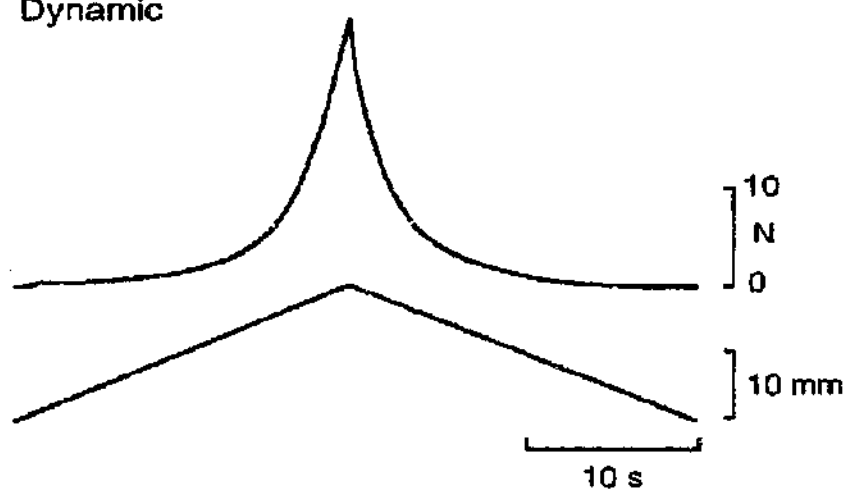
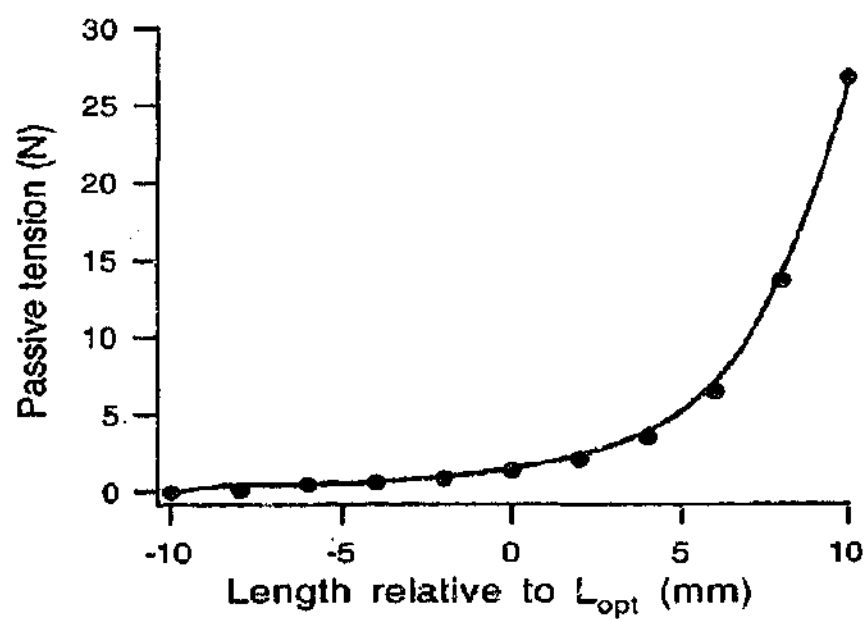The muscle was then slowly stretched (0.2 mm s^{-1}) by 2 mm and held at that length for 10 s. This procedure was repeated over a range of muscle lengths from L_{opt}-10 mm to L_{opt}+10 mm. At each length, passive tension was measured by averaging tension over the final 1 s of the 10 s hold period. The second method (dynamic passive tension) was used as both a measure of passive tension and work absorbed by the passive muscle (see Figure 4.5 B). Here, the muscle was shortened to L_{opt}-10 mm and a conditioning isometric contraction (15pps, 100ms duration) of the whole muscle was performed. This ensured that passive properties were in a defined, mechanical state. The muscle then underwent a series of 5 successive stretch-shortening cycles at a rate of 1 mm s^{-1} over a 20 mm length range. Following the eccentric contractions, relative changes to the dynamic passive length-tension relationship were calculated using an iterative analysis procedure. The tension measured at each muscle length after the eccentric contractions, was divided by the tension measured at the corresponding muscle length before the eccentric contractions, and then expressed as a percentage. This provided a continuous trace of the change in passive tension plotted against length.

The work absorbed during each stretch-shortening cycle was calculated from the area contained within a passive length-tension loop (see Figure 4.8). This value was then expressed as a percentage of the work done on the muscle, which was calculated from the area underneath the lengthening phase of each cycle.

Eccentric contractions

The muscle was subjected to 150 eccentric contractions, spaced 20 s apart. Each eccentric contraction consisted of a 400 ms tetanic contraction at 80 pps, with a 6 mm stretch at 50 mm s^{-1}, starting 150 ms after the onset of stimulation. The stretch covered a length range that lay symmetrically about L_{opt}, that is, it began at L_{opt}-3 mm and finished at L_{opt}+3 mm (Figure 4.2).

Study 2: Experimental procedure

At the start of each experiment, the dorsal roots were divided into a number of filaments, each containing a single functional Golgi tendon organ afferent fibre. Tendon organs are served by group 1b afferent nerve fibres, which conduct at a velocity between 70 to 120 m s^{-1} (Hunt, 1954). Afferent conduction velocity was measured by dividing the conduction distance, the path length between stimulating

Figure 4.2 *Active length-tension relation and eccentric contractions.*

(A) An example of an active length-tension curve for MG measured before eccentric contractions. The thin line is a Gaussian curve fitted to the measured length-tension values (open circles), and the thick line indicates the length range over which the muscle was stretched during the eccentric contractions. The arrow indicates the optimum length for peak tension, as determined by the computer fit. This value was very close to the measured optimum length (L_{opt}). (B) Examples of eccentric contractions of MG for one experiment. Upper traces, tension, middle trace, length, and bottom trace, period of stimulation at 80 pps. Tension measurements are shown for the 1st (blue), 10th (red) and 150th (green) eccentric contractions.

A

Active tension (N) vs Length relative to L_{opt} (mm)

B

50 N

5 mm

0.1 s
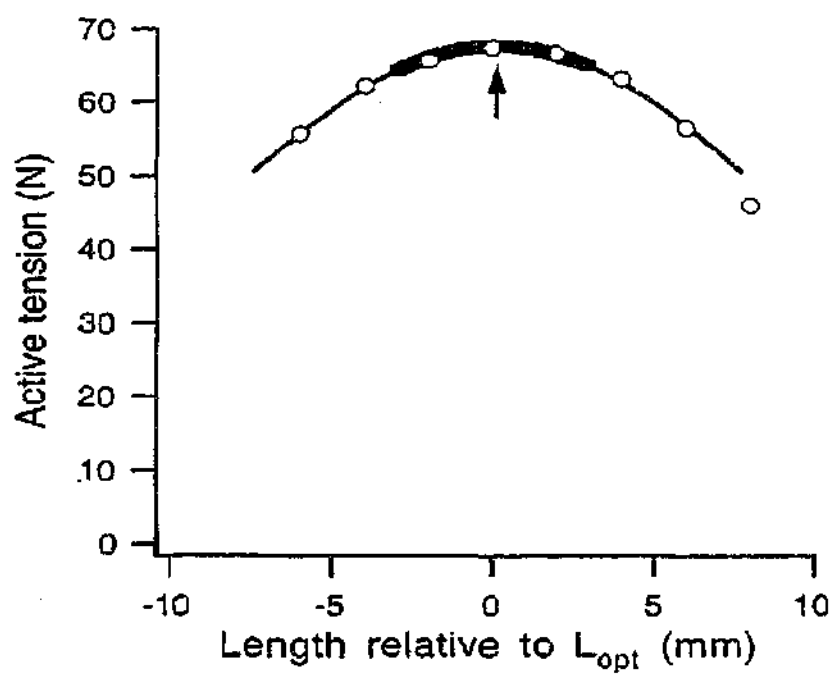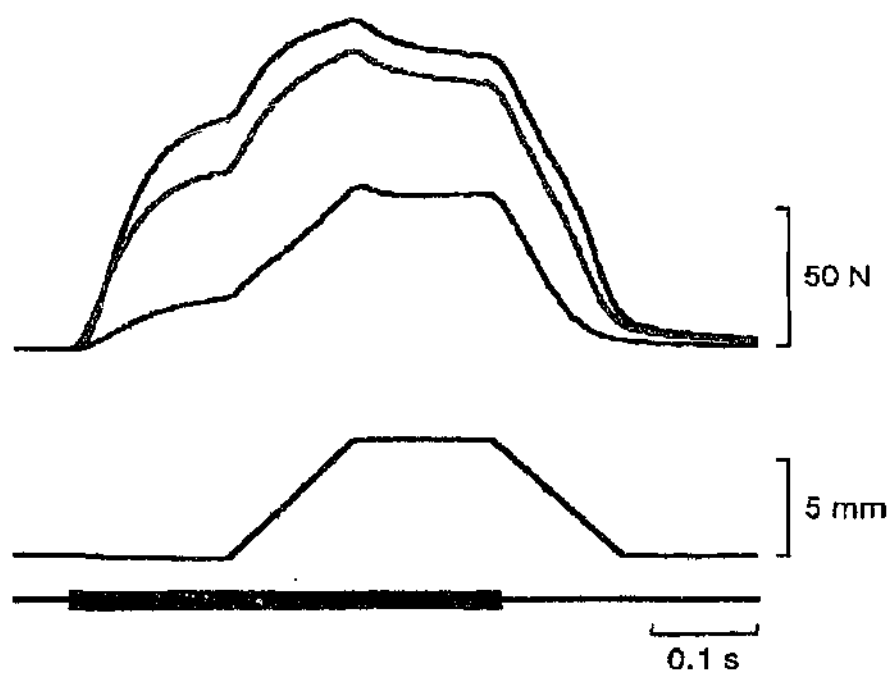
and recording sites, by the latency of the evoked, all or none action potential in the filament of dorsal root in response to muscle nerve stimulation. Tendon organ singularity was determined by the regular pattern of discharge during passive stretch and contraction (Figure 4.3). Tendon organs were distinguished from muscle spindles by their 'in series' responses during muscle contraction. That is, as tension increased during a muscle twitch, tendon organ firing rate became progressively greater (see Figure 4.3), whereas muscle spindles became silent. The dorsal root filaments, typically 4 to 6 per experiment, were mounted on a multiple, monopolar electrode array. Nerve impulses were processed via an A-D converter (PCI-MIO-16E-4, National Instruments, Austin, TX, USA) and custom-designed software written in the program Igor Pro (Wavemetrics, Lake Oswego, OR, USA).

The experimental protocol was essentially the same as that described for Study 1. However, there were a few modifications. After the active length-tension curve had been measured, passive length-tension measurements were made, while at the same time the discharge rate of the Golgi tendon organ afferents was recorded. Passive tension was measured during stretch-shortening movements, as described above. Here, the lengthening phase of the passive movement was of most interest, as it was predicted that after the eccentric contractions, the tendon organs would commence firing at a shorter length during passive stretch and would show a greater response during the stretch. The eccentric contractions were carried out in the same way as for Study 1, however in some experiments, the initial length before the stretch was up to 6 mm shorter than L_{opt}. Also, the number of eccentric contractions varied between 50 and 150.

Data Analysis

For both studies, all parameters were calculated as the mean $\pm$ S.E.M., with the significance level set at $P < 0.05$. A two or three-factor ANOVA with interactions was used to test for significant changes to variables after the eccentric contractions. The common factors for each test were animal and condition (before or after eccentric contractions). Where the ANOVA was significant, LSD *post hoc* tests were to identify individual points of significance. The statistical program used was Data Desk (Ithaca, NY, USA).

Figure 4.3 *Determination of tendon organ singularity during passive stretch and contraction.*

Raw records showing typical responses of a tendon organ during (A) a passive stretch and (B) an isometric twitch. In both (A) and (B), tension is the red trace, while the tendon organ discharge response, as recorded from a dorsal root filament, is shown by the second top blue trace. This was then used to calculate the instantaneous discharge rate of the receptor (top blue traces). In (A), the black trace represents the length change during the passive stretch, which was 5 mm in amplitude at a velocity of 1 mm s^{-1}. The initial length before the stretch and isometric twitch was L_{opt}.

A

50
imp s⁻¹
0

200 mV

2 N

5 mm

5 s

B

200
imp s⁻¹
0

2 μV

20 N

50 ms
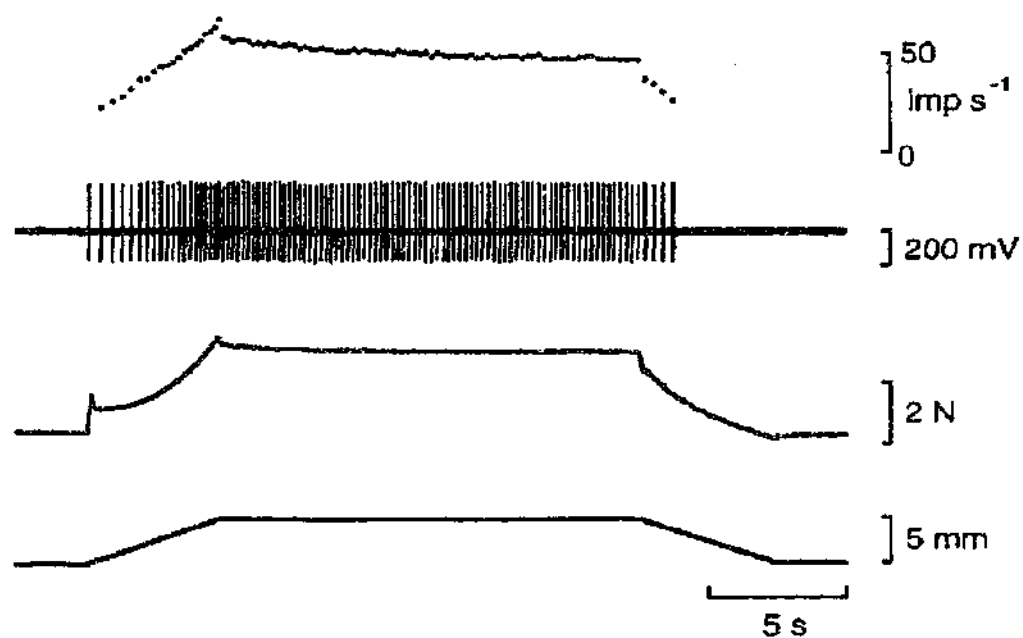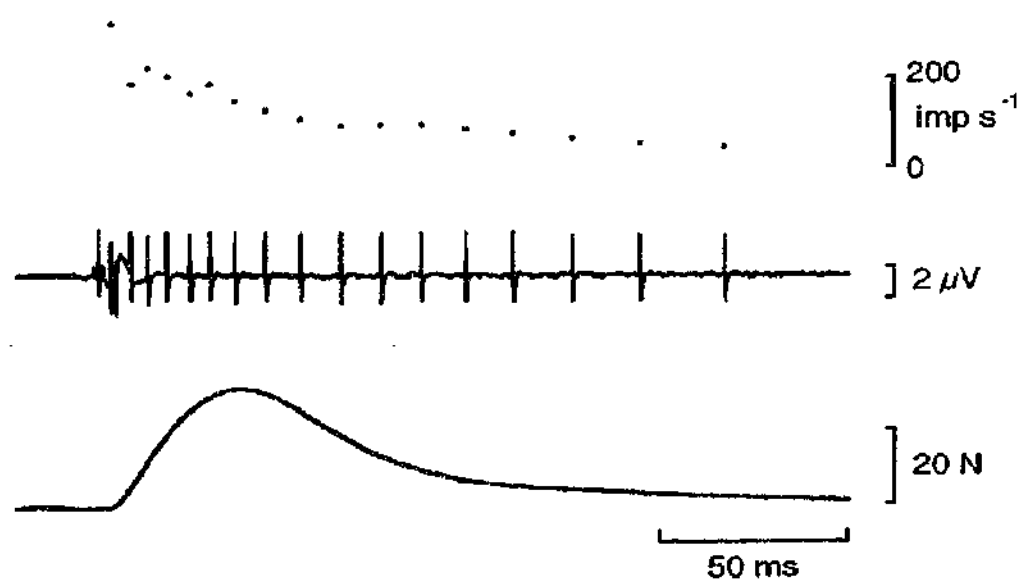
Results

Study 1

The main purpose of these experiments was to investigate changes to the passive mechanical properties of MG after a series of eccentric contractions. Muscle damage was also assessed by examining changes to active length-tension properties. At the end of the series of 150 eccentric contractions, the isometric tension measured during the final contraction before stretch onset, had fallen by 75.3% ($\pm$ 1.2) of the corresponding value measured during the first eccentric contraction (see Figure 4.2). This value, however, represented an overestimate of the tension drop since it did not take into account the shift of the active length-tension relation to longer muscle lengths, as shown for one experiment in Figure 4.4. The mean shift in optimum length was 3.85 mm ($\pm$ 0.4) and, after moving to the new optimum length, isometric tension dropped by 58% ($\pm$ 4.8) of its value before the eccentric contractions.

Static and dynamic passive length-tension measurements were made before and immediately after eccentric contractions, over the same range of muscle lengths (L_{opt}-10 mm to L_{opt}+10 mm), where L_{opt} refers to the measured pre-eccentric contractions optimum length (Figure 4.5 A). Here it can be seen that passive tension increased over most of the length range after the eccentric contractions. An example of the amount by which passive tension increased at each length is shown in Figure 4.6. For this experiment, the absolute increase in passive tension became greater with length, to a maximum of about 15 N for dynamic lengthening movements. However, when passive tension measured after eccentric contractions was plotted as a percent increase above that measured before the contractions, the differences were found to be length-dependent; increasing with muscle length to a maximum at about L_{opt}+4 mm before decreasing but still remaining above control values at longer lengths (Figure 4.6 B). This was a consistent finding in all five experiments. The magnitude of the rise in passive tension was slightly lower for static compared to dynamic lengthening measurements, but maximum values for both measurements occurred over a similar range of muscle lengths, between L_{opt} and L_{opt}+4 mm. When values were pooled for the five experiments, the mean maximum increase in passive tension after the eccentric contractions was 162.5% ($\pm$ 21.9) for static measurements, and 169.3% ($\pm$ 25.1) for dynamic lengthening measurements. Statistical analysis

Figure 4.4 *Active length-tension curves before and after eccentric contractions.*

Active length-tension curves of MG before (blue) and immediately after (red) 150 eccentric contractions. Tension (filled circles) is plotted against muscle length, relative to the optimum length measured before the eccentric contractions (L_{opt}). Gaussian curves (lines) are fitted to the measured values, and the arrows indicate the optimum length for peak tension as determined by the fit.
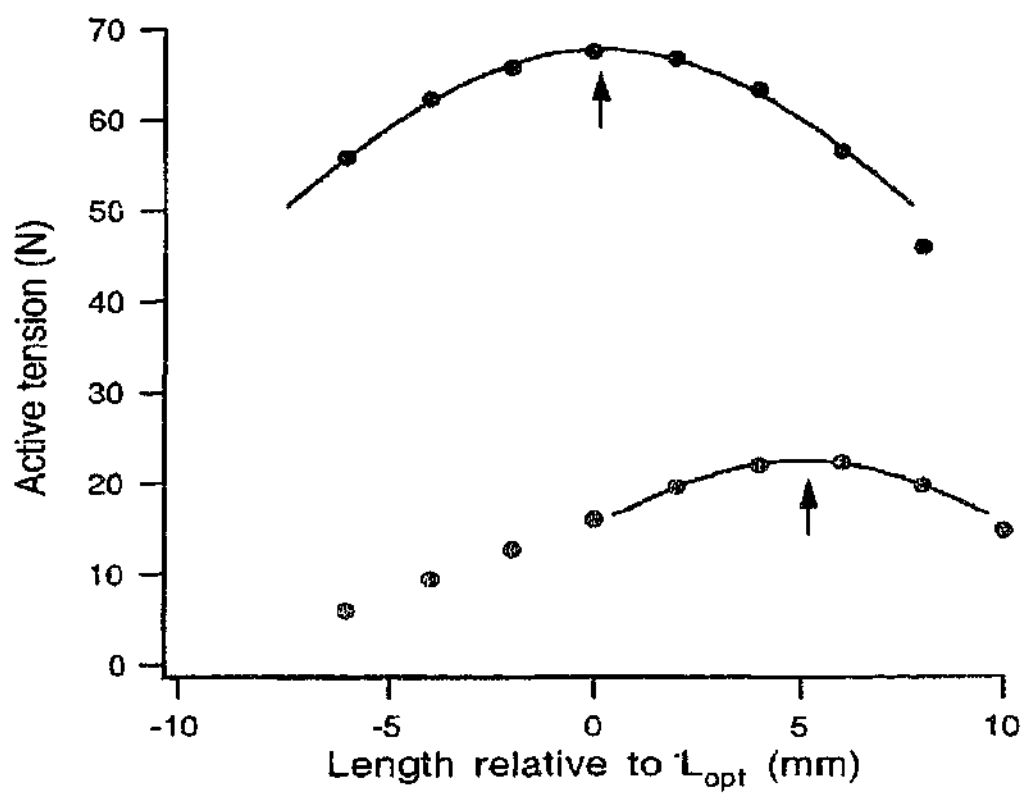
Figure 4.5 *Passive length-tension measurements before and after eccentric contractions.*

(A) Passive length-tension curves as measured before (blue) and after (red) 150 eccentric contractions using the static method of measurement. (B) Passive length-tension curves for dynamic lengthening (continuous lines) and shortening movements (dotted lines), as measured before (blue) and after (red) 150 eccentric contractions. For both (A) and (B), tension is plotted against muscle length relative to the optimum length measured before the eccentric contractions (L_{opt}).

A

B

Length relative to L$_{opt}$ (mm)
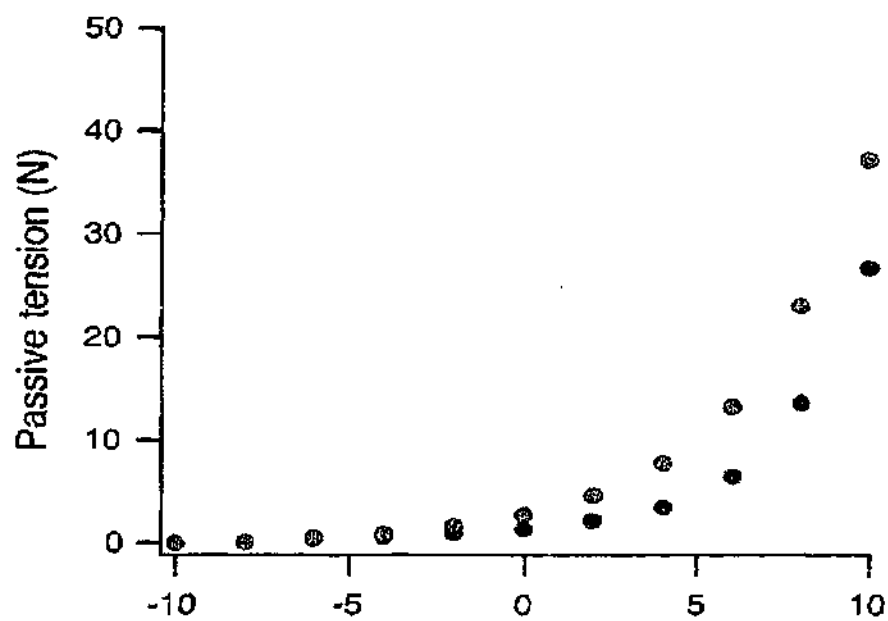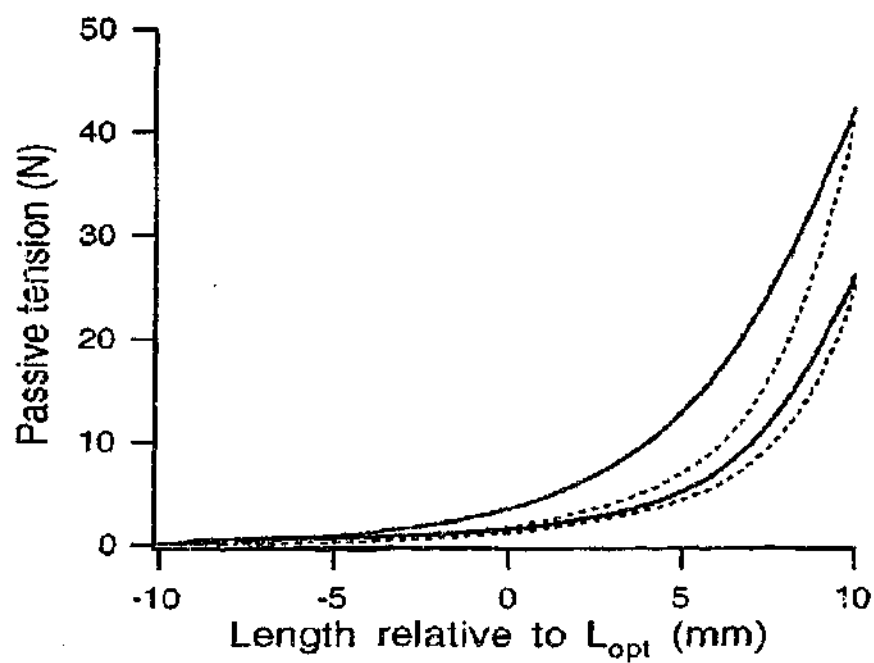
Figure 4.6 *Length-dependence of the increase in passive tension after eccentric contractions.*

The increase in passive tension after eccentric contractions as a function of muscle length, expressed as the absolute increase (A) and the percent increase (B) above the tension measured before the eccentric contractions. Filled symbols, static measurements; continuous line, dynamic lengthening measurements; dashed line, dynamic shortening movements.

A

Passive tension increase (N)

B

Passive tension increase (%)

Length relative to L_opt (mm)
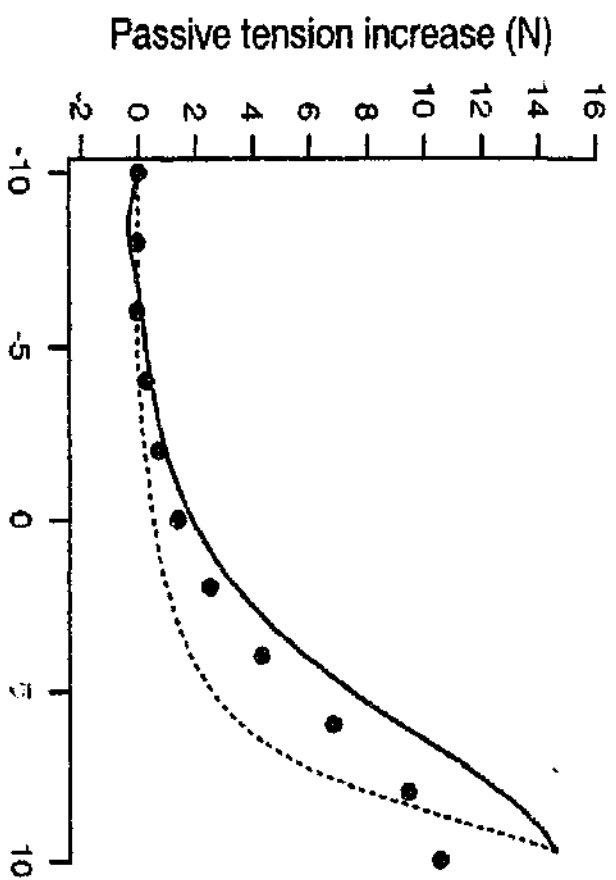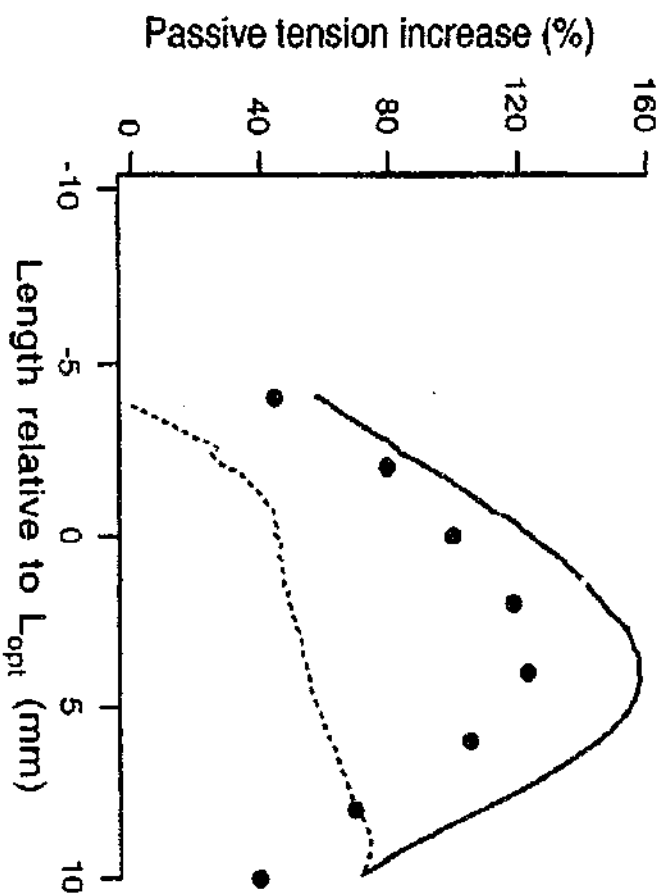
(three-factor ANOVA) performed on static measurements showed that there was a significant increase in passive tension at all muscle lengths from L_{opt} to $L_{opt}+10$ mm ($P < 0.05$, LSD *post hoc* test). An interesting finding was that the percent increase in passive tension after eccentric contractions, measured during dynamic shortening movements, was significantly less, with values reaching a maximum at muscle lengths close to $L_{opt}+10$ mm and then progressively decreasing at shorter lengths (Figure 4.6 B, dotted line). Here the mean maximum rise in passive tension was 75.7% ($\pm$ 9.8). The differences in passive tension between lengthening and shortening movements were proposed to reflect the effects of history-dependent changes to passive tension as a result of muscle damage from the eccentric contractions (see Discussion).

While passive tension was higher after the eccentric contractions over most of the measured length range, this was not the case at short muscle lengths, where the tendon lay slack and passive tension was close to zero. Before the eccentric contractions, the tension response to the passive stretch was biphasic; initially increasing steeply, attributed to the short-range component (see Chapter 2), followed by a more gradual change. In comparison, after the eccentric contractions, the initial increase was less steep, but the subsequent slope was much steeper than before the eccentric contractions. In addition, after the eccentric contractions, the initial rise in tension during the first stretch was delayed, so that passive tension was found to be lower over the first 2 to 3 mm of the length change than beforehand (Figure 4.7). It was thought that this might be attributable to the presence of disrupted, overextended sarcomeres in damaged muscle fibres, leading to an increase in whole muscle compliance (see Discussion).

Further analysis was performed to explore this idea. It was decided to measure the length at which passive tension was first generated during the slow stretch, both before and after the eccentric contractions. The method of Close (1981) was used to calculate these values, as it provided a means of determining the length at which passive tension began to rise, independent of differences in the shapes of the tension traces (see Figure 4.7). It involved performing a series of successive linear regression analyses on the tension trace, starting at $L_{opt}-10$ mm and gradually moving towards longer lengths, until a point was reached where the tension trace was no longer linear, and it began to rise more steeply (short-range component). This point,

Figure 4.7 *The change in onset length of passive tension.*

(A) Passive length-tension curves measured during dynamic lengthening movements before (blue) and after (red) eccentric contractions. The thick lines are from stretch cycle 1 and the thin lines are from cycle 2. Note that the two tension traces overlap for measurements made before the eccentric contractions (B) An expanded view of the tension traces during lengthening in the region enclosed by the dotted line in (A). The onset length of passive tension (see text for details) was measured before (blue) and after (red) eccentric contractions, for cycle 1 (arrows pointing down) and cycle 2 (arrows pointing up).
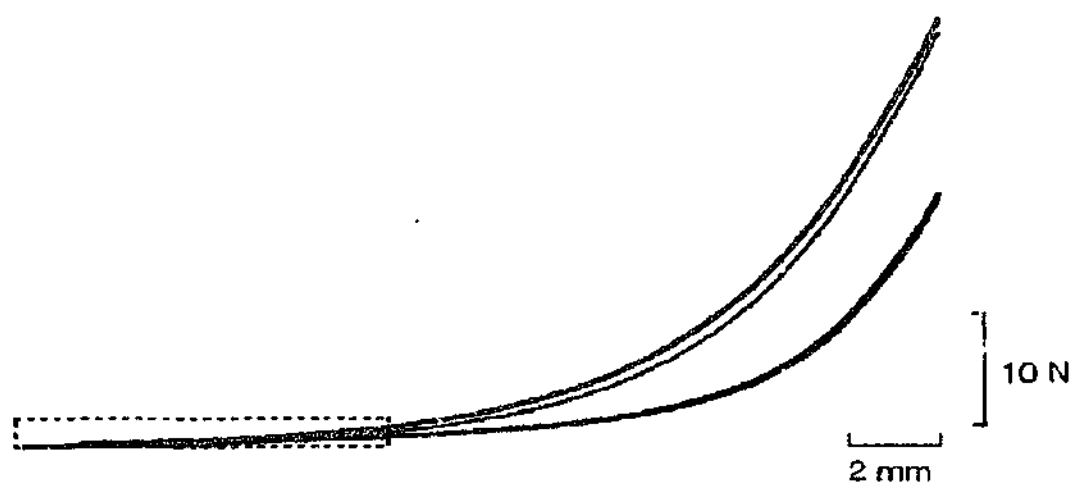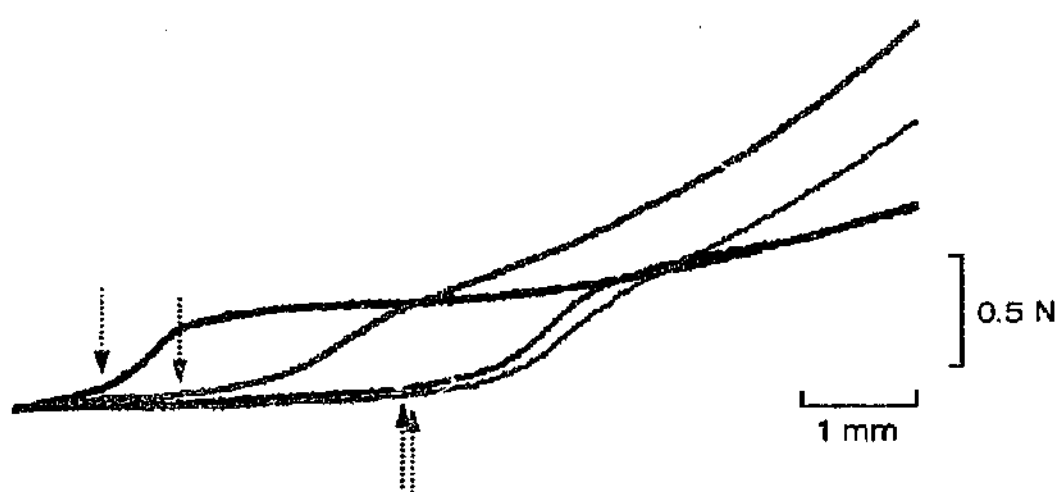
called the onset length of passive tension, was determined by the standard error of the slope of the fitted line reaching a minimum. The onset length was determined for both stretch cycles 1 and 2 (Figure 4.7, arrows). The mean onset length was L_{opt}-9.14 mm (± 0.16) before and L_{opt}-8.2 mm (± 0.24) after the eccentric contractions. This equated to a shift to longer lengths of close to 1 mm after the eccentric contractions. For stretch cycle 2, the onset length shifted further both before and after the eccentric contractions, however the difference between values was less (see Figure 4.7). In two experiments, the onset length after the eccentric contractions was at a length shorter than before. The mean value for the 5 experiments was L_{opt}-7.2 mm (± 0.42) before the eccentric contractions and L_{opt}-6.8 mm (± 0.40) afterwards, representing a difference of 0.4 mm.

The amount of work absorbed by the passive muscle was measured during each of 5 consecutive stretch-shortening cycles, as shown for one experiment in Figure 4.8 A. Work absorption was calculated as the area contained within each loop (Figure 4.8 B). Measurement of work absorption enabled changes in passive tension over the full range of muscle lengths to be calculated. In order to account for the fact that passive tension had increased after the eccentric contractions, values of work absorption were expressed as a percentage of the work done on the passive muscle, which was calculated from the area underneath the lengthening phase of each cycle. Before the eccentric contractions, the muscle absorbed an average of 21.4% (± 2.2) of the work done on it during the first stretch-shortening cycle. This value increased to 34.8% (± 2.1) immediately after the eccentric contractions (Figure 4.9). Work absorption measured during the second and subsequent cycles fell, both before and after the eccentric contractions. The largest reduction in work absorption occurred between cycles 1 and 2, with a greater mean drop occurring after (4.5% ± 0.6) than before (1.84% ± 0.6) eccentric contractions. For each of the five cycles, work absorption was significantly higher after the eccentric contractions compared with that measured beforehand ($P < 0.01$, three-factor ANOVA).

Study 2

The changes in active length-tension properties after eccentric contractions for Study 2 were similar to those for Study 1. The mean shift in optimum length was 4.4 mm (± 0.3) and peak active tension dropped by 52% (± 3.0). The mean maximum

Figure 4.8 *Work absorption during passive stretch-shortening movements.*

(A) An example of the tension (upper traces) and length (lower trace) recorded during a series of 5 successive passive stretch-shortening cycles before (blue) and after (red) 150 eccentric contractions. (B) Plots of muscle length against tension for the first stretch-shortening movement shown in (A) before (blue) and after (red) eccentric contractions. The arrows indicate the lengthening phase of the movement in each case. Work absorption was measured from the area contained within these length-tension figures.

A

20 N

20 mm

20 s

B

10 N

5 mm
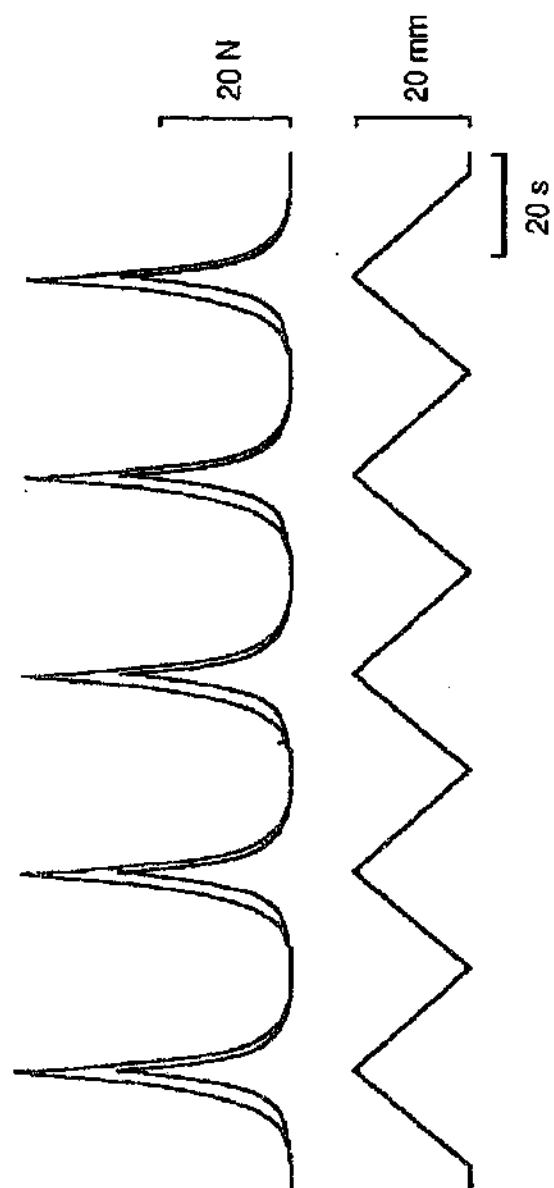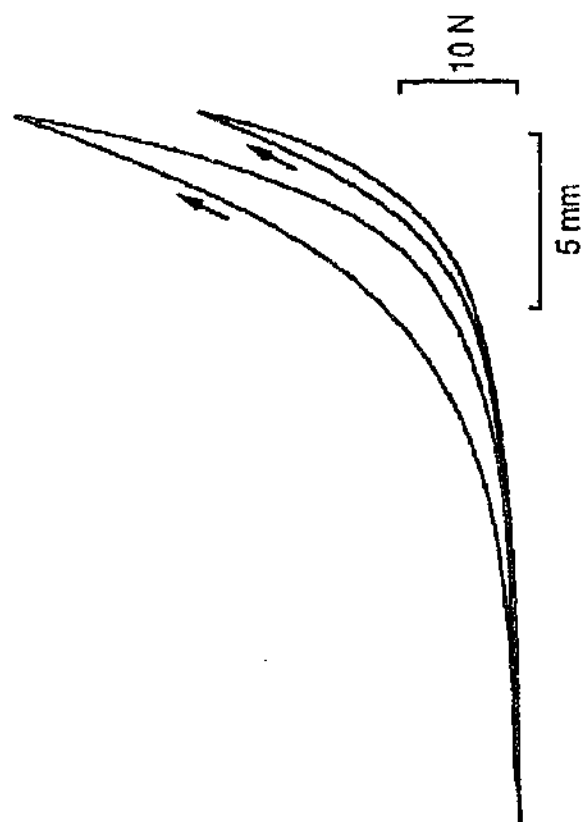
Figure 4.9 *Changes in work absorption after eccentric contractions.*

The mean ($\pm$ S.E.M.) work absorbed by the muscle during each of 5 passive stretch-shortening cycles before (blue) and after (red) eccentric contractions. For each cycle, work absorption was measured from the area contained within a length-tension figure and expressed as a percentage of the work done on the muscle. Asterisks indicate a significant difference between pre and post-eccentric contraction values ($P < 0.05$, LSD *post hoc* test).

Work absorption (% of work done)
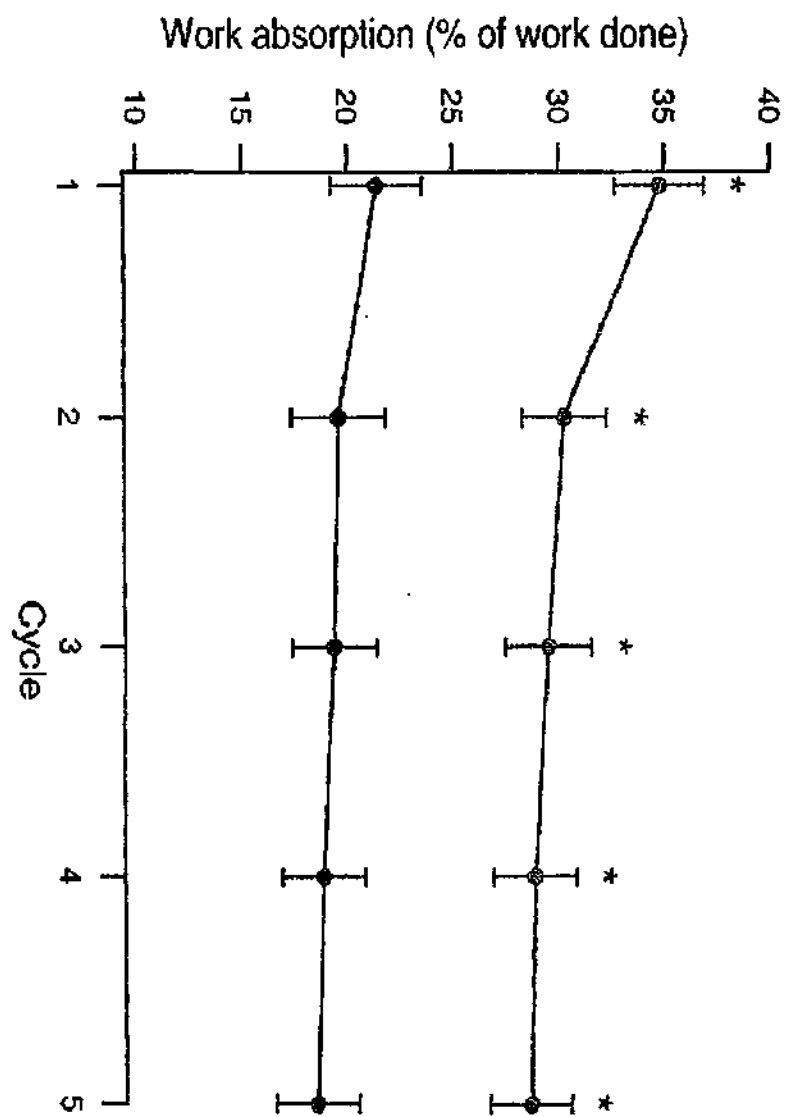
increase in passive tension for dynamic lengthening movements was 148.4% ($\pm$ 28.3), which was similar to that found in Study 1.

The responses of tendon organs were recorded from their afferent fibres, during passive movements before and after the eccentric contractions (Figure 4.10). For almost all tendon organs, firing commenced at a shorter muscle length but at a similar level of tension after the eccentric contractions as before (Figure 4.10, arrows). It can also be seen that after the eccentric contractions, the tendon organ maintained a higher discharge rate throughout the stretch, accompanying the rise in passive tension.

For each tendon organ, the firing rate measured during passive movements was plotted against both muscle length and tension (Figure 4.11). This provided a means of measuring the length and tension thresholds, that is, the length and tension at which the tendon organ began to respond to the passive stretch. Of the 40 tendon organs, where the whole muscle underwent eccentric contractions, all but 2 showed a shift in length threshold to shorter muscle lengths (Figure 4.12), with values ranging between 0.3 mm to 5.7 mm. One tendon organ showed no change while another increased its threshold to longer lengths by 0.3 mm. The mean change in tendon organ length threshold was 2.4 mm ($\pm$ 0.2) in the direction of shorter muscle lengths. This change was found to be significant ($P < 0.02$, two-factor ANOVA).

The tension thresholds measured before the eccentric contractions ranged between 0.1 N and 12.5 N, with a mean of 4.0 N ($\pm$ 0.5). In order to account for differences in muscle size between animals, tension thresholds for each experiment were normalised to the peak passive tension, measured at $L_{opt}+10$ mm. After the eccentric contractions, there was no consistent change in tension threshold, and the changes were small, less than 10% of the peak passive tension (Figure 4.12). The mean change in tension threshold was an increase of 0.6% ($\pm$ 0.6), and this was found to be not significant.

In three experiments, it was decided to record the responses of a tendon organ in which the muscle fibres acting on the receptor did not undergo eccentric contractions. These measurements were carried out in order to test the hypothesis that only tendon organs supplied by muscle fibres damaged by the eccentric contractions would show a change in length threshold. The procedure involved

Figure 4.10 *Response of a tendon organ during a passive stretch-shortening movement.*

An example of the response of a tendon organ during a passive stretch-shortening movement, before (blue traces) and after (red traces) a series of eccentric contractions. The top traces show the firing rate of the tendon organ, shown as an instantaneous frequency display. The middle traces show the measured passive tension and the bottom trace represents the length change. The arrows indicate the lengths at which the tendon organ began discharging before and after the eccentric contractions.

100
imp s⁻¹
0

10 N

20 mm

10 s
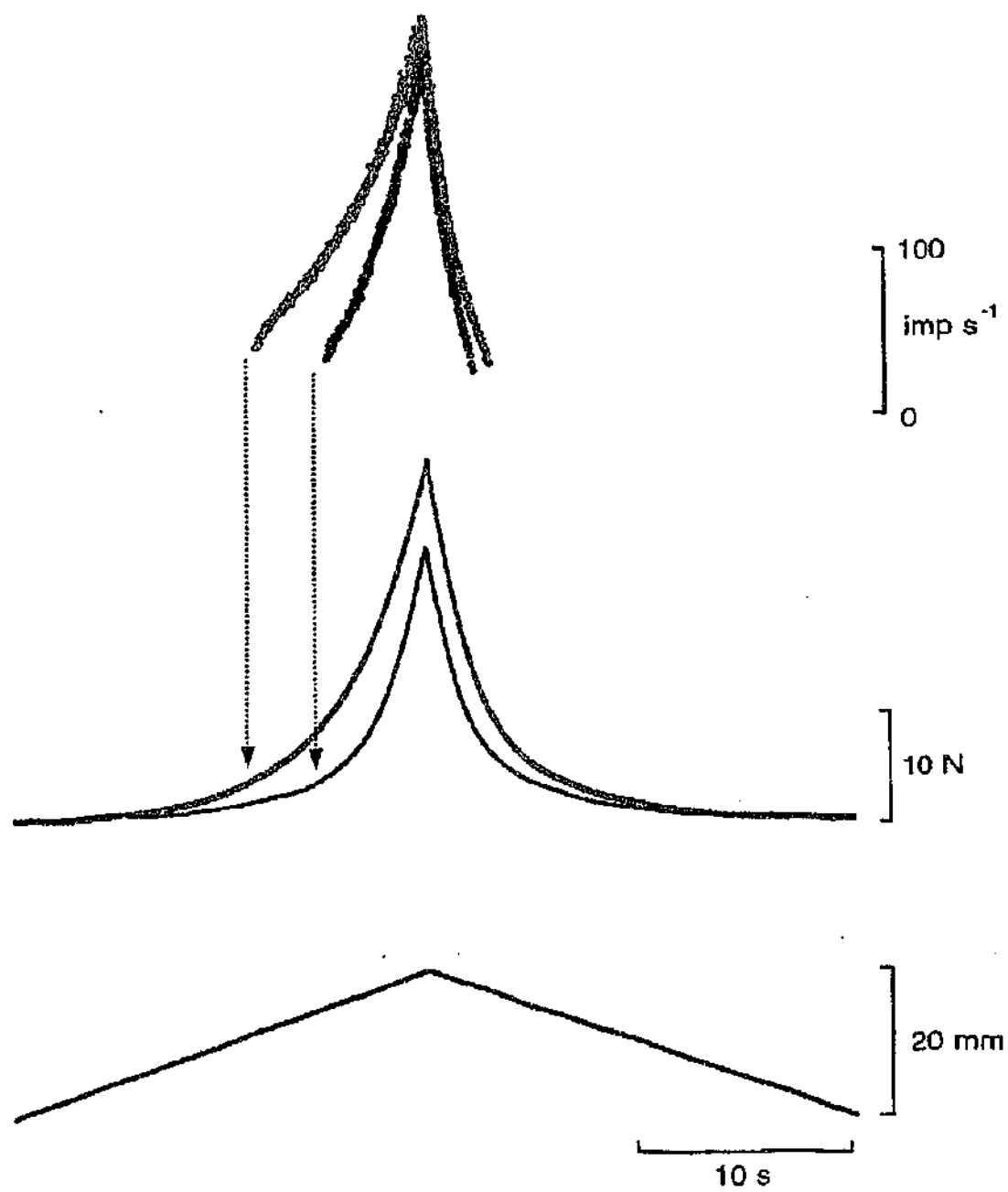
Figure 4.11 *Impulse frequency versus length and tension relations for a tendon organ.*

An example of the impulse frequency of a tendon organ plc ted against muscle length (A) and passive tension (B) during stretch-shortening movements before (blue) and after (red) the eccentric contractions. Note that after the eccentric contractions the length threshold for passive stretch had shifted by about 4 mm towards shorter muscle lengths (arrows) but there was little change in the tension threshold (arrows).

A

Impulse frequency (imp s^{-1})

Length relative to L$_{opt}$ (mm)

B

Impulse frequency (imp s^{-1})

Passive tension (N)
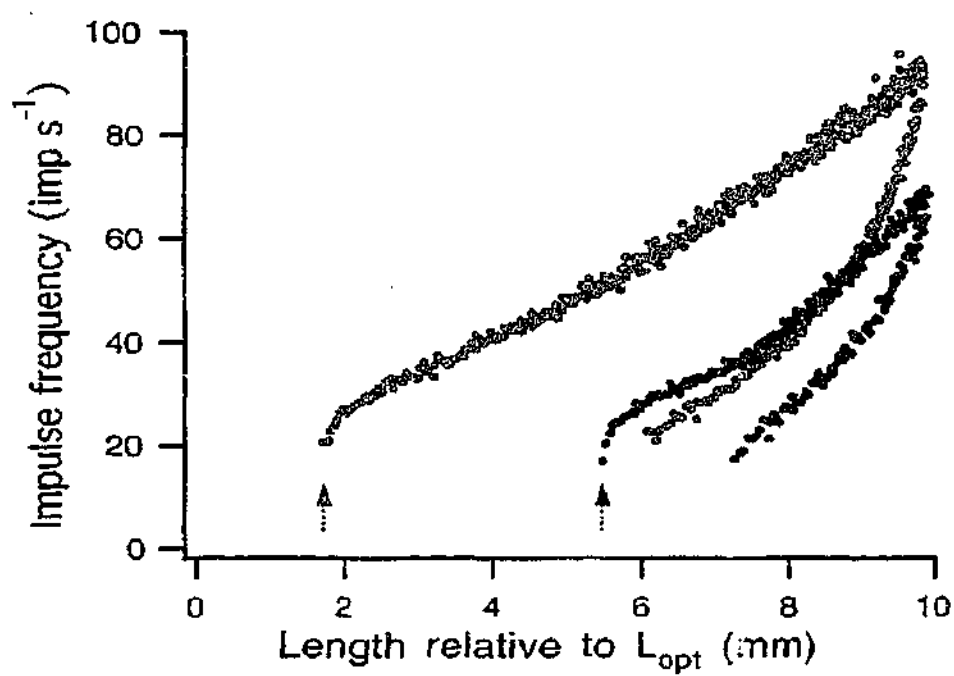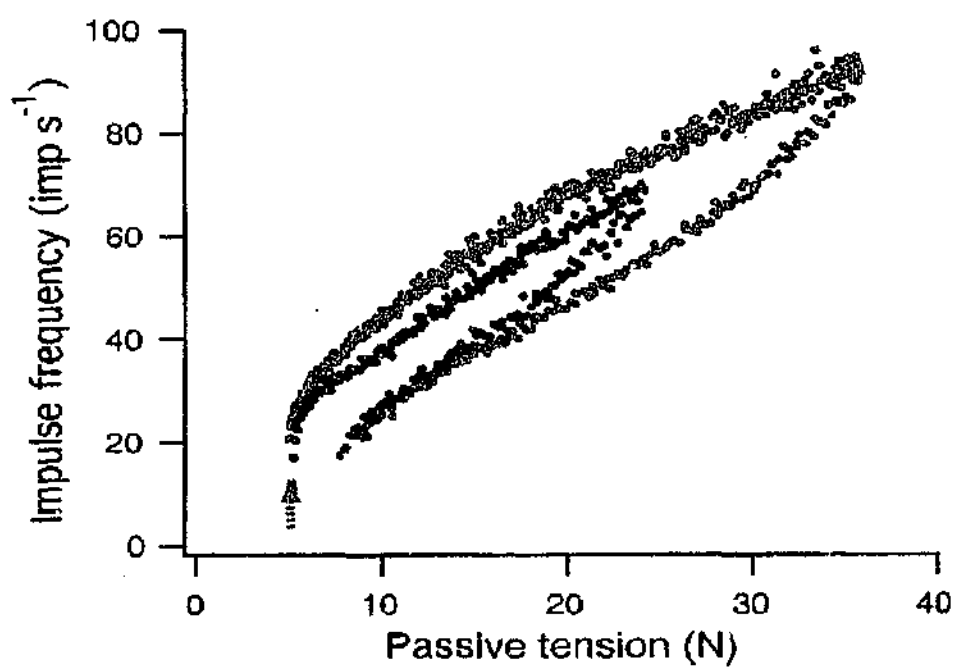
splitting the ventral roots repeatedly into fine filaments, and noting which filaments, when stimulated, produced a strong response from the receptor. This process enabled any motor units with muscle fibres inserting into the tendon organ to be identified and separated out, so that they were not stimulated during the eccentric contractions. The three tendon organs used for these experiments showed essentially no change in length threshold after the eccentric contractions (Figure 4.12, filled circles).

During a passive stretch, the sensitivity of each tendon organ, that is, the relationship between tension and tendon organ discharge rate, was calculated before and after the eccentric contractions. The sensitivity was measured as the slope of a straight line fitted to the plot of tendon organ firing rate versus tension (an example of which is shown in Figure 4.11 B), over the region of increasing tension from threshold up to the peak tension measured before the eccentric contractions. The sensitivities of each receptor before and after eccentric contractions are shown in Figure 4.13. Overall, there was a small reduction in sensitivity with values dropping from 2.93 impulses s^{-1} N^{-1} ($\pm$ 0.15) before the eccentric contractions, to 2.58 impulses s^{-1} N^{-1} ($\pm$ 0.11) afterwards. To determine whether this change was significant, the ratio of the sensitivity measured after the eccentric contractions to that before the eccentric contractions was calculated, with values ranging between 0.44 and 1.54. The mean ratio was 0.91 ($\pm$ 0.03), suggesting a small reduction in sensitivity after the eccentric contractions, which was close to being statistically significant ($P = 0.06$, two-factor ANOVA).

Figure 4.12 *Changes in tendon organ length and tension thresholds after eccentric contractions.*

The change in length threshold is plotted against the change in tension threshold after the eccentric contractions for each of 40 tendon organs (open circles). Values for each experiment are shown by a different colour. A negative length threshold indicates that the tendon organ commenced firing at a shorter muscle length after the eccentric contractions. The tension threshold values have been normalised to the peak tension recorded during the passive stretch for each experiment, because of differences in passive length-tension relations between animals. A negative tension threshold indicates that the tendon organ began firing at a lower level of passive tension after the eccentric contractions. The filled circles show threshold changes for three tendon organs, where the motor units with a specific excitatory action on the tendon organ had not undergone eccentric contractions. The mean (± S.E.M.) length and tension threshold for the 40 receptors is shown by the open square. The dotted lines indicate zero change in tension and length thresholds.
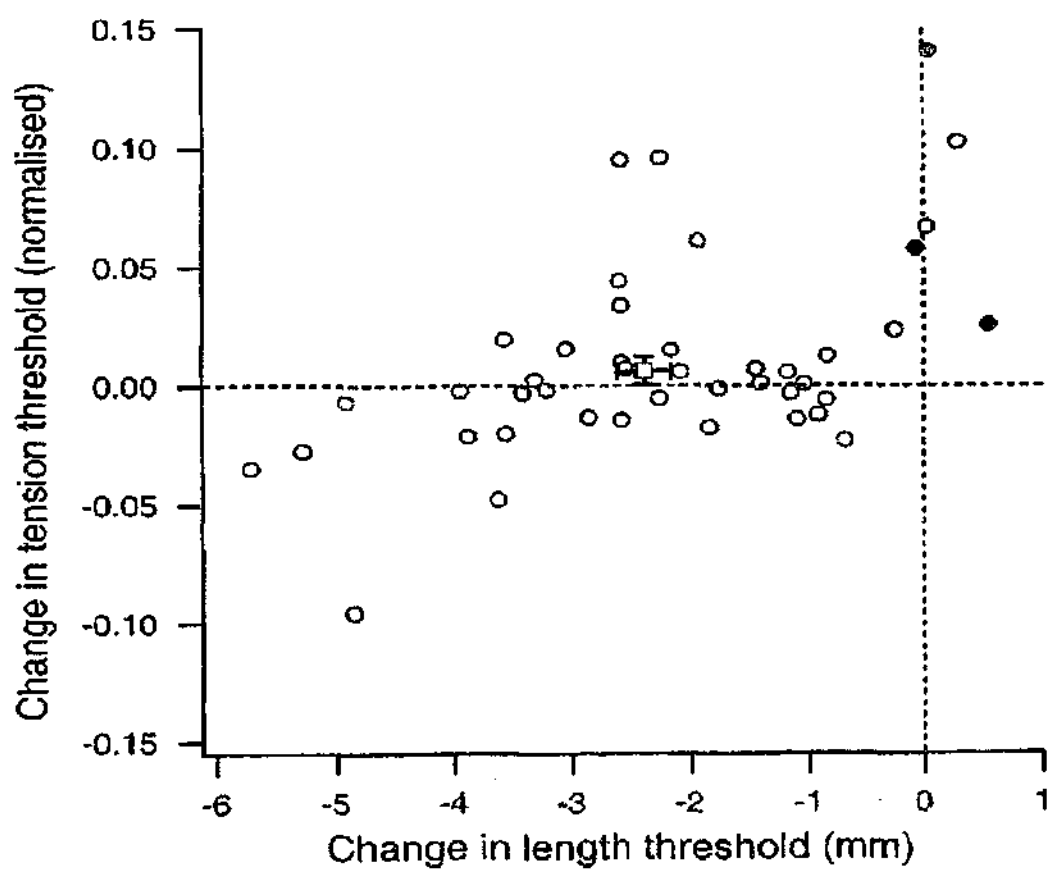
Figure 4.13 *Tendon organ sensitivity during passive stretch, before and after eccentric contractions.*

The sensitivity of tendon organs during passive stretch (open circles) was estimated from the relationship between impulse frequency and tension. This was measured as the slope of a straight line fitted to plots, such as the one shown in Figure 4.11 B. The sensitivities for 40 tendon organs (open circles) are shown before and after eccentric contractions, with each colour representing values from one experiment. The filled circles show values for three tendon organs where the motor units with a specific excitatory action on the tendon organ were not subjected to eccentric contractions. A line of proportionality is shown (dotted line).

Discussion

The experiments carried out in this chapter examined the effects of a series of eccentric contractions on changes to the passive mechanical properties of mammalian muscle. It was hypothesised that sarcomere non-uniformities would lead to overextension and disruption of some sarcomeres during eccentric contractions, and that during repeated eccentric contractions, the regions of disruption would spread until membranous structures became damaged and there was a disturbance of calcium homeostasis in the fibre (Morgan & Allen, 1999). It was envisaged that a rise in the resting intracellular calcium concentration would be localised to regions of damage in muscle fibres, resulting in the development of a contracture in some muscle fibres, and thereby raising the level of passive tension produced by the muscle.

Study 1

The findings from this series of experiments supported the above proposal, since there was a large increase in passive tension immediately after the eccentric contractions, with an average peak increase of more than 160%. Further evidence was provided by the increase in work absorption of 15% after the eccentric contractions, which was proposed to reflect the stretch of actively cycling cross-brides in muscle fibres undergoing contracture. Muscle damage was also assessed by changes to active mechanical properties, such as a shift to longer lengths of the optimum length for active tension and a reduction in peak active tension (Jones et al. 1997; Talbot & Morgan, 1998; Whitehead et al. 1998). The mean shift in optimum length was about 4 mm, or 20% of the length range used in these experiments. As described in Chapter 1, a shift in optimum length is proposed to be due to the presence of disrupted sarcomeres in the muscle, which have a longer resting length and lead to an increase in series compliance during activation. The reduction in peak isometric tension was also significant, with values falling by close to 60% after the eccentric contractions. It was estimated that muscle fatigue could account for approximately 10 to 15% of the drop in tension, based on findings to be presented in Chapter 5, so that the remaining 50% or so was presumably due to other effects associated with damage to muscle fibres (see Chapter 1).

Discussion

The experiments carried out in this chapter examined the effects of a series of eccentric contractions on changes to the passive mechanical properties of mammalian muscle. It was hypothesised that sarcomere non-uniformities would lead to overextension and disruption of some sarcomeres during eccentric contractions, and that during repeated eccentric contractions, the regions of disruption would spread until membranous structures became damaged and there was a disturbance of calcium homeostasis in the fibre (Morgan & Allen, 1999). It was envisaged that a rise in the resting intracellular calcium concentration would be localised to regions of damage in muscle fibres, resulting in the development of a contracture in some muscle fibres, and thereby raising the level of passive tension produced by the muscle.

Study 1

The findings from this series of experiments supported the above proposal, since there was a large increase in passive tension immediately after the eccentric contractions, with an average peak increase of more than 160%. Further evidence was provided by the increase in work absorption of 15% after the eccentric contractions, which was proposed to reflect the stretch of actively cycling cross-brides in muscle fibres undergoing contracture. Muscle damage was also assessed by changes to active mechanical properties, such as a shift to longer lengths of the optimum length for active tension and a reduction in peak active tension (Jones et al. 1997; Talbot & Morgan, 1998; Whitehead et al. 1998). The mean shift in optimum length was about 4 mm, or 20% of the length range used in these experiments. As described in Chapter 1, a shift in optimum length is proposed to be due to the presence of disrupted sarcomeres in the muscle, which have a longer resting length and lead to an increase in series compliance during activation. The reduction in peak isometric tension was also significant, with values falling by close to 60% after the eccentric contractions. It was estimated that muscle fatigue could account for approximately 10 to 15% of the drop in tension, based on findings to be presented in Chapter 5, so that the remaining 50% or so was presumably due to other effects associated with damage to muscle fibres (see Chapter 1).

An interesting observation was that the percentage increase in passive tension after the eccentric contractions varied with muscle length, in a similar way to that shown for human muscle in Chapter 3. For both static and dynamic lengthening movements, passive tension increased with muscle length to a maximum at about $L_{opt}+4$ mm and then began to fall but still remained above control values at long lengths (see Figure 4.6 B). Since this length-dependent relationship mirrored the active length-tension relationship of the whole muscle, measured after the eccentric contractions (see Figure 4.4), it was initially thought that the changes in passive tension with length might reflect the active length-tension properties of contracture clots in damaged muscle fibres.

Intuitively, though, it seemed more likely that because of its high stiffness, a region of contracture in a damaged muscle fibre would largely resist the passive stretch, so that the vast majority of the movement would be taken up by remaining undamaged, passive sarcomeres in the same fibre. It was proposed that during the passive stretch, the tension in fibres might rise to a level where the passive sarcomeres were stretched to long lengths and titin filaments yielded. With further stretch, the sarcomere would undergo a period of lengthening where tension remained constant until other intermediate filament structures, such as desmin, began to bear tension (Wang et al. 1993). If this process were to occur non-uniformly, as for active stretches (Morgan, 1990), then tension in the fibre would be expected to increase progressively less steeply throughout the remainder of the passive stretch, as titin filaments in each sarcomere, from weakest to strongest, became overstretched and yielded. This scenario would then provide one plausible explanation for the finding that the increase in passive tension became gradually less at lengths longer than about $L_{opt}+4$ mm, although more experiments are required to further explore this idea. Interestingly, this process could also explain part of the increase in work absorption after the eccentric contractions, since stretching isolated fibres (Wang et al. 1993) or myofibrils (Minajeva et al. 2001) to sarcomere lengths beyond the yield point for titin, has been shown to increase the amount of hysteresis during stretch-shortening movements.

While localised regions of contracture have been favoured here as the cause of the rise in passive tension and work absorption after eccentric contractions, the current evidence, though, based on changes to passive mechanical properties, remains

indirect. While there is other experimental evidence in support of this view, as described in Chapter 3 (see also Chapter 1), there is, however, some evidence of a small, more generalised increase in resting calcium concentration in muscle fibres following eccentric contractions (Balnave *et al.* 1997; Ingalls *et al.* 1998). In one of these studies, it was shown that the rise in resting calcium was distributed uniformly throughout the fibre, although the authors suggested that the resolving power of their imaging technique may not have been sufficient to detect more localised, large increases in calcium concentration (Balnave *et al.* 1997). It is conceivable that these small increases in resting calcium concentration could activate the contractile machinery, leading to an increase in passive tension. Thus, it would be important in future single fibre experiments, using more sensitive imaging techniques, to determine whether or not localised areas of raised resting calcium concentration exist.

While passive tension was shown to increase after the eccentric contractions, over most of the measured length range, closer inspection revealed that this was not case at short muscle lengths, at the beginning of the passive stretch. Here, there were a number of interesting observations (refer to Figure 4.7). Firstly, it was observed that before the eccentric contractions, the initial rise in tension during the first stretch was steeper, and began at a length 1 mm shorter than after the eccentric contractions. These findings are proposed to reflect history-dependent changes in passive properties as a result of damage from eccentric contractions. It was envisaged that in damaged muscle fibres, disrupted sarcomeres would have a longer resting length. This would tend to delay the onset of passive tension during stretch, as a greater length change would be required to remove both slack from the tendon and the added compliance from disrupted sarcomeres. If the disrupted sarcomeres failed to reinterdigitate upon relaxation, the number of stable cross-bridges in the resting muscle would most likely be reduced, providing an explanation for why the initial rise in tension was less steep after the eccentric contractions. However, as the muscle was stretched further, tension increased much more steeply after the eccentric contractions. This was attributed to the higher level of tension generated by actively cycling cross-bridges in muscle fibres undergoing contracture.

At the start of the second passive stretch, tension began to rise at a longer length than for the first stretch, both before and after the eccentric contractions. This was

.umably due to the incorporation of slack into the muscle and tendon from the
.tening phase of the first cycle, which had to be taken up by the second stretch
.re tension was generated. However, the difference in the onset length of passive
.ion before and after the eccentric contractions was now less. It was suggested
this might be the result of some slack being taken up by the regions of injury
.racture. A final point was that before the eccentric contractions, the tension
.es for the first and second stretches merged, while afterwards they remained
.rated over the whole length range. This provided an explanation for the greater
. in work absorption from the first to second stretch-shortening cycles after the
.ntric contractions. It also supported the earlier proposal, that during the first
.ch, sarcomeres become overextended and titin filaments yielded, so that tension
.ained lower throughout the second stretch because the passive length-tension
.ionship had shifted to longer lengths (Wang *et al.* 1993).

.ly 2

.cond series of experiments examined the effects of eccentric contractions on the
.cle's tension receptors, the Golgi tendon organs, during passive length-tension
.surements. The study was initiated, in part, to investigate a possible role of
.on organs in proprioceptive perturbations (see Gregory *et al.* 2002), which has
. previously shown to occur in humans after eccentric exercise (Saxton *et al.*
.; Brockett *et al.* 1997). Here though, the main focus of these experiments was
.ovide additional support for the contracture hypothesis. Since tendon organs are
.vn to have muscle fibres inserting directly into their capsule (Zelena, 1994) it
.ed plausible that an increase in passive tension after eccentric contractions, due
.amaged muscle fibres with regions of contracture, would be signalled by these
.tors.

.nsistent finding from these experiments was that almost all tendon organs
.nenced firing at a shorter muscle length after the eccentric contractions than
.e. This was accompanied by no consistent change in the tension threshold and
. a small reduction in sensitivity of the tendon organs during passive stretch,
.esting that the sensitivity of the receptors to passive tension was largely
.fected by the eccentric contractions. It was proposed that the change in length
.hold after eccentric contractions was due to the rise in passive tension from
.aged fibres in contracture. Further support for this idea was provided by the

results from three experiments, in which motor units with muscle fibres acting on a single tendon organ were not subjected to eccentric contractions. For these tendon organs, there was no change in length threshold, while other tendon organs in the same muscle, whose motor units had undergone eccentric contractions, showed the usual shift in length threshold. This finding argued against effects other than damage to muscle fibres, such as muscle swelling or changes to tendon properties, as the likely cause of the shift in length threshold after eccentric contractions.

An interesting finding was the range of length threshold changes, up to 5 mm, for different tendon organs from the same muscle. This suggested that the number of fibres and/or the extent of the damage to these fibres varied somewhat amongst the tendon organ population. One possible reason for this observation might be that some of the fibres inserting into a particular tendon organ were more susceptible to damage than others. A recent report by Brockett *et al.* (2002) provides some support for this proposal. It was found that the range of initial optimum lengths for motor units of cat MG was about 5 mm, and that those motor units with shorter optimum lengths before the eccentric contractions showed evidence of greater damage afterwards.

An important question arising from the experiments in both Study 1 and 2 was; how extensive were the regions of damage-induced contracture throughout the muscle? The fact that almost all (40 out of 42) tendon organs showed a change in length threshold can be used to give an estimate of the minimum amount of damage after the eccentric contractions. It has been shown for cat MG, that about 20 muscle fibres from 10 motor units insert into a tendon organ (Barker, 1974; Gregory, 1990). Thus, if it was assumed that a contracture in one of these fibres was sufficient to alter the threshold of the receptor, then at least 5% of the fibres in the muscle were likely to have been undergoing contracture. However, based on the large reduction in active tension, it seemed probable that the damage was much more widespread, involving a greater number of muscle fibres with a region of contracture. It was envisaged that fibres undergoing contracture, with damaged membranes and raised intracellular calcium concentration, were also responsible for a large fraction of the reduction in active tension. This idea was supported the study by the study of Warren *et al.* (1995). They reported that following eccentric contractions of mouse soleus, the number of muscle fibres containing swollen regions with a raised calcium

concentration, obvious signs of contracture, was correlated with the magnitude of the reduction in active tension. In the current experiments, the size of the active tension drop due to muscle damage was about 50%. Thus, based on the result of Warren *et al.* (1995), this value (50%) could be used as a rough estimate of the percentage of the muscle undergoing contracture. This would suggest that the rise in passive tension after eccentric contractions was produced by only about half of the muscle, so that the actual increase in passive tension for this half was likely to have been twice the measured increase for the whole muscle. In the example shown in Figure 4.5, passive tension measured before the eccentric contractions reached a maximum of 27 N, while afterwards it ˙ increased to 43 N. Thus, after the eccentric contractions, if half of the muscle had the same passive length-tension relationship as before the eccentric contractions, then this part of the muscle would generate about 13.5 N (27 N × 0.5). Therefore, the remaining tension, about 30 N, would be produced by half of the muscle, comprising damaged fibres in contracture. In other words, an equivalent tension produced by the whole muscle would be twice this value (60 N). This represents a level of passive tension two times higher than that measured before the eccentric contractions.

While this analysis involves a number of assumptions that were not formally tested in these experiments, it does, however, highlight the fact that muscle fibres with regions of contracture are likely to be generating considerable tension. This adds further support to the proposal put forward earlier, that during a passive stretch, undamaged sarcomeres in series with contracture segments might become overextended and disrupted because of the high tension in the fibre. Since such a high level of passive tension in muscle fibres could potentially have an effect on the extent of damage to the muscle following eccentric contractions, as well as on the subsequent repair and adaptation processes, it would be interesting to investigate this issue further in future experiments (see Chapter 6).

C h a p t e r   F i v e

Changes to passive properties of cat medial gastrocnemius following eccentric contractions at different lengths

Introduction

In Chapter 4, an increase in passive tension was observed immediately after eccentric contractions carried out over a length range that lay symmetrically about the optimum length for active tension. However, previous studies have shown that the amount of muscle damage is length-dependent, that is, more extensive muscle damage occurs following eccentric contractions at long than at short muscle lengths (Newham et al. 1988; Talbot & Morgan, 1998). This chapter tested the hypothesis that the rise in passive tension would also be length-dependent, being greater after eccentric contractions carried out further down the descending limb of the active length-tension curve, since there were likely to be more damaged fibres with regions of injury- contracture throughout the muscle.

It was proposed that muscle damage from eccentric contractions would manifest itself in two ways. Where the damage to fibre membranes had led to the development of regions of contracture, there would be an increase in whole muscle passive tension. However, where the damage remained restricted to disrupted sarcomeres in series with normal functioning sarcomeres, and had not progressed to the stage of membrane damage and contracture formation, such overextended sarcomeres would lead to a reduction in passive tension (see Chapter 4). It was postulated that changes to the passive length-tension relationship following eccentric contractions, carried out at different lengths, would depend on the balance of these two opposing factors.

In Chapter 4, it was shown that the rise in passive tension after eccentric contractions was labile, in the sense that a single passive stretch-shortening cycle led to a reduction in passive tension and work absorption during the second and subsequent cycles. Thus, some of the experiments carried out in this chapter investigated the

effect of passive stretches on the increase in passive tension from eccentric contractions.

Methods

The experiments were carried out on a total of 12 cats of both sexes, with a weight range of 2.0 to 4.5 kg. The experiments were undertaken with approval from the Monash University Committee for Ethics in Animal Experimentation. Animals were anaesthetised and surgical procedures were carried out in the same way as described in Chapter 2. The experiments were performed on the medial gastrocnemius muscle (MG).

Experimental procedure

Three parts of the muscle: Initial experiments

The initial series of experiments was carried out on 5 animals. Here, the aim was to measure changes to the passive and active length-tension relationships after 3 sets of 50 eccentric contractions, each performed over a different length range on three functionally separate portions of the muscle (see below). The procedure involved splitting the ventral root supply to MG (L7-S1) into three approximately equal parts. This was determined by recording the tension generated by each portion during a twitch and brief tetanic stimulation of the three ventral root fragments. These measurements were made at the optimum length of the whole muscle (L_{opt}). The division of the muscle in this way provided a means of performing eccentric contractions over three different length ranges within the same muscle. Thus, the effect of the eccentric contractions on the passive properties of each part of the muscle could be measured as a change in the passive length-tension relationship of the whole muscle. The experiment was also designed in this way, as opposed to performing whole muscle eccentric contractions at different lengths, to minimise the number of animals used.

Active length-tension measurements

The active length-tension relationship for each third of the muscle was measured before and after eccentric contractions using the same stimulation parameters as previously described for the whole MG (see Chapters 2 and 4). The measured optimum length for each part of the muscle was 1 to 2 mm shorter than that of the whole muscle. This was attributed to the fact that activation of part of a muscle does

not stretch the series elastic component as much as activation of the whole. Thus, there would be less internal shortening during part activation and for a given muscle-tendon length, the muscle fibres would be at a longer length, resulting in a shorter optimum length (Sandercock, 2000). In order to calculate the optimum length and peak active tension, Gaussian curves were fitted to tension values above 75% of the maximum tension.

In one additional experiment, it was decided to estimate the drop in active tension from muscle fatigue, as distinct from damage. The motor supply to the muscle was divided into two approximately equal halves and each was subjected to a series of 50 isometric contractions, followed 2 s later by a passive stretch, using the same stimulus (400 ms duration at 80 pps) and stretch (6 mm at 50 mm s^{-1}) parameters as for the eccentric contractions (see below). This procedure was carried out at a starting length of L_{opt}-3 mm for one half of the muscle and L_{opt}+3 mm for the other. Active length-tension curves were measured before and after the isometric contractions.

Passive length-tension measurements

Passive tension and work absorption were measured using the protocol described in Chapter 4. This involved two continuous stretch-shortening cycles at a velocity of 1 mm s^{-1}, over muscle lengths ranging from L_{opt} −10 mm to L_{opt} +10 mm. It was decided here to use only two cycles because the greatest drop in work absorption occurs between cycles 1 and 2, with little change occurring thereafter (see Chapter 4). The muscle was conditioned with an isometric contraction (80 pps, 100 ms duration) and held at a constant length (L_{opt} −10 mm) for 30 seconds before the onset of cycle 1.

Changes to the passive length-tension relationship following eccentric contractions of each third of the muscle were calculated using an iterative analysis procedure, as described in Chapter 4. The change in tension at each muscle length was calculated and divided by the tension, measured at the corresponding muscle length, before the eccentric contractions of Part 1. Values were then expressed as a percentage. This provided a continuous trace of the change in passive tension plotted against length (see Figure 5.3 B). Work absorption was measured as the area contained within the stretch-shortening loop and expressed as a percentage of the work done on the

muscle (as described in Chapter 4). The change in work absorption after each set of eccentric contractions was then calculated.

Eccentric contractions

Fifty eccentric contractions were carried out, one every 40 seconds, on each of the three parts of the muscle. For each eccentric contraction, the muscle was stimulated at 80 pps for 400 ms. At 150 ms after the onset of stimulation, the muscle was stretched by 6 mm at a velocity of 50 mm s^{-1}. The eccentric contractions were carried out over a different region of the length-tension relation for each of the three parts. It was decided to refer to each length range in terms of the final length at the end of the stretch, since it was considered important to indicate whether or not the eccentric contractions involved the descending limb of the length-tension relation. For the 5 experiments, the lengths at which the stretch ended during the eccentric contractions were: L_{opt}–3 mm (n=4 parts), L_{opt} (n=3 parts), L_{opt}+3 mm (n=3 parts), L_{opt}+6 mm (n=1 part) and L_{opt}+9 mm (n=4 parts). In general, the length range of the eccentric contractions for the three parts was in the order of short to long lengths, so that they ended at ended at L_{opt}–3 mm for Part 1, L_{opt} or L_{opt}+3 mm for Part 2, and L_{opt}+6 mm or L_{opt}+9 mm for Part 3. This order was chosen, since muscle damage from eccentric contractions at short lengths (Part 1) was expected to be minor, so that any further changes would have a negligible effect on passive properties measured after eccentric contractions at longer lengths (Parts 2 and 3). It was assumed, though, that changes in passive tension brought about by the eccentric contractions of each third would remain more or less constant, independent of one another, and not be affected by passive stretches applied subsequently during eccentric contractions to other parts. This assumption was formally tested in a series of control experiments carried out on three animals (see below).

Three parts of the muscle: Control experiments

For these experiments, the muscle was again divided into three parts. In the first experiment, performed on two animals, the eccentric contractions were carried out over the same length range for each part, ending at L_{opt}+3 mm. Here, it was expected that the damage to each part of the muscle from the eccentric contractions would be similar, so that changes in passive tension should also be comparable. The second experiment, carried out on one animal, examined the effect of a series of passive stretches imposed on the muscle after the eccentric contractions, on changes to the

passive length-tension relationship. This involved 50 passive stretch-shortening movements, each separated by 40 s, and of the same amplitude (6 mm) and velocity (50 mm s^{-1}) as the eccentric contractions.

Whole muscle experiments

In a further 3 experiments, the aim was to compare changes to passive properties, after eccentric contractions performed on the whole muscle at intermediate or longer muscle lengths. These experiments did not require a laminectomy. Here, MG of both hindlimbs was used, so two leg dissections were carried out (see Chapter 2, Methods). Smaller cats, weighing between 2 and 2.5 kg, were chosen for these experiments, so that the servomotor could withstand the tension generated during whole muscle eccentric contractions. There were two main objectives of these experiments. The first was to examine changes to passive properties after whole muscle eccentric contractions at intermediate or long muscle lengths. The second aim was to investigate, after the eccentric contractions, the effects of passive movements, and time, on changes to the passive length-tension relationship. For these experiments, the eccentric contractions and passive movements were carried out in the same way as described previously, except that the stretches were 5 mm rather than 6 mm in amplitude because the physiological length range of the muscle for these smaller cats was expected to be less. Similarly, the passive length-tension measurements were performed over a slightly shorter length range, from $L_{opt}-10$ mm to $L_{opt}+8$ mm.

Data Analysis

For all parameters measured, the mean ($\pm$ S.E.M.) was calculated. Statistical significance was set at $P < 0.05$. A two-factor analysis of variance (ANOVA) with interactions was used to determine whether changes after the eccentric contractions at different lengths were significant. The factors used were animal, and the length range of the eccentric contractions. Where significance was found, an LSD *post hoc* test was used. For some variables, a linear regression analysis was also performed. The statistical program used was Data Desk (Ithaca, NY, USA).

Results

Three parts of the muscle: Initial experiments

Active length-tension changes

In Figure 5.1 A, sample records from one experiment, of the 1st and 50th eccentric contractions for each of the three parts of the muscle are shown. It can be seen that when the eccentric contractions ended at longer lengths, the reduction in isometric tension became progressively greater. Mean values of the drop in isometric tension from the 1st to 50th eccentric contraction, ranged from 54.7% ($\pm$ 8.6) to 80.5% ($\pm$ 2.1) when the eccentric contractions ended at L_{opt}-3 mm and L_{opt}+9 mm, respectively.

Figure 5.1 B shows active length-tension curves measured before and after the eccentric contractions for the same three parts of the muscle in Figure 5.1 A. Note that as the eccentric contractions ended at longer lengths, there was a progressively larger shift in optimum length towards longer lengths and drop in peak active tension. Mean values for the shift in optimum length ranged from 2.1 mm ($\pm$ 0.5) to 7.1 mm ($\pm$ 0.3) for eccentric contractions ending at L_{opt}-3 mm and L_{opt}+9 mm, respectively (Figure 5.2 A). The mean drop in peak active tension ranged from 19% ($\pm$ 3.5) when the eccentric contractions ended at L_{opt}-3 mm to 68.6% ($\pm$ 2.8) when they ended at L_{opt}+9 mm (Figure 5.2 A). Analysis showed a significant effect of the length range of the eccentric contractions on both the shift in optimum length and active tension drop (P < 0.01, two-factor ANOVA). There was also a strong linear relationship between these parameters (r^2 = 0.87, P < 0.01) (Figure 5.2 B).

In one additional experiment, the amount of fatigue, independent of muscle damage was estimated from isometric contractions. The motor supply of the muscle was divided into two parts. Each part was given 50 isometric contractions using the same stimulus parameters as for the eccentric contractions. Two seconds after each contraction, the muscle underwent a passive stretch, in order to mimic the length change that had occurred during the eccentric contractions. The isometric contractions were carried out at L_{opt}-3 mm for the first half, and L_{opt}+9 mm for the second half, with passive stretches also beginning at these lengths. Active length-tension measurements were made before and after the contractions. It was found that peak active tension dropped by 11% and 15.3%, after the isometric contractions of

Figure 5.1 *Changes in the active length-tension relationship after eccentric contractions of three parts of the muscle.*

(A) Raw records of eccentric contractions for the three parts of the muscle from one experiment. Measurements of tension are shown for the 1st (continuous line) and 50th (dotted line) eccentric contractions for each part. The colour of each tension trace refers to the length range of the eccentric contractions for each part, as indicated by the hatched bars in (B). The length change (second bottom trace) and period of stimulation (solid bar, bottom trace) are shown. (B) Active length-tension curves as measured before (open circles) and after (filled circles) 50 eccentric contractions for each of the three parts of the muscle shown in (A). Tension is plotted against the optimum length measured before the eccentric contractions (L_{opt}). The length range of the eccentric contractions for each part is indicated (hatched bars). The arrows indicate the optimum length for peak tension. For measurements made before the eccentric contractions only a single arrow is shown, as there were very small differences in optimum length between the three parts.

A

Part 1

Part 2

Part 3

]20 N

]5 mm

0.1 s

B

50

40

Active tension (N)

30

20

10

0

Part 1   Part 2   Part 3

-10   -5   0   5   10

Length relative to L$_{opt}$ (mm)
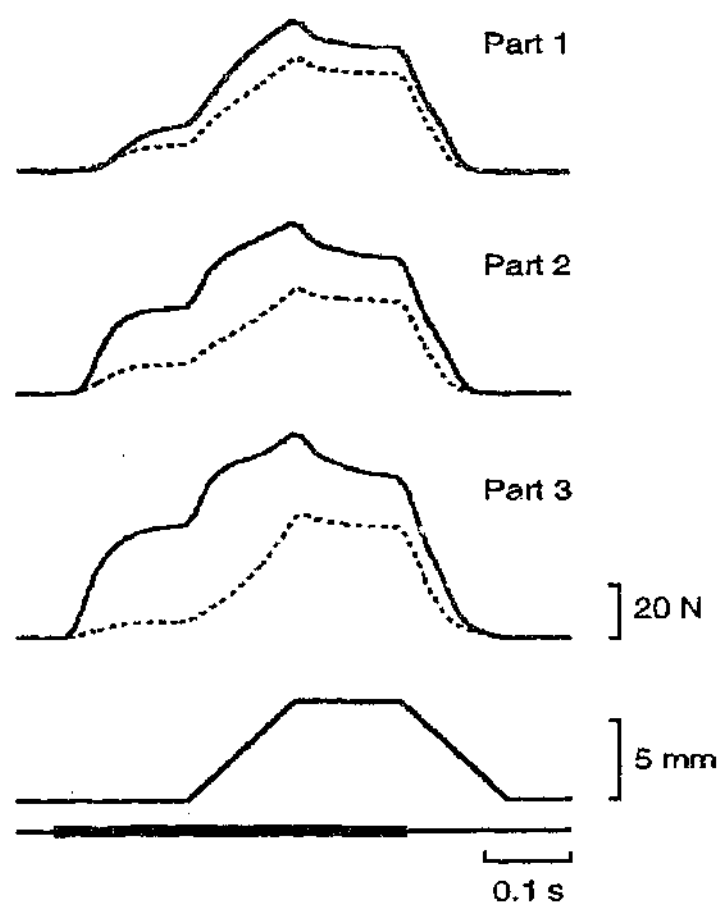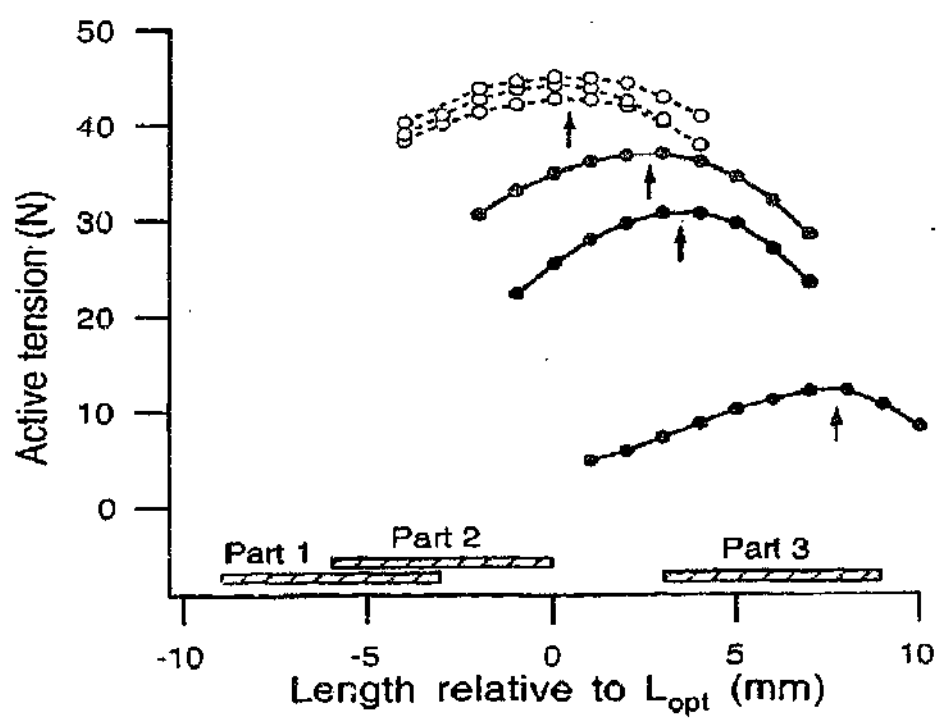
Figure 5.2 *The shift in optimum length and active tension drop after eccentric contractions of three parts of the muscle.*

(A) The mean (± S.E.M.) shift in optimum length (filled circles) and active tension drop (open circles), as calculated from active length-tension curves before and after eccentric contractions of each part. Values are plotted against the length at which the eccentric contractions ended relative to the optimum length ($L_{o,j,n}$) for each part. (B) A plot showing the relationship between the shift in optimum length and the drop in peak active tension after eccentric contractions of a third of the muscle, carried out at different lengths. A regression line is also shown.

A

Active tension drop (%)

Final length of stretch (mm)

Shift in optimum length (mm)

B

Shift in optimum length (mm)

Active tension drop (%)
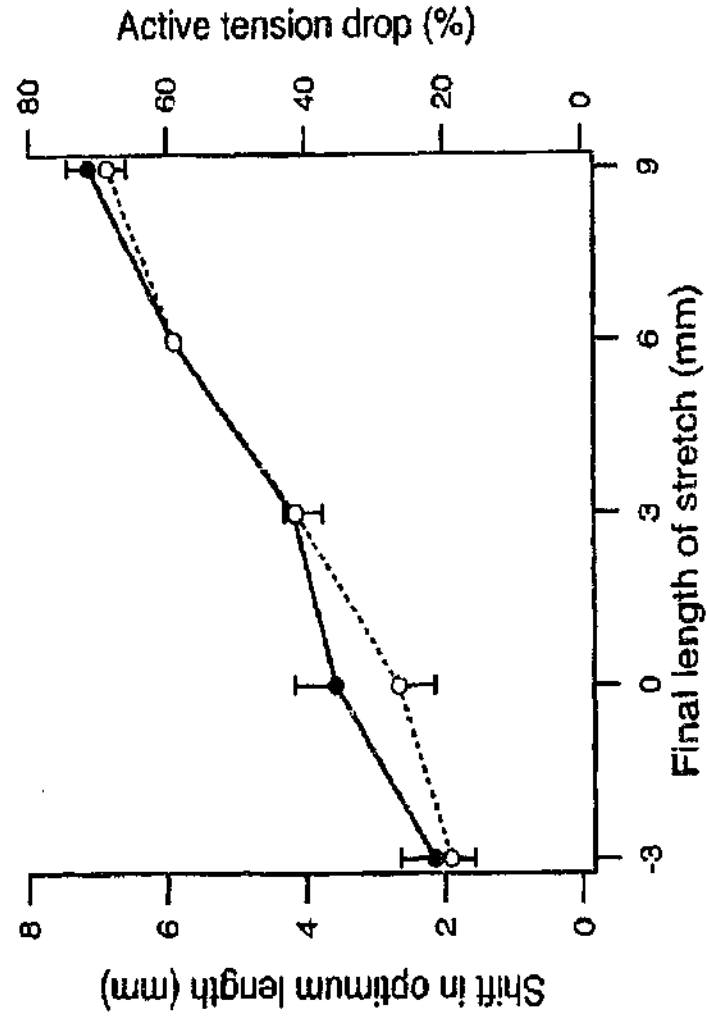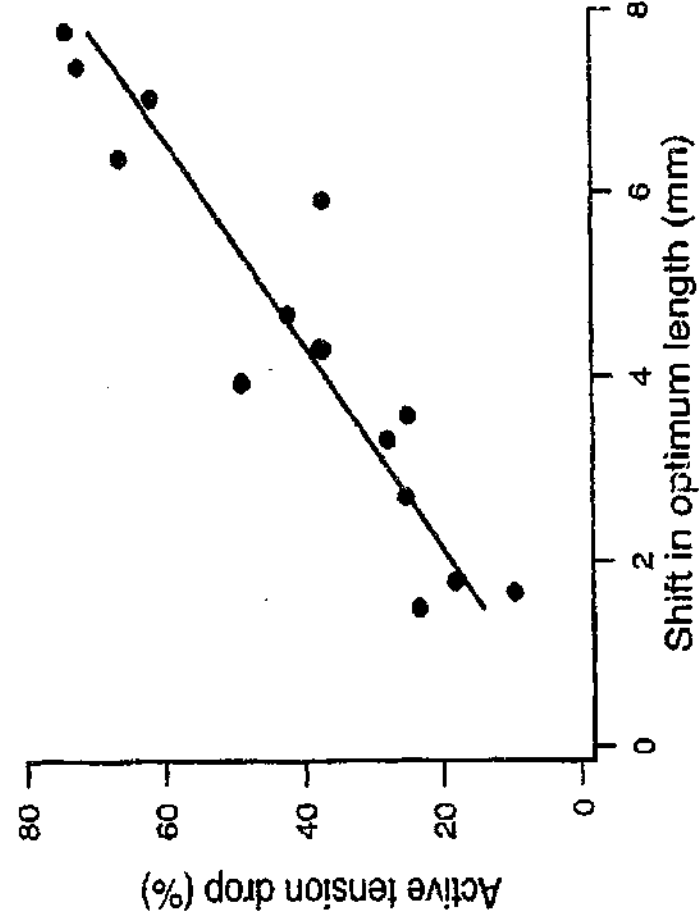
the first and second half, respectively. For both halves, there was only a small shift in the optimum length to longer lengths of 0.65 mm for Part 1 and 0.62 mm for Part 2.

Changes to the passive length-tension relationship

An example of dynamic passive length-tension curves before and after eccentric contractions of each third of the muscle is shown in Figure 5.3 A. It can be seen that the measured passive tension was dependent on the length range over which the eccentric contractions were carried out. This was highlighted more clearly when the percent change in passive tension after the eccentric contractions for each part was plotted against muscle length (Figure 5.3 B). In accord with the findings of Chapter 4, the increases in passive tension observed here varied with muscle length, with values increasing to a maximum before decreasing at longer lengths. As the eccentric contractions ended at progressively longer muscle lengths, there were two main effects. Firstly, the increase in passive tension became less, and secondly, the maximum increase in tension occurred at a longer muscle length (Figure 5.3 B, arrows). For some experiments, following the eccentric contractions ending at $L_{opt}+9$ mm, passive tension had decreased over most of the measured length range.

When values for the maximum increase in passive tension for the 5 experiments were pooled, the peak value was 50.9% ($\pm$ 12.7), recorded after eccentric contractions ending at $L_{opt}-3$ mm, although similar values were also recorded after eccentric contractions ending at L_{opt} and $L_{opt}+3$ mm (Figure 5.4 A). However, when the eccentric contractions were carried out at longer lengths, values became less, with a maximum increase of only 3.2% ($\pm$ 5.3), following eccentric contractions ending at $L_{opt}+9$ mm (Figure 5.4 A). Overall, there was a significant effect of the length range of the eccentric contractions on maximum increases in passive tension ($P < 0.05$, two-factor ANOVA). Pooled data showed that the length corresponding to the maximum increase in passive tension, ranged from $L_{opt}-1.6$ mm ($\pm$ 0.8) to $L_{opt}+6$ mm ($\pm$ 2.7) after eccentric contractions ending at $L_{opt}-3$ mm and $L_{opt}+9$ mm, respectively. Here, the effect of the length range of the eccentric contractions just failed to reach statistical significance ($P < 0.08$, two-factor ANOVA).

The dynamic passive length-tension relationship was also used to measure work absorption, which was calculated from the area contained within the stretch-shortening loop, and expressed as a percentage of the work done on the muscle

Figure 5.3 *Changes to the passive length-tension relationship after eccentric contractions of three parts of the muscle.*

(A) Passive length-tension curves as measured during dynamic lengthening before (black trace) and after eccentric contractions of each of the three parts of the muscle. The colours of the tension traces correspond to length range of the eccentric contractions for each part (hatched bars). (B) The increase in passive tension after eccentric contractions of each part plotted against muscle length. Increases in passive tension of each part above the previous part, were expressed as a percentage of the corresponding values measured before eccentric contractions of the first part (dotted line). Again, the colour of the trace corresponds to a particular part of the muscle, as in (A). The arrows indicate the length at which the maximum increase in passive tension occurred.

A

B

Length relative to L_{opt} (mm)
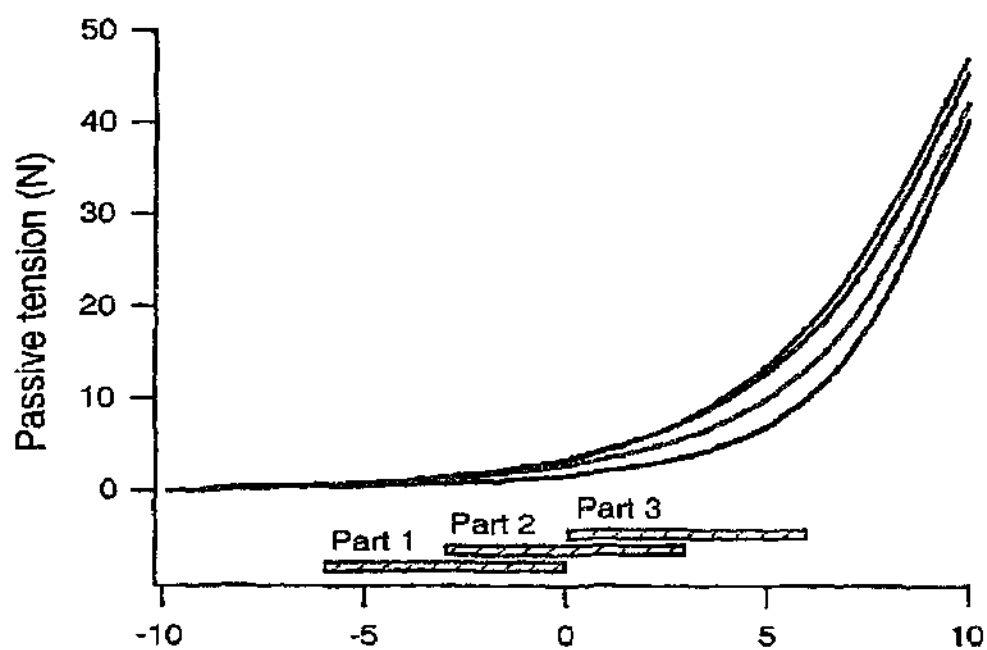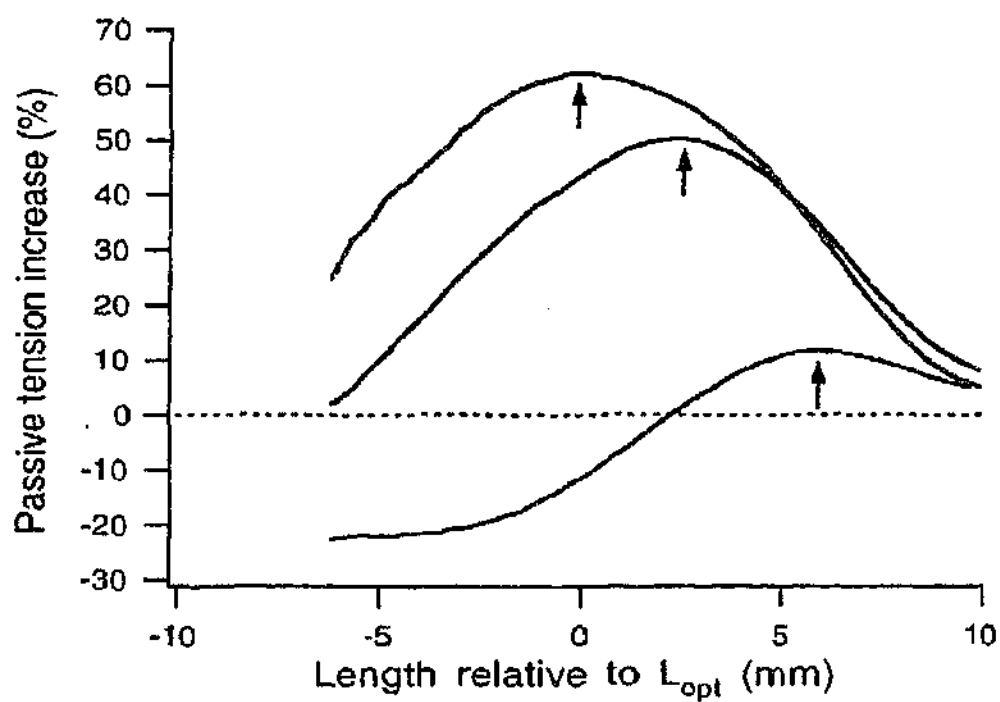
Figure 5.4 *Mean increases in passive tension and work absorption after eccentric contractions at different lengths.*

(A) The mean (± S.E.M.) maximum increase in passive tension, expressed as a percentage, plotted against the length at which the eccentric contractions ended for each third of the muscle. The maximum increase in passive tension was measured from plots like those shown in Figure 5.3 B. (B) The mean (± S.E.M.) increase in work absorption plotted against the final length of the eccentric contractions. Values of work absorption were expressed as a percentage of the work done on the muscle during the lengthening phase of the passive stretch-shortening movement.

A

Passive tension increase (%)

B

Work absorption increase (%)

Final length of stretch (mm)
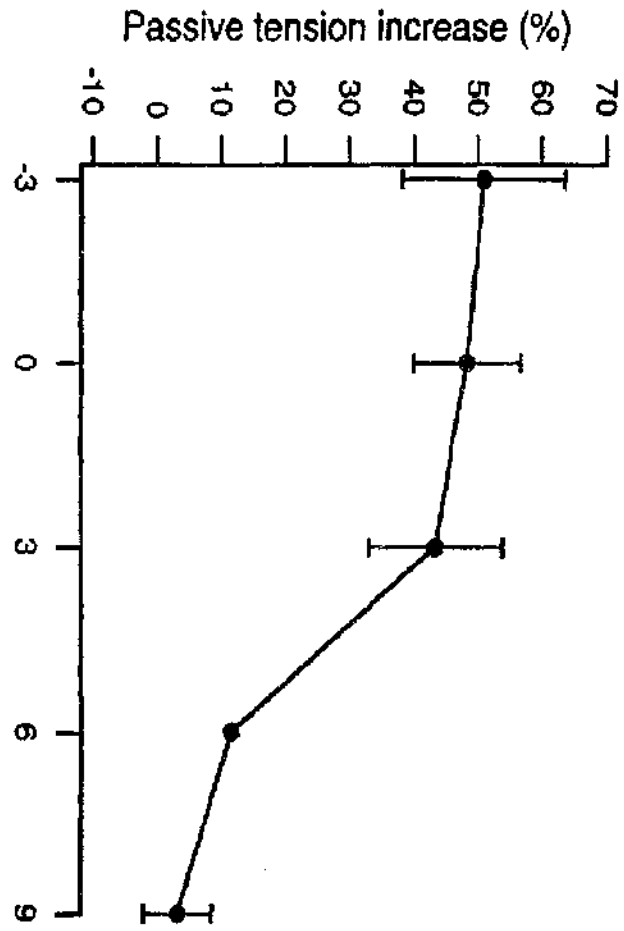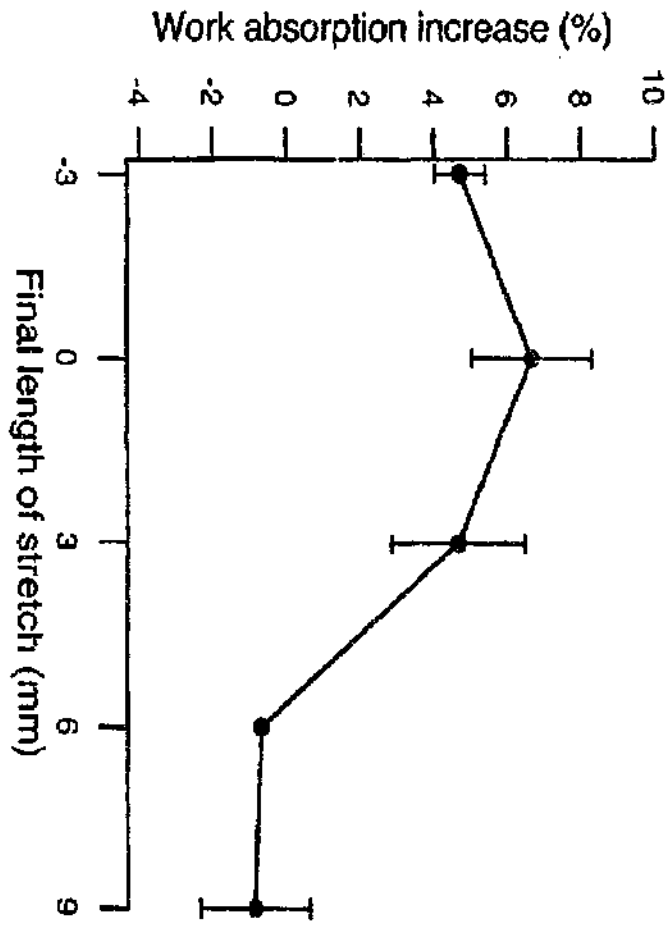
during the stretch (see Chapter 4). When values were pooled across the 5 experiments, the change in work absorption showed a similar trend to the increase in passive tension (Figure 5.4 B). Values ranged from an increase of 6.68% ($\pm$ 1.63) to a decrease of 0.83% ($\pm$ 1.49) following eccentric contractions ending at L_{opt} and $L_{opt}+9$ mm, respectively. Analysis showed a significant effect of the length range of the eccentric contractions on changes in work absorption ($P < 0.05$, two-factor ANOVA).

Since passive tension and work absorption had not increased as expected for the eccentric contractions carried out at longer lengths, the possibility was considered that these values might have been underestimated. Here, the concern was that the stretch accompanying an eccentric contraction of one part of the muscle might alter the measured level of passive tension in another previously stimulated part. This was because the whole muscle was being stretched, not just a third. Therefore, a series of control experiments was carried out to test this idea.

Three parts of the muscle: Control experiments

These experiments were performed on three animals in which the motor supply of MG was again divided into three parts. The results of these experiments are presented in Table 5.1.

In the first experiment, on two animals, the eccentric contractions were carried out over the same length range for each third of the muscle, with the stretch ending at $L_{opt}+3$ mm. The main result of these experiments was that the increase in passive tension and work absorption became significantly less after the eccentric contractions of Parts 2 and 3, compared to that measured after Part 1. For example in one experiment, the maximum increase in passive tension was almost 140% after eccentric contractions of Part 1, while identical eccentric contractions of Parts 2 and 3 produced further rises of 97% and 52%, respectively. Values for the other experiment were similar, so the pooled data from both experiments is shown in Table 5.1. The shift in optimum length and active tension drop for each of the three parts was similar, both within and across the 2 experiments, with a range of 4.1 to 5 mm for the shift in optimum length, and 39.4 to 44.6% for the active tension drop. Again, mean values are shown in Table 5.1. The similarities between the three parts, in terms of changes to active length-tension properties after eccentric contractions,

Table 5.1 *Changes to active and passive length-tension measurements for the 'three parts' control experiments.*
For experiment 1, the three parts of the muscle all underwent eccentric contractions (Ecc) over the same length range, with the stretch ending at $L_{opt}+3$ mm. For this experiment, the mean values from 2 animals are shown.
For experiment 2, the eccentric contractions (Ecc) were carried out over a different length range for each of the three parts of the muscle. After the eccentric contractions on Part 1, ending at $L_{opt}-3$ mm, two sets of 50 passive stretches (Pass) were performed on the whole muscle. Here, the first set of stretches ended at $L_{opt}+3$ mm, while the second set ended at $L_{opt}+9$ mm. After the eccentric contractions on Part 2, ending at $L_{opt}+3$ mm, one set of 50 passive stretches was carried out, ending at $L_{opt}+9$ mm. Finally, eccentric contractions were carried out on Part 3, where the stretch ended at $L_{opt}+9$ mm. For both experiments, positive values indicate a shift to longer muscle lengths in the optimum length, and an increase in passive tension and work absorption.

Control Experiments

	PART	Final length of stretch	Shift in Optimum length (mm)	Active tension drop (%)	Passive tension change (%)	Work absorption change (%)
Experiment 1	1	L_{opt}+3mm (Ecc)	4.36	43.69	127.49	11.19
(mean of 2)	2	L_{opt}+3mm (Ecc)	4.34	42.02	70.08	3.96
	3	L_{opt}+3mm (Ecc)	4.75	41.07	47.35	1.68
Experiment 2	1	L_{opt}-3mm (Ecc)	1.57	14.96	52.05	10.58
		L_{opt}+3mm (Pass)	-	-	-30.59	-3.01
		L_{opt}+9mm (Pass)	-	-	-18.5	-2.98
	2	L_{opt}+3mm (Ecc)	5.84	34.79	60.72	7.93
		L_{opt}+9mm (Pass)	-	-	-38.89	-4.56
	3	L_{opt}+9mm (Ecc)	7.71	52.28	38.97	6.84

argued against differences in the degree of muscle damage as the cause of the smaller increase in passive tension and work absorption for Parts 2 and 3. It suggested that the smaller increases might be the result of concurrently altering the effect of the previous part(s).

It was shown in Chapter 4, that a single passive stretch was sufficient to reduce the increase in passive tension and work absorption during the second and subsequent stretches. This led to the realisation that during the eccentric contractions of one third of the muscle, the inactive, remaining parts of the muscle were, at the same time, being passively stretched. Thus, it seemed possible that these passive movements might reduce any previous increase in passive tension. A second control experiment examined the effects of passive stretches on changes to passive tension after the eccentric contractions had been performed on Parts 1 and 2. The aim was to mimic the protocol for the initial series of experiments, so that the passive stretches covered the same length ranges as the eccentric contractions in the initial experiments.

The important finding of this experiment was that the increase in passive tension after eccentric contractions was significantly reduced by a subsequent series of passive stretches (see Table 5.1). For example, after eccentric contractions to Part 1, ending at L_{opt}-3 mm, passive tension increased by a maximum of 52.1%. This value then dropped by 30.6% after 50 passive stretches ending at L_{opt}+3 mm, representing the length range over which the muscle would have been stretched if Part 2 had been subjected to eccentric contractions. Passive tension then fell by a further 18.5% following another 50 passive stretches, ending at L_{opt}+9 mm, representing the length range over which the eccentric contractions for Part 3 would have been performed. This meant that passive tension was now only 3% above that measured before the eccentric contractions. Passive tension then increased by 60.7% after eccentric contractions to Part 2, ending at L_{opt}+3 mm, but dropped by 38.9% following passive stretches ending at L_{opt}+9 mm, again representing the length range of the eccentric contractions for Part 3. Finally, after eccentric contractions to Part 3, ending at L_{opt}+9 mm, passive tension increased by 39%. These findings suggested that for the initial 'three parts of the muscle' experiments, increases in passive tension and work absorption had been greatly underestimated for Part 2, and particularly Part 3, where the eccentric contractions were performed at long lengths (see Figure 5.4).

By adding to the mean values shown in Figure 5.4, the amount by which passive tension was reduced from the passive stretches, as measured in the control experiment, it was estimated that passive tension would have increased by 74% (43 + 30.6%) after eccentric contractions ending at $L_{opt}+3$ mm, and 60% (3 + 18.5 + 38.9%) after eccentric contractions ending at $L_{opt}+9$ mm. These adjusted values suggested that passive tension increased significantly following eccentric contractions at long lengths and, tended to be a little higher than values measured after eccentric contractions at short lengths. However, there is obviously some degree of uncertainty with these estimates, so to avoid the complications associated with the 'three parts' protocol, a further series of experiments was carried out involving eccentric contractions of the whole muscle (see below).

Whole muscle experiments

A total of 3 experiments were performed on MG of both legs, so that measurements were made on a total of 6 muscles. However, in one experiment, the distal tendon of MG became damaged during the eccentric contractions, so that the results from one muscle had to be discarded.

Changes to the active length-tension relationship

An example of the active length-tension curves measured before and after eccentric contractions for two muscles from the same animal is shown in Figure 5.5. Notice the similar length-tension curves for the two muscles before the eccentric contractions. It can be seen that after eccentric contractions carried out over an intermediate length range, ending at $L_{opt}+2.5$ mm, the shift in optimum length to longer lengths was about 4 mm, and peak active tension had dropped by 24%. In comparison, following eccentric contractions at the longer length, ending at $L_{opt}+7.5$ mm, the optimum length had shifted more (5 mm) and there was a greater drop in active tension (47%). Values at each length range were similar in the other two experiments (Figure 5.6 A). A plot of the shift in optimum length against the drop in active tension showed a significant relationship ($r^2 = 0.76$, P = 0.055) (Figure 5.6 B).

Changes to the passive length-tension relationship

Passive length-tension curves for both muscles from the same animal, before and after eccentric contractions at two lengths, are shown in Figure 5.7. In both

Figure 5.5 *Active length-tension curves before and after eccentric contractions of the whole muscle at two different lengths.*

Active length-tension curves as measured for both MG muscles from the same animal before (blue circles) and after (red circles) 50 eccentric contractions of the whole muscle at intermediate (**A**) and long (**B**) muscle lengths. In each case, the length range of the eccentric contractions is indicated (hatched bar). Tension is plotted against the optimum length measured before the eccentric contractions (L_{opt}). The arrows indicate the optimum length for peak tension.

A
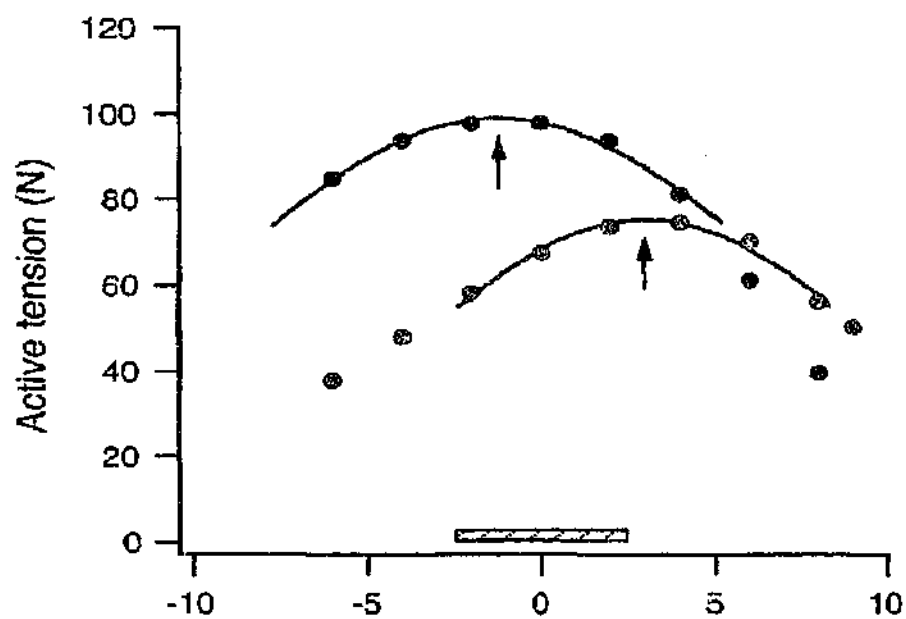
B
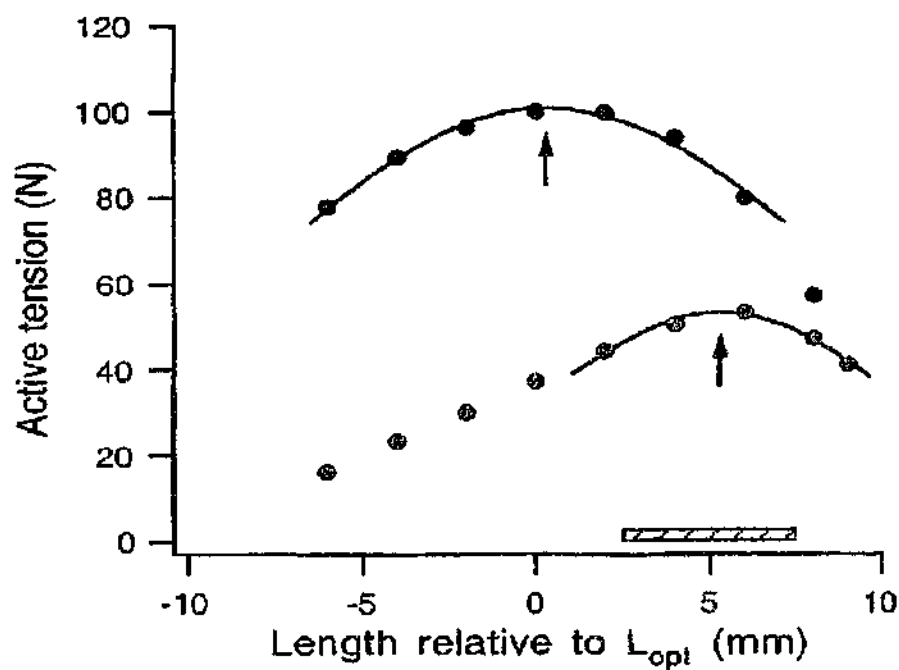
Figure 5.6 *The shift in optimum length and active tension drop after eccentric contractions of the whole muscle at two different lengths.*

(A) Data from 3 experiments showing the shift in optimum length (filled circles) and peak drop in active tension (open circles), as measured after eccentric contractions of the whole muscle at two different lengths. Values are plotted against the length at which the eccentric contractions ended. Each colour represents values from one animal. (B) A plot of the relationship between the shift in optimum length and the drop in peak tension from values measured after the eccentric contractions. Each colour corresponds to values from one animal as indicated in (A). A regression line is also shown.

A
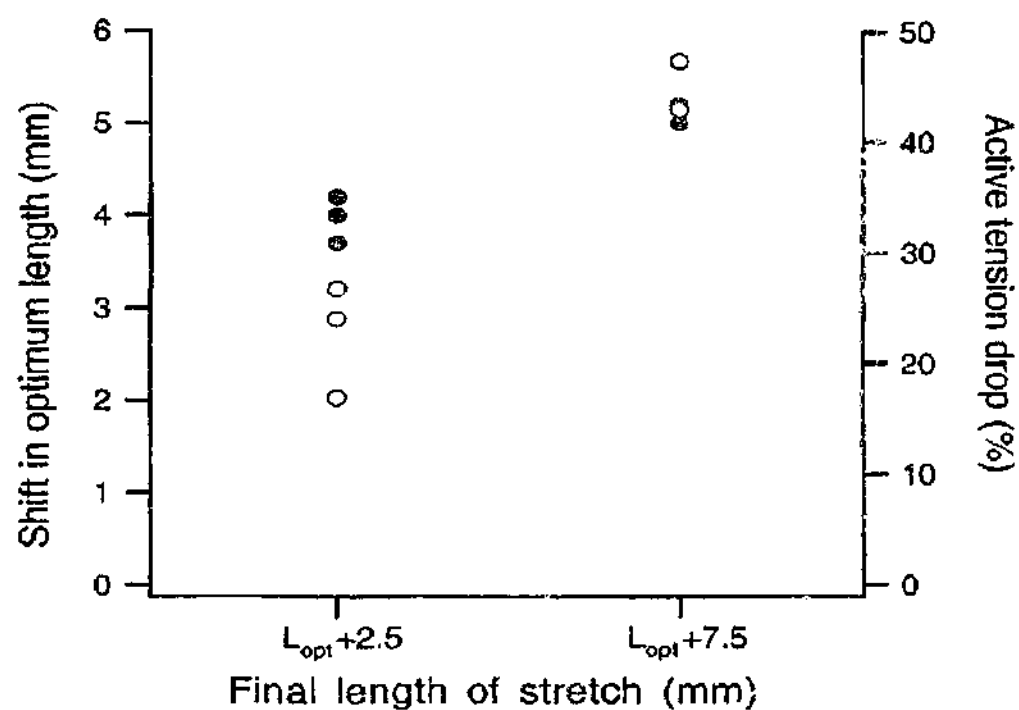
B
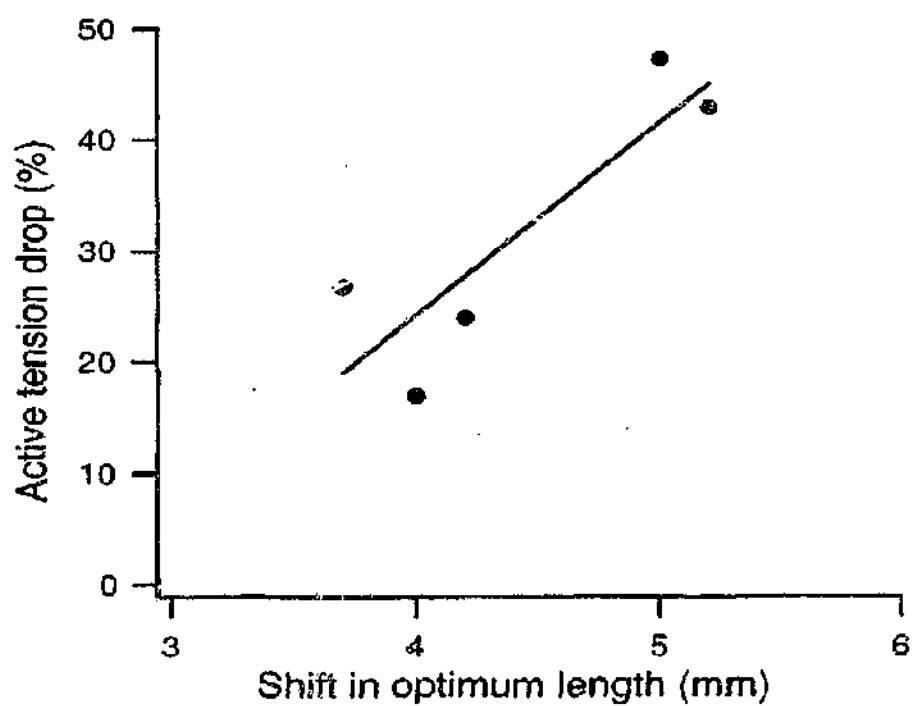
Figure 5.7 *Passive length-tension curves before and after eccentric contractions of the whole muscle at two different lengths.*

Passive length-tension curves as measured for both MG muscles from the same animal before (blue) and after (red) 50 eccentric contractions of the whole muscle at intermediate (A) and long (B) muscle lengths. The length range over which the eccentric contractions were carried out is indicated for each muscle (hatched bar). Tension is plotted against the optimum length measured before the eccentric contractions (L_{opt}).

A

B

Length relative to L_opt (mm)
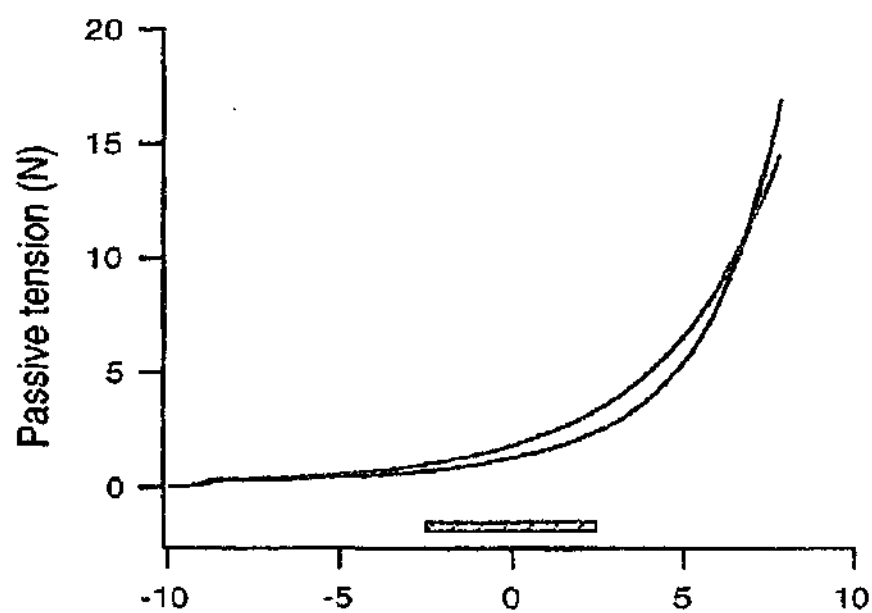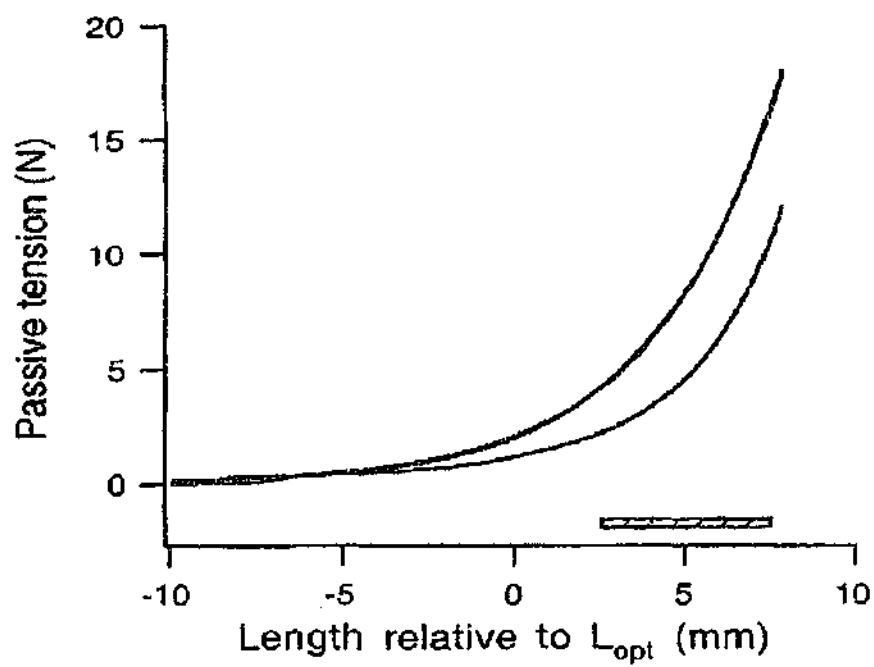
examples, passive tension increased after the eccentric contractions, although the rise was greater following eccentric contractions ending at $L_{opt}+7.5$ mm. The maximum increase in passive tension after eccentric contractions at the longer length was twice that measured after contractions at the intermediate length (93% versus 45%). However, in another experiment, a higher value of passive tension was found after the eccentric contractions over the intermediate length range. The individual and mean values at each length are shown in Figure 5.8 A. These findings highlighted an important point. That is, passive tension increased significantly after whole muscle eccentric contractions at long lengths, which argued against the results of the initial 'three parts' experiments. The effect of 50 passive stretches on changes to passive tension after eccentric contractions was also examined in these experiments. It was found that passive stretches ending at $L_{opt}+7.5$ mm led to a greater drop in passive tension (ranging from 33 to 35%) compared with stretches ending at $L_{opt}+2.5$ mm (ranging from 8.5 to 16%) (Figure 5.8 B).

It was proposed that there would be a greater number of disrupted sarcomeres in muscle fibres after eccentric contractions at longer lengths. Thus, the shift in onset length of passive tension was examined here, shown for one experiment in Figure 5.9 A. Here, it can be seen that after the eccentric contractions at intermediate lengths, ending at $L_{opt}+2.5$ mm, there was a small shift in onset length of 0.16 mm, while after eccentric contractions ending at longer lengths, $L_{opt}+7.5$ mm, a larger shift of 1.4 mm was observed. A similar trend was found for all the three experiments (Figure 5.9 B). The shift onset length was also significantly related to the shift in optimum length of active length-tension relationship ($r^2 = 0.79$. $P < 0.05$).

Since passive stretches were found to reduce passive tension, it seemed possible that changes in passive tension after eccentric contractions might vary depending on the time at which the measurement was made. Thus, in one experiment, after eccentric contractions and passive stretches at the longer length, an additional passive length-tension measurement was made following a delay of about 40 minutes. In the intervening period, the muscle remained relaxed at a short length. It was found that immediately after the eccentric contractions, ending at $L_{opt}+7.5$ mm, passive tension had increased by a maximum of 93%, while after the 40 minute delay, this value had doubled (186%) (Figure 5.10).

Figure 5.8 *Changes in passive tension after eccentric contractions and passive stretches of the whole muscle at two different lengths.*

(A) The maximum percent increase in passive tension after eccentric contractions of the whole muscle at intermediate or long lengths. Data for all 3 experiments is shown, with each coloured symbol representing measurements made on both MG muscles from the same animal. Values are plotted against the length at which the eccentric contractions ended. The mean ($\pm$ S.E.M.) of the 3 values at L_{opt}+2.5 mm, and the mean of the 2 values at L_{opt}+7.5 mm are also shown (open squares). (B) The drop in maximum passive tension from a series of 50 passive stretches at intermediate or long lengths, after eccentric contractions carried out over the same length range. The different colours correspond to values from the same animal as shown in (A). Again, the mean ($\pm$ S.E.M.) of the 3 values at L_{opt}+2.5 mm, and the mean of the 2 values at L_{opt}+7.5 mm are shown (open squares).

A

B

Final length of stretch (mm)
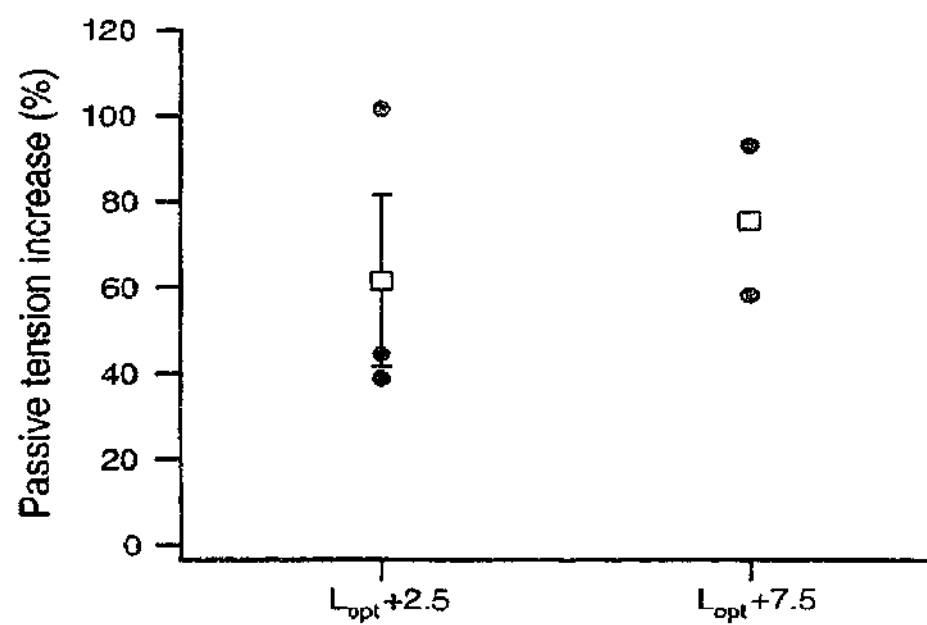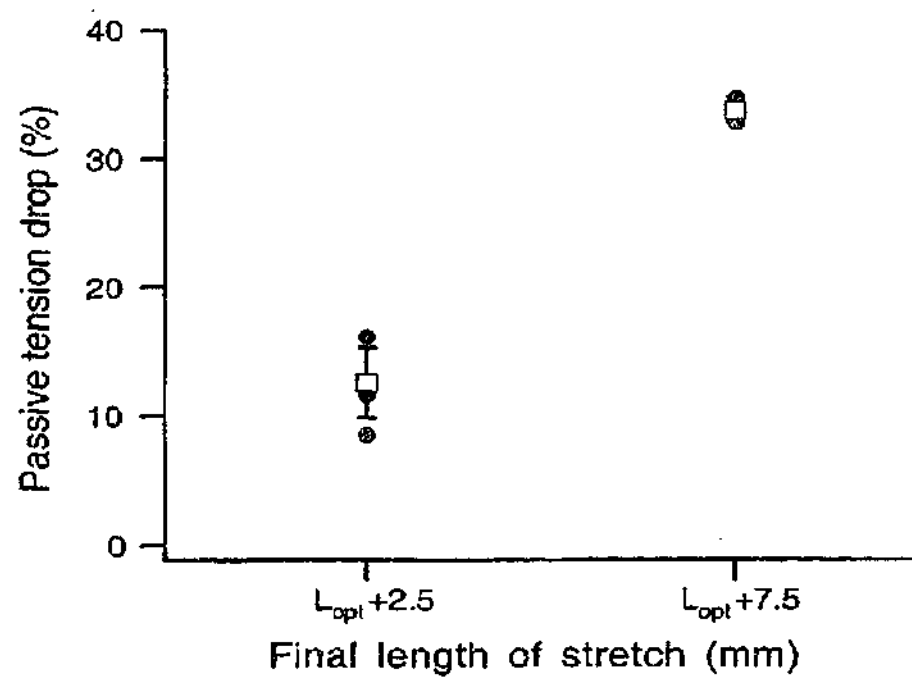
Figure 5.9 *The shift in onset length of passive tension after eccentric contractions of the whole muscle at two different lengths.*

(A) Tension measured during the initial 5 mm of a passive stretch before (blue traces) and after (red traces) eccentric contractions at an intermediate length, ending at L_{opt}+2.5 mm (upper panel) or at a long length, ending at L_{opt}+7.5 mm (lower panel). The corresponding coloured arrows indicate the onset length of passive tension (see Chapter 4 for details) for each tension trace. (B) Values from all 3 experiments showing the shift in onset length of passive tension after the eccentric contractions at the two different muscle lengths. All shifts were in the direction of longer muscle lengths. The different colours correspond to values from both muscles from the same animal.
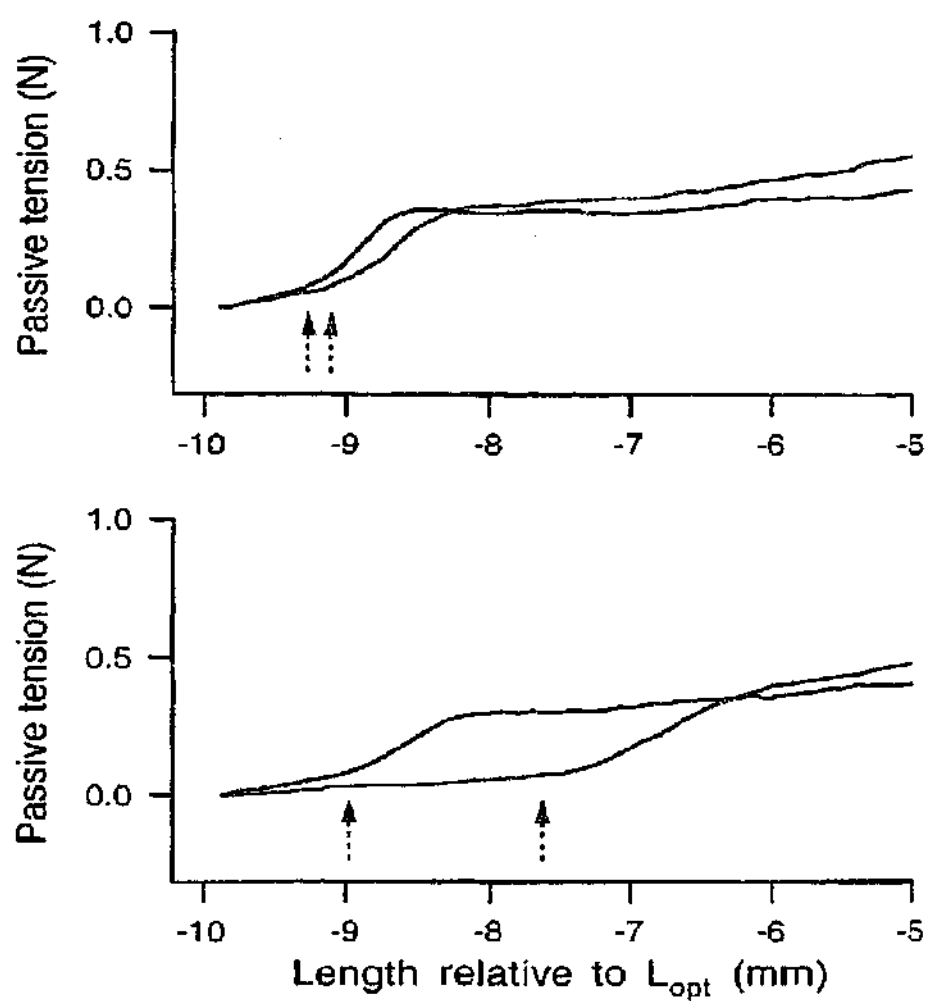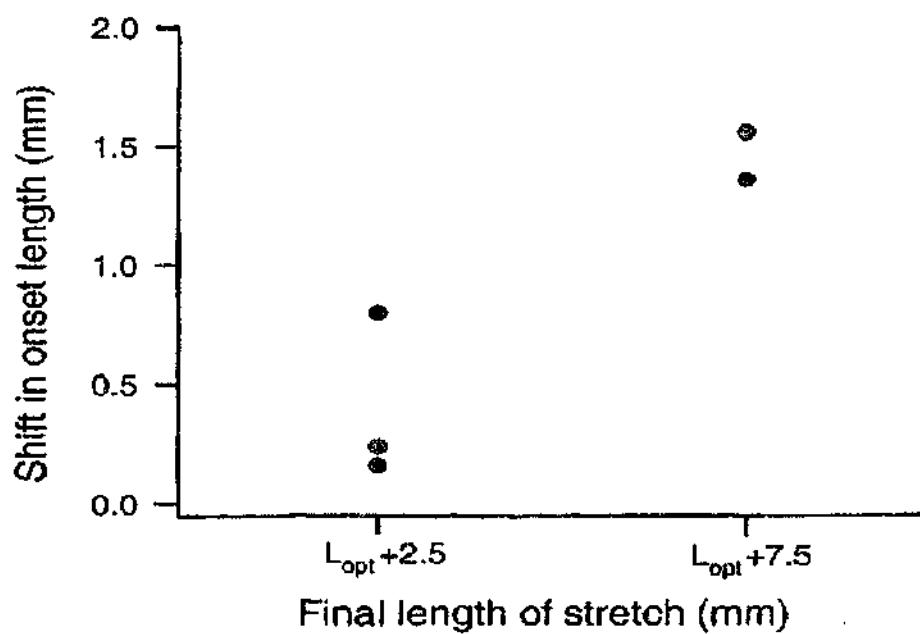
Figure 5.10 *Changes to the passive length-tension relationship after eccentric contractions at a long length, and the effects of a 40 minute delay.*

(A) Passive length-tension curves measured before (blue trace), after 50 eccentric contractions (red trace), and a subsequent delay of about 40 minutes (green trace). The eccentric contractions were carried out a long length (hatched bar). (B) Plots of the increase in passive tension after eccentric contractions (red trace) or 40 minute delay (green trace). The increase in tension was expressed as a percentage of the corresponding value measured before the eccentric contractions. These traces were constructed from the curves shown in (A).

A

Passive tension (N)

B

Passive tension increase (%)

Length relative to L_{opt} (mm)
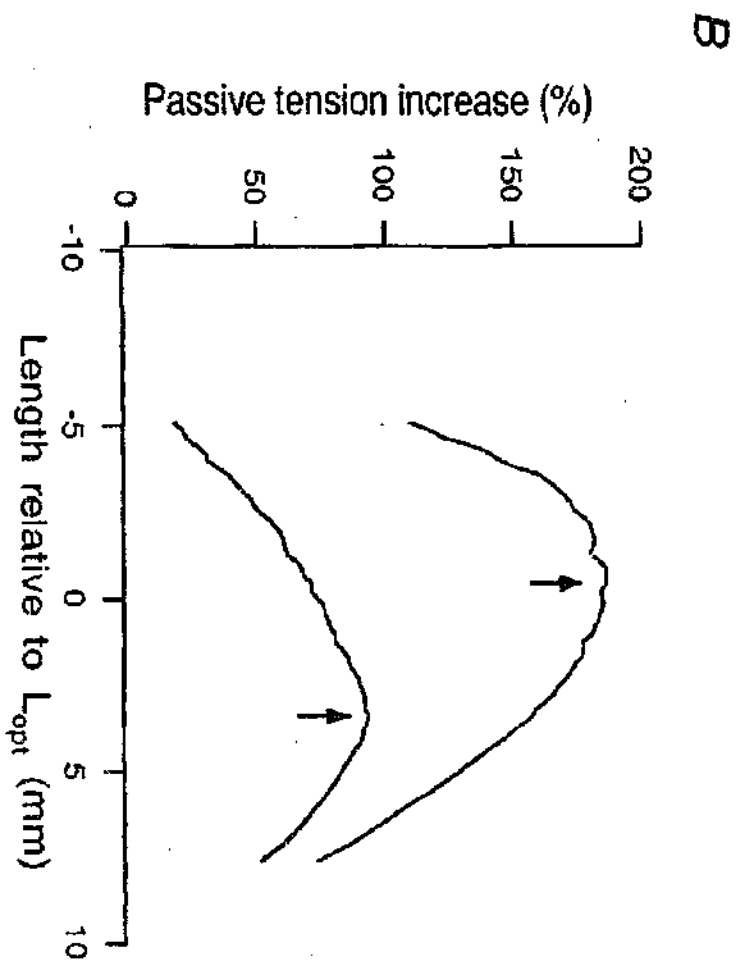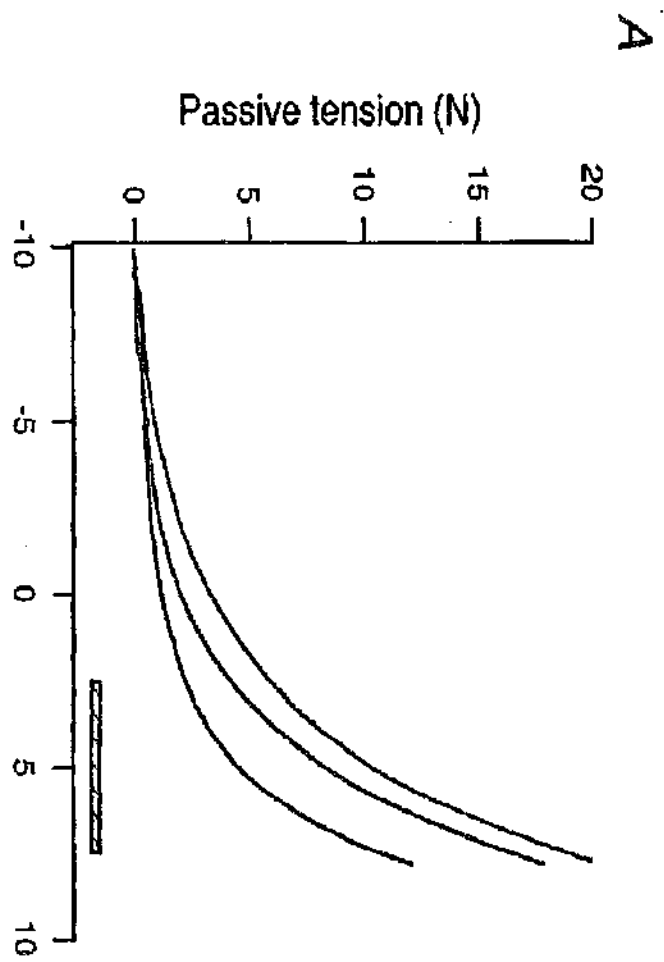
Discussion

Three parts of the muscle experiments

The main aim of these experiments was to investigate the effect of eccentric contractions, performed over different regions of the length-tension relation, on changes to passive mechanical properties of the muscle. In the initial series of five experiments, it was found that the changes in passive tension were dependent on the length range over which the eccentric contractions were carried out. This was shown by the increases in passive tension and work absorption becoming significantly less as the eccentric contractions were carried out at longer muscle lengths. That is, after eccentric contractions on a third of the muscle, where the stretch ended at $L_{opt}+9$ mm, passive tension increased by a maximum of only 3%, compared with 50% or more after eccentric contractions at short to intermediate lengths. Changes in work absorption followed a similar trend. These findings were unexpected as other measures of muscle damage, the shift in optimum length and reduction in active tension, showed that more extensive muscle damage had occurred after eccentric contractions at longer lengths. It seemed likely that the shift in optimum length and drop in active tension for each part of the muscle were reliable measurements, assuming that at the level of the ventral root, motor axons were freely mixed so that the proportions of fatigue resistant and fatiguable motor units (Burke *et al.* 1973) were similar in each third. While this assumption was not formally tested it was considered reasonable, given that in one experiment, isometric contractions of two halves of the muscle produced a similar amount of fatigue.

It was found that the maximum increase in passive tension occurred after eccentric contractions ending on the ascending limb of the active length-tension relationship ($L_{opt}-3$ mm). It had been expected that there would be little damage to the muscle following eccentric contractions over this length range, which was supported by the small shift in optimum length (~2 mm) and drop in active tension (~20%). Since the results from one experiment showed that the fatigue component of the active tension drop at this length was about 10%, it meant that only 10% of the total drop in tension was likely to be due to muscle damage. Thus, the relatively large increase in passive tension and work absorption (~50%) were not consistent with this minor damage to the muscle. It was proposed that changes in passive tension measured after eccentric contractions over this length range were found to be higher, because values measured

after eccentric contractions at longer lengths were significantly underestimated. This was subsequently confirmed by the results of a series of control experiments (see below).

From the results of the initial 'three parts' experiments, the possibility was considered that the increase in passive tension from eccentric contractions to Part 1, was altered by subsequent eccentric contractions to other parts of the muscle. This proposal was examined through two control experiments. In the first control experiment, carried out on two animals, measurements were made before and after eccentric contractions of three parts of the muscle, in which the length range of the stretch was kept the same for each part. Here, it was shown that the increase in passive tension and work absorption were greater following eccentric contractions of Part 1 than Parts 2 and 3, despite each part of the muscle showing a similar degree of muscle damage by active length-tension measurements (see Table 5.1). This implied that the level of passive tension measured after eccentric contractions of one third of the muscle was altered by eccentric contractions given to another third.

A clue as to the underlying cause of this problem was provided by the results of another control experiment. Here, it was shown that a series of passive stretches performed after eccentric contractions to a third of the muscle, led to a significant drop in passive tension and work absorption. Moreover, the effect of the passive stretches in reducing passive tension was greater when they were carried out at longer lengths. This was consistent with the results presented in Chapter 4, where the increase in passive tension and work absorption after eccentric contractions fell from the first to second stretch-shortening movement. These findings implied that in the initial 'three parts' experiments, increases in passive tension after eccentric contractions of Parts 2 and 3 were greatly underestimated, because passive stretches reduced the increase in passive tension for parts of the muscle that had already undergone eccentric contractions. Importantly, when the reduction in passive tension from the passive stretches was added to the mean, maximum increase in passive tension measured in the initial 'three parts' experiments, it was found that the rise in passive tension was now greater for Part 2 and particularly Part 3, following eccentric contractions at long lengths. While these adjusted values (75% and 60%) were found to be higher than those measured following eccentric contractions ending

at L_{opt}-3 mm (50%), no firm conclusions can be drawn because of the degree of uncertainty in these estimates.

Whole muscle experiments

These experiments confirmed that the changes in passive tension under the 'three parts' protocol were underestimated. Furthermore, they provided some important new insights into the measurement of passive properties after eccentric contractions, and the significant effect of passive stretches on the increase in passive tension. The postulated sequence of events leading to these changes, incorporating some of the findings from Chapter 4, is shown in Figure 5.11.

In a series of three experiments, eccentric contractions were carried out on the whole muscle at intermediate muscle lengths, or at longer lengths. In agreement with the initial 'three parts' experiments, more extensive damage occurred after eccentric contractions at longer lengths, as shown by a greater shift in optimum length and drop in peak active tension. However, in contrast with results of the initial 'three parts' experiments, it was shown for the whole muscle that passive tension increased significantly after eccentric contractions at long lengths, by more than 90% in one experiment, as had originally been anticipated. While there was some variability in the size of the increase in passive tension, there was a trend, when values were pooled, for it to be slightly larger after eccentric contractions carried out at the longer length (see Figure 5.8). However, owing to the small sample size, this point deserves further consideration in future experiments, as it is important to determine whether the rise in passive tension correlates with other indicators of muscle damage, which, as described earlier, become greater following eccentric contractions performed at progressively longer lengths.

Based on the results of the 'three parts' control experiments, it was proposed that the increase in passive tension, after eccentric contractions at different lengths, would be reduced by passive stretches. This idea was supported by the observation that a series of 50 passive stretches, performed after the eccentric contractions, led to a significant reduction in passive tension, about 35% when the stretch ended at L_{opt}+7.5 mm. This value was about half of the initial increase after eccentric contractions at the same length (70%). In comparison, the drop in passive tension after passive stretches ending at L_{opt}+2.5 mm was less, about 12%, which represented

a fifth of the increase in passive tension measured after eccentric contractions at this length (60%). In other words, the effectiveness of a passive stretch in reducing the increase in passive tension, produced by a previous series of eccentric contractions, was determined by the length range over which the muscle was stretched.

An interesting finding of these experiments was that following eccentric contractions carried out at longer lengths, ending at $L_{opt}+7.5$ mm, there was a much greater shift in the onset length of passive tension towards longer lengths, than when the eccentric contractions ended at $L_{opt}+2.5$ mm (see Figure 5.10). In Chapter 4, it was proposed that such a shift in the onset length of passive tension was due to the presence of disrupted sarcomeres in damaged muscle fibres. Thus, the current finding of a correlation between the shift in onset length and the shift in optimum length provided an independent piece of evidence, based on changes in passive properties of the muscle, to support the view that a shift in optimum length of the active length-tension relation was due to an increase in series compliance of the muscle as a result of disrupted sarcomeres (Proske & Morgan, 2001).

An important new observation from one of these experiments was that the rise in passive tension was time-dependent. That is, allowing the muscle to relax at a short length for about 40 minutes led to a doubling of the increase in passive tension measured immediately after the eccentric contractions. This seemed to be a characteristic of muscle damaged by eccentric contractions, since measurements made before eccentric contractions indicated that any small drop in passive tension could be rapidly reversed within a few minutes (data not shown). The identification of such a time-dependent effect after eccentric contractions was important, as it now provided some new insights into the events taking place within a damaged muscle fibre. Here, it must be remembered that the 40 minute delay period occurred after a series of 50 passive stretches. It was postulated that there were two likely scenarios to explain such time-dependent changes.

Firstly, it is proposed that if during the eccentric contractions, muscle fibres became damaged to the point where they could no longer propagate an action potential, as reported by McBride et al. (2000), these damaged fibres would then be passively stretched for the remaining eccentric contractions. If these same fibres were undergoing contracture, then passive stretches might act to reduce the size of the

increase in passive tension by causing overextension of sarcomeres and yielding of titin filaments, or possibly breaking up of contracture clots. An additional series of 50 passive stretches would serve to exacerbate this process, leading to a further drop in passive tension. Thus, the additional rise in passive tension after the 40 minute delay might reflect the return of sarcomeres with an longer resting length to normal lengths, so that passive tension begins to rise earlier during the stretch. Interestingly, there is some experimental support for this idea, as it has been shown in rabbit psoas fibres that the process of titin yielding is reversible with time, after a delay of about 20 minutes (Wang *et al.* 1993).

A second scenario is that at least part of the additional rise in passive tension is due to spread of the damage as a result of the passive stretches. This might occur, for example, if passive stretch of a damaged fibre caused further overextension and disruption of sarcomeres, tearing of more muscle membranes, and thereby leading to the development of new regions of contracture. There may also be some additional effects on the already existing regions of contracture, exacerbating the damage and leading to further spread of contracture segments across or along the fibre. It is clear that further research is required in an attempt to differentiate between these possibilities. This could be done experimentally by measuring changes in passive tension immediately after eccentric contractions and following a delay, compared with those measured after the same delay period following both eccentric contractions and passive stretches. It would also be useful to measure changes in passive tension after different delay times, within the first few hours after the eccentric contractions.

In summary, the results described in this chapter have emphasised the fact that careful consideration of several factors must be taken into account when making measurements of passive mechanical properties of muscle after eccentric contractions, and interpreting the changes that occur. However, these findings have also provided new insights into the aetiology of the damage process. The fact that passive stretches can significantly reduce the increase in passive tension from eccentric contractions might be of particular relevance in future experiments, for investigating the longer time-course of muscle damage in humans, and the associated repair and adaptation processes.

Figure 5.11 *Postulated sequence of events leading to damage and changes in passive and active properties of the muscle after eccentric contractions.*

The proposed sequence of events by which: (a) eccentric contractions lead to muscle damage and changes in passive and active mechanical properties of the muscle (red arrows), (b) passive stretches reduce the increased passive tension after eccentric contractions (blue arrows) and (c) the effects of passive stretches are reversible with time (green arrows). In (a) it is proposed that during eccentric contractions, the weakest sarcomeres in muscle fibres become overextended and with repeated eccentric contractions may become disrupted. This, in turn results in an increase in series compliance in the muscle, causing a shift in optimum length to longer lengths. If the region of disruption spreads along or across the fibre, membrane damage occurs. This may impair the E-C coupling process, so that active tension drops. Also, an influx of calcium into the cell might lead to the development of localised regions of contracture, which increases passive tension. The proposition presented in (b), is based on the results described in Chapters 4 and 5. Here, it is envisaged that in fibres with an injury contracture, during passive stretches, particularly at long lengths, normal sarcomeres in these fibres will become overextended, so that titin filaments yield. This increases the rest length of these disrupted sarcomeres, causing a shift to longer lengths of the onset of passive tension, and a decrease in passive tension and work absorption. Finally (c), it is suggested that the effects of passive stretches are reversible with time because disrupted sarcomeres, with an increased rest length, gradually return to normal lengths, thereby increasing passive tension.
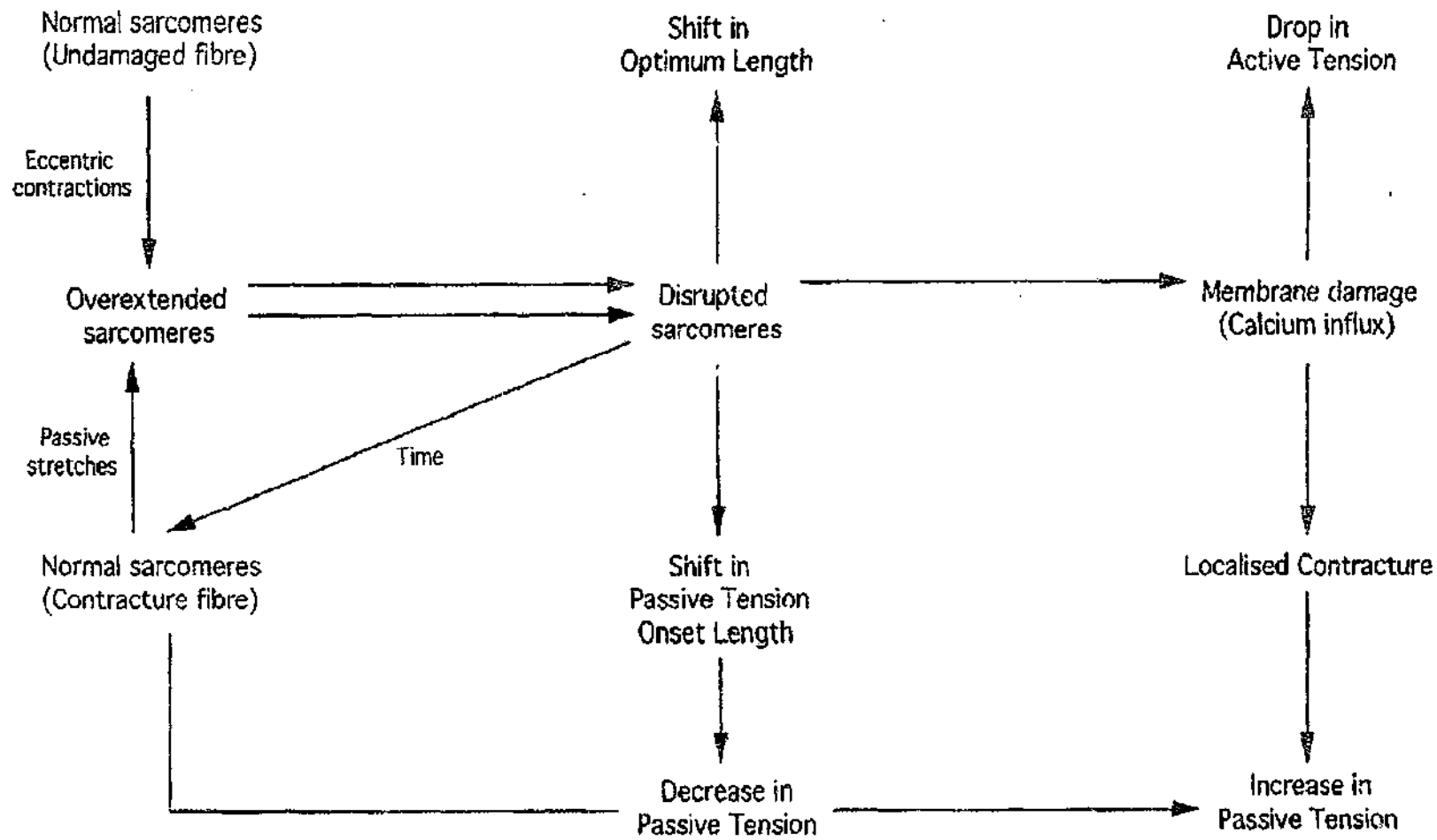
C h a p t e r   S i x

General Discussion

The work undertaken in this thesis investigated the passive mechanical properties of muscle, in relation to its thixotropic behaviour and, in particular, the effects of muscle damage from eccentric contractions. It was observed that the measurement and interpretation of passive properties is a complicated process, depending on both the muscle's previous mechanical history and length. It was also found that examining the mechanism underlying the increase in passive tension following a series of eccentric contractions, provided a means of gaining important new insights into the damage process.

History-dependence of passive tension

In Chapter 2, experimental evidence was provided in support of the hypothesis that a major component of the resting tension at short lengths, and the steep rise in tension during a slow passive stretch, were manifestations of the mechanical properties of stable cross-bridges, present in the resting muscle (Hill, 1968; Campbell & Lakie, 1998). It was also shown that these passive properties were dependent on the type of conditioning imposed on the muscle and the length at which the measurements were made. While these observations may be important in terms of gaining an understanding of passive properties from a mechanical point of view, as discussed in Chapter 2, a pertinent question is; do these findings have any functional significance in terms of everyday movements? After all, the history-dependent component of resting tension at short muscle lengths represented only a very small percentage, less than 0.5%, of the maximum isometric tension produced by the muscle. While this may be so, an important functional consequence of thixotropy, at short to intermediate lengths, is the incorporation of slack into the muscle-tendon unit, following conditioning in which the muscle is shortened (Proske *et al.* 1993). Importantly, the presence of slack in muscle appears to be a physiologically relevant phenomenon during normal movements in vivo (Elek *et al.* 1990; Herbert & Gandevia, 1995; Herbert *et al.* 2002). In fact, Herbert & Gandevia (1995) reported that the passive, human brachialis muscle and tendon remained slack over most of the physiological working range. They suggested that this could impose constraints .

on the active mechanical properties of muscle. The muscle would need to shorten to lengths less than optimum in order to sufficiently stretch the slack tendon to a length where it could maintain the tension produced during a voluntary contraction. This idea is supported by the fact that after shortening a single muscle fibre so that it falls slack, there is a subsequent delay in the rise in tension during an isometric contraction, due to the time taken to remove the slack (Proske *et al.* 1993). In addition, the presence of slack in muscle spindles, due to thixotropic behaviour, has been proposed to have effects on the reflex control of muscle contraction and on proprioception, the ability to sense limb position (see Proske *et al.* 1993).

The history-dependence of the short-range component, during stretch of a passive muscle, also has potentially important functional consequences. As suggested by Campbell & Lakie (1998), an inherent short-range stiffness would provide a mechanism for maintaining postural stability during unexpected perturbations of the muscle, without compromising its ability to execute repeated voluntary movements over a wide range of muscle lengths. It has been proposed that muscle stiffness acts as a load compensation mechanism, providing stability and postural control in a functionally similar way to the stretch reflex, but without the time delays associated with neuromuscular transmission (Grillner, 1972). The effect of thixotropy on the degree of resistance to stretch of a passive antagonist muscle, has recently been shown to alter the neuromuscular activity of the muscle acting as the prime mover (Axelson & Hagbarth, 2001). In this study, it was reported that during repeated voluntary movements, there was a close relationship between the short-range stiffness of the passively stretched antagonist muscle and the EMG activity of the actively shortening agonist. That is, the large short-range stiffness during the first stretch was accompanied by a large burst of EMG activity, while subsequent stretches produced a smaller short-range stiffness and EMG response. This highlights the fact that the motor system must account and appropriately compensate for the history-dependence of the short-range stiffness of the antagonist muscle group in order to precisely control voluntary movements (Axelson & Hagbarth, 2001). It seems likely that this process would also be influenced by muscle length, based on the results of Chapter 2. Here, too, it is important to consider the history-dependent behaviour of the muscle at long lengths, which was proposed to reflect the mechanical properties of titin filaments (see Chapter 2). Since passive tension during

stretch rises quite steeply at long lengths, even beyond the yield point of the short-range component, it might mean that ongoing adjustments by the motor control system are required throughout the movement. The underlying mechanism by which the motor control system adjusts to these history and length-dependent effects on passive mechanical properties is an important issue that is yet to be elucidated (see Axelson & Hagbarth, 2001).

Finally, it has been recently reported that actively contracting muscle displays thixotropic behaviour qualitatively similar to that of passive muscle (Campbell & Moss, 2000; Campbell & Moss, 2002). These studies showed that the size of the short-range tension and stiffness during stretch, and the time course of their re-development, were increased as the calcium concentration of the bathing solution was raised from a level just sufficient to activate the contractile machinery to one that produced maximal tension. On this basis, the authors concluded that the thixotropic properties of both the resting and actively contracting muscle were likely to share a common cross-bridge origin. Thus, investigating the passive mechanical properties of muscle might also have wider implications in terms of gaining new insights into the active contractile function of muscle.

Effect of eccentric contractions on passive properties

The main focus of this thesis was to examine the effect of eccentric contractions on the passive mechanical properties of muscle and, in particular, to gain an understanding of the mechanism underlying the rise in passive tension. The findings presented in Chapter 3 on human TS were qualitatively similar to those of previous studies on human elbow flexors (Howell et al. 1993; Chleboun et al. 1998), in which passive torque (or stiffness) increased immediately after eccentric exercise and was maintained, at more or less similar levels, over the next 4 days. It was proposed that the immediate increase in passive torque was not attributable to neural activation, as indicated by the lack of any EMG activity, nor was it the result of muscle swelling, which was negligible at this time. A similar conclusion was reached by Chleboun et al. (1998). In addition, the passive angle-torque relationship remained essentially unchanged after concentric exercise, suggesting that the rise in passive torque after eccentric exercise was a manifestation of muscle damage. Importantly, the increase in passive torque was correlated with a shift in optimum angle and drop in peak

active torque, highlighting the fact that passive mechanical properties can be used, alongside changes to active properties, as a measure of the degree of muscle damage following eccentric exercise. Adding to this point, it was proposed in Chapter 4 that a delay in the onset of passive tension during a slow stretch provided support for the view that a shift to longer lengths of the optimum length for active tension, was due to the presence of disrupted sarcomeres in muscle fibres from eccentric contractions. That is, disrupted sarcomeres have two effects; they increase the rest length of sarcomeres and increase the series compliance of muscle fibres.

The changes to passive properties measured immediately after eccentric contractions were considered to be of particular importance, in terms of gaining insight into the damage process. This was investigated more formally in Chapter 4 on cat MG. These experiments were designed to test the hypothesis that the increase in passive tension from eccentric contractions was attributable to regions of injury-contracture in some muscle fibres, due to localised damage to muscle membranes and a resultant influx of calcium into the cell. Support for this proposal was provided by the large increase in passive tension and work absorption immediately after the eccentric contractions. The mean increase in passive tension for cat MG (170%) was more than four times greater than the mean increase in passive torque for human TS (40%). This was likely to reflect the fact that the eccentric contractions for human subjects involved submaximal voluntary contractions, whereas cat MG was stimulated synchronously at a maximal rate. Importantly, the percent rise in passive tension was found to vary with muscle length in a similar way for both cat and human muscle. This implied that the same underlying mechanism was responsible for the rise in passive tension in both species, the presence of a contracture in damaged muscle fibres.

In Chapter 4, another approach was tried to test the contracture hypothesis. Here, the responses of Golgi tendon organs were measured during a slow passive stretch, before and after eccentric contractions. Since tendon organs are known to have muscle fibres that insert directly into their capsule (Zelena, 1994), it was envisaged that these receptors would detect and appropriately signal any increase in passive tension from fibres undergoing contracture. Support for this proposal was provided by the fact that after the eccentric contractions, most tendon organs responded to the

rise in passive tension, showing a shift in their response threshold to shorter muscle lengths.

Measuring the responses of tendon organs during passive length-tension measurements offers an important new method of investigating the effects of muscle damage from eccentric contractions on the mechanical properties of the muscle. It has been shown that only one or two muscle fibres from a given motor unit act on a single tendon organ in cat MG (Barker, 1974; Gregory, 1990). Thus, by performing eccentric contractions on a single motor unit, with a strong excitatory action on a tendon organ, it would be possible to effectively measure the rise in passive tension in one or two muscle fibres, as signalled by a tendon organ. In other words, this technique provides a unique opportunity to measure the passive mechanical properties of muscle fibres in vivo, following eccentric contractions of a single motor unit. Preliminary experiments of this kind have been recently carried out in this laboratory. The early results suggest that both tendon organ firing rate and passive tension increase in response to as few as 1 or 2 eccentric contractions of a motor unit, reaching a maximum after about 20 eccentric contractions (Gregory, Morgan & Proske; unpublished observations). The data supports the view that the damage process is progressive, each eccentric contraction exacerbating the damage produced by the one beforehand. Future experiments, using this procedure, are aimed at investigating the time course of changes to passive tension after eccentric contractions and the effects of passive stretches, both of which were shown to have significant effects on the rise in passive tension after eccentric contractions (see Chapter 5). Furthermore, by measuring the tendon organ response to active tension of a motor unit, this technique also has the potential to explore, in vivo, whether or not damaged muscle fibres lose excitability following eccentric contractions. This is an important issue in terms of the mechanism of the reduction in active tension after eccentric contractions, which presently remains unresolved (see Allen, 2001). While the preliminary data suggests that a fibre can show a rise in passive tension and still continue to respond to nerve stimulation (Gregory, Morgan & Proske; unpublished observations), it will be interesting to see future developments from these experiments as they unfold.

In Chapter 4, the issue was raised as to whether or not the rise in resting intracellular calcium concentration following eccentric contractions, is localised to regions of the

fibre adjacent to sites of membrane damage, or is more uniformly distributed across the fibre. While a small, generalised increase in resting calcium concentration in muscle fibres, as shown in some studies (Balnave *et al.* 1997; Lynch *et al.* 1997; Ingalls *et al.* 1998), could feasibly raise the level of tension in muscle fibres, it seems likely that the magnitude of the passive tension rise would be considerably less than for a fibre undergoing an uncontrolled contracture. In Chapter 4, it was estimated, based on some assumptions, that damaged fibres were producing significant passive tension, therefore favouring the contracture hypothesis. Nevertheless, this issue still remains unresolved, and in future single fibre experiments, it would be worth trying to find a relationship between the increase in resting calcium concentration and the rise in passive tension. Having available such information, at the single fibre level, would greatly assist in the interpretation of changes to passive mechanical properties after eccentric contractions of whole muscles.

In Chapter 5, the increase in passive tension after eccentric contractions was reduced when a subsequent series of passive stretches was imposed on the muscle, and it was proposed that this complicated the interpretation of increases in passive tension following eccentric contractions carried out at different lengths. It was suggested that this effect might be attributable to the overextension of sarcomeres in fibres, lying in series with the ongoing contracture, and the subsequent yielding of titin filaments in these sarcomeres. However, alternatively, it could represent the breaking up and redistribution of contracture clots along the fibre. In terms of the process of muscle damage from eccentric contractions, this is certainly an important issue that warrants further investigation. In one experiment, it was shown that passive tension increased immediately after eccentric contractions, fell following a series of passive stretches, and then after a 40 minute delay, increased again to a value twice that measured immediately after the eccentric contractions. As previously suggested in Chapter 5, passive stretches might act to exacerbate the damage from the eccentric contractions, by initiating the development of new regions of contracture and/or extending existing regions, thereby leading to a further rise in passive tension. However, an alternative possibility is that passive stretches reduce the level of passive tension by causing some sarcomeres to overextend and titin filaments to yield, and that the additional rise in passive tension is due to the return of these sarcomeres to normal resting lengths. From a broader perspective, it is

envisaged that such a process might be of relevance for individuals, particularly elite athletes, who regularly perform passive stretching exercises as a means of 'warming down' after a period of strenuous activity. If, for example, the exercise involved eccentric contractions, which caused some muscle damage, then based on the above scenarios, passive stretches could potentially either cause more widespread damage to the muscle or alternatively, they could provide some therapeutic benefits by temporarily reducing passive tension.

Finally, the work undertaken in this thesis has provided evidence that passive mechanical properties can be utilised as a new, important indicator of muscle damage from eccentric contractions. However, in light of the fact that the increase in passive tension remains for a period of several days following eccentric exercise in humans, it is also worth considering whether such a maintained rise in passive tension provides a stimulus for muscle adaptation which, ultimately, offers protection against damage from subsequent eccentric exercise. It is well known that stretch provides an important mechanical stimulus for the addition of sarcomeres to muscle fibres, both in series and parallel (Goldspink, 1999). In addition, it has been reported that increased passive tension stimulates protein synthesis in cultured muscle fibres (Etlinger et al. 1981). Therefore, based on these experimental findings, and the fact that muscle fibres undergoing contracture are likely to be producing considerable tension, as discussed earlier, it is tempting to speculate that a maintained increase in passive tension might provide a trigger for the initiation of sarcomere addition in fibres, as has been shown following eccentric exercise training in rats (Lynn & Morgan, 1994). By manipulating the level of passive tension with, for example, passive stretches, the degree of this adaptation might also be altered. Given that changes to the active length-tension relationship following such an adaptation have been proposed to provide protection against further damage from subsequent eccentric contractions (Brockett et al. 2001), this certainly remains an issue of future importance.

References

ALLEN, D. G. (2001). Eccentric muscle damage: mechanisms of early reduction of force. *Acta Physiologica Scandinavica* **171**, 311-319.

ARMSTRONG, R. B., OGILVIE, R. W. & SCHWANE, J. A. (1983). Eccentric exercise-induced injury to rat skeletal muscle. *Journal of Applied Physiology* **54**, 80-93.

ARMSTRONG, R. B. (1984). Mechanisms of exercise induced delayed onset muscular soreness. *Medicine and Science in Sports and Exercise* **16**, 529-538.

ARMSTRONG, R. B., WARREN, G. L. & WARREN, J. A. (1991). Mechanisms of exercise-induced muscle fibre injury. *Sports Medicine* **12**, 184-207.

ASMUSSEN, E. (1953). Positive and negative muscular work. *Acta Physiologica Scandinavica* **28**, 364-382.

AXELSON, H. W. & HAGBARTH, K. E. (2001). Human motor control consequences of thixotropic changes in muscular short-range stiffness. *Journal of Physiology* **535**, 279-288.

BAGNI, M. A., CECCHI, G., COLOMO, F. & GARZELLA, P. (1995). Absence of mechanical evidence for attached weakly binding cross-bridges in frog relaxed muscle fibres. *Journal of Physiology* **482**, 391-400.

BALNAVE, C. D. & THOMPSON, M. W. (1993). Effect of training on eccentric exercise induced muscle damage. *Journal of Applied Physiology* **75**, 1545-1551.

BALNAVE, C. D. & ALLEN, D. G. (1995). Intracellular calcium and force in single mouse muscle fibres following repeated contractions with stretch. *Journal of Physiology* **488**, 25-36.

BALNAVE, C. D. & ALLEN, D. G. (1996). The effect of muscle length on intracellular calcium and force in single fibres from mouse skeletal muscle. *Journal of Physiology* **492**, 705-713.

BALNAVE, C. D., DAVEY, D. F. & ALLEN, D. G. (1997). Distribution of sarcomere length and intracellular calcium in mouse skeletal muscle following stretch-induced injury. *Journal of Physiology* **502**, 649-659.

BANUS, M. G. & ZETLIN, A. (1938). The relation of isometric tension to length in skeletal muscle. *Journal of Cellular and Comparative Physiology* 12, 403-420.

BÁRÁNY, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *Journal of General Physiology* 50, 197-218.

BARKER, D. (1974). The morphology of muscle receptors. In *Handbook of Sensory Physiology* ed. C. C. Hunt, III/2. pp 1-190. Berlin: Springer-Verlag.

BARTOO, M. L., LINKE, W. A. & POLLACK, G. H. (1997). Basis of passive tension and stiffness in isolated rabbit myofibrils. *American Journal of Physiology* 273, C266-276.

BELCASTRO, A. N. (1993). Skeletal muscle calcium-activated neutral protease (calpain) with exercise. *Journal of Applied Physiology* 74, 1381-1386.

BELCASTRO, A. N., SHEWCHUK, L. D. & RAJ, D. A. (1998). Exercise-induced muscle injury: a calpain hypothesis. *Molecular and Cellular Biochemistry* 179, 135-145.

BERNE, R. M. & LEVY, M. N. (1993). *Physiology*. St Louis: Mosby-Year Book.

BORG, T. K. & CAULFIELD, J. B. (1980). Morphology of connective tissue in skeletal muscle. *Tissue and Cell* 12, 197-207.

BOURGEOIS, J., MACDOUGALL, D., MACDONALD, J. & TARNOPOLSKY, M. (1999). Naproxen does not alter indices of muscle damage in resistance-exercise trained men. *Medicine and Science in Sports and Exercise* 31, 4-9.

BROCKETT, C., WARREN, N., GREGORY, J. E., MORGAN, D. L. & PROSKE, U. (1997). A comparison of the effects of concentric versus eccentric exercise on force and position sense at the human elbow. *Brain Research* 771, 251-258.

BROCKETT, C. L., MORGAN, D. L. & PROSKE, U. (2001). Human hamstring muscles adapt to eccentric exercise by changing optimum length. *Medicine and Science in Sports and Exercise* 33, 783-790.

BROCKETT, C. L., MORGAN, D. L., GREGORY, J. E. & PROSKE, U. (2002). Damage to different motor units from active lengthening of the medial gastrocnemius muscle of the cat. *Journal of Applied Physiology* 92, 1104-1110.

BROWN, L. M. & HILL, L. (1991). Some observations on variations in filament overlap in tetanized muscle fibres and fibres stretched during a tetanus, detected in the electron microscope after rapid fixation. *Journal of Muscle Research and Cell Motility* 12, 171-182.

BURKE, R. E., LEVINE, D. N., TSAIRIS, P. & ZAJAC, F. E. (1973). Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *Journal of Physiology* 234, 723-748.

BYRNES, W. C., CLARKSON, P. M. & KATCH, F. I. (1985a). Muscle soreness following resistance exercise with and without eccentric contractions. *Research Quarterly for Exercise and Sport* 56, 283-285.

BYRNES, W. C., CLARKSON, P. M., WHITE, J. S., HSIEH, S. S., FRYKMAN, P. M. & MAUGHAN, R. J. (1985b). Delayed onset muscle soreness following repeated bouts of downhill running. *Journal of Applied Physiology* 59, 710-715.

BYRNES, W. C. & CLARKSON, P. M. (1986). Delayed onset muscle soreness and training. *Clinics in Sports Medicine* 605-614.

CAMPBELL, K. S. & LAKIE, M. (1998). A cross-bridge mechanism can explain the thixotropic short-range elastic component of relaxed frog skeletal muscle. *Journal of Physiology* 510, 941-962.

CAMPBELL, K. S. & MOSS, R. L. (2000). A thixotropic effect in contracting rabbit psoas muscle: prior movement reduces the initial tension response to stretch. *Journal of Physiology* 525, 531-548.

CAMPBELL, K. S. & MOSS, R. L. (2002). History-dependent mechanical properties of permeabilized rat soleus muscle fibers. *Biophysical Journal* 82, 929-943.

CHLEBOUN, G. S., HOWELL, J. N., CONATSER, R. R. & GIESEY, J. J. (1998). Relationship between muscle swelling and stiffness after eccentric exercise. *Medicine and Science in Sports and Exercise* 30, 529-535.

CLARKSON, P. M. & TREMBLAY, I. (1988). Exercise-induced muscle damage, repair and adaptation in humans. *Journal of Applied Physiology* 65, 1-6.

CLARKSON, P. M., NOSAKA, K. & BRAUN, B. (1992). Muscle function after exercise-induced muscle damage and rapid adaption. *Medicine and Science in Sports and Exercise* 24, 512-520.

CLARKSON, P. M. & NEWHAM, D. J. (1995). Associations between muscle soreness, damage, and fatigue. *Advances in Experimental Medicine and Biology* 384, 457-469.

CLEAK, M. J. & ESTON, R. G. (1992). Muscle soreness, swelling, stiffness and strength loss after intense eccentric exercise. *British Journal of Sports Medicine.* 26, 267-272.

CLOSE, R. I. (1972). Dynamic properties of mammalian skeletal muscles. *Physiological Reviews* 52, 129-197.

CLOSE, R. I. (1981). Activation delays in frog twitch muscle fibres. *Journal of Physiology* 313, 81-100.

CROISIER, J. L., CAMUS, G., MONFILS, T., DEBYDUPON, G., FAFCHAMPS, F., VENNEMAN, I., CRIELAARD, J. M., JUCHMESFERIR, A., LHERMEROUT, C., LAMY, M. & DEBY, C. (1996). Piroxicam fails to reduce myocelluar enzyme leakage and delayed onset muscle soreness induced by isokinetic eccentric exercise. *Mediators of Inflammation* 5, 230-254.

CURTIN, N. A. & DAVIES, R. E. (1973). Chemical and mechanical changes during stretching of activated frog skeletal muscle. *Cold Spring Harbour Symposia on Quantitative Biology* 37, 619-626.

DARR, K. C. & SCHULTZ, E. (1987). Exercise-induced satellite cell activation in growing and mature skeletal muscle. *Journal of Applied Physiology* 63, 1816-1821.

DAVIES, C. T. M. & WHITE, M. J. (1981). Muscle weakness following eccentric work in man. *Pflugers Archiv- European Journal of Physiology* 392, 168-171.

DENNY-BROWN, D. (1929). On the nature of postural reflexes. *Proceedings of the Royal Society of London B* 104, 252-301.

DONNELLY, A. E., CLARKSON, P. M. & MAUGHAN, R. J. (1992). Exercise-induced muscle damage: effects of light exercise on damaged muscle. *European Journal of Applied Physiology and Occupational Physiology* 64, 350-353.

DUAN, C., DELP, M. D., HAYES, D. A., DELP, P. D. & ARMSTRONG, R. B. (1990). Rat skeletal muscle mitochondrial [Ca2+] and injury from downhill walking. *Journal of Applied Physiology* **68,** 1241-1251.

DULHUNTY, A. F. & FRANZINI-ARMSTRONG, C. (1975). The relative contributions of the folds and caveolae to the surface membrane of frog skeletal muscle fibres at different sarcomere lengths. *Journal of Physiology* **250,** 513-539.

EDMAN, K. A., ELZINGA, G. & NOBLE, M. I. (1978). Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. *Journal of Physiology* **281,** 139-155.

ELEK, J., PROCHAZKA, A., HULLIGER, M. & VINCENT, S. (1990). In-series compliance of gastrocnemius muscle in cat step cycle: do spindles signal origin-to-insertion length? *Journal of Physiology* **429,** 237-258.

ENDO, M. (1973). Length dependence of activation of skinned muscle fibers by calcium. *Cold Spring Harbour Symposia on Quantitative Biology* **37,** 505-510.

ETLINGER, J. D., KAMEYAMA, T., VAN DER WESTHUYZEN, D., ERLIJ, D. & MATSUMOTO, K. (1981). Roles of calcium and tension in the regulation of protein turnover in muscle. In *Mechanism of Muscle Adaptation to Functional Requirements* ed. F. Guba, G. Marechal and O. Takacs, pp 241-253. New York: Pergamon.

FAULKNER, J. A., BROOKS, S. V. & OPITECK, J. A. (1993). Injury to skeletal muscle fibers during contractions: Conditions of occurrence and prevention. *Physical Therapy* **73,** 911-921.

FENN, W. O. & MARSH, B. S. (1935). Muscle force at different speeds of shortening. *Journal of Physiology* **85,** 227-297.

FLITNEY, F. W. & HIRST, D. G. (1978). Cross-bridge detachment and sarcomere 'give' during stretch of active frog's muscle. *Journal of Physiology* **276,** 449-465.

FLUCHER, B. E. (1992). Structural analysis of muscle development: transverse tubules, sarcoplasmic reticulum, and the triad. *Developmental Biology* **154,** 245-260.

FREUND, H. J. (1983). Motor unit and muscle activity in voluntary motor control. *Physiological Reviews* **63,** 387-436.

FRIDÉN, J., SJOSTROM, M. & EKBLOM, B. (1981). A morphological study of delayed muscle soreness. *Experientia* **37**, 506-507.

FRIDÉN, J., SJOSTROM, M. & EKBLOM, B. (1983). Myofibrillar damage following intense eccentric exercise in man. *International Journal of Sports Medicine* **4**, 170-176.

FRIDÉN, J., KJORELL, U. & THORNELL, L.-E. (1984). Delayed muscle soreness and cytoskeletal alterations: An immunocytological study in man. *International Journal of Sports Medicine* **5**, 15-18.

FRIDÉN, J. & LIEBER, R. L. (1996). Ultrastructural evidence for loss of calcium homeostasis in exercised skeletal muscle. *Acta Physiologica Scandinavica* **158**, 381-382.

FRIDÉN, J. & LIEBER, R. L. (1998). Segmental muscle fibre lesions after repetitive eccentric contractions. *Cell and Tissue Research* **293**, 165-171.

FRIDÉN, J. & LIEBER, R. L. (2001). Serum creatine kinase level is a poor predictor of muscle function after injury. *Scandinavian Journal of Medicine & Science in Sports* **11**, 126-127.

FUNATSU, T., HIGUCHI, H. & ISHIWATA, S. (1990). Elastic filaments in skeletal muscle revealed by selective removal of thin filaments with plasma gelsolin. *Journal of Cell Biology* **110**, 53-62.

FÜRST, D. O., OSBORN, M., NAVE, R. & WEBER, K. (1988). The organization of titin filaments in the half-sarcomere revealed by monoclonal antibodies in immunoelectron microscopy: a map of ten nonrepetitive epitopes starting at the Z line extends close to the M line. *Journal of Cell Biology* **106**, 1563-1572.

GOLDSPINK, G. (1999). Changes in muscle mass and phenotype and the expression of autocrine and systemic growth factors by muscle in response to stretch and overload. *Journal of Anatomy* **194**, 323-334.

GORDON, A. M., HUXLEY, A. F. & JULIAN, F. J. (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *Journal of Physiology* **184**, 170-192.

GRANZIER, H., HELMES, M., CAZORLA, O., MCNABB, M., LABEIT, D., WU, Y., YAMASAKI, R., REDKAR, A., KELLERMAYER, M., LABEIT, S. & TROMBITAS, K. (2000). Mechanical properties of titin isoforms. *Advances in Experimental Medicine and Biology* **481**, 283-300.

GREGORY, J. E. (1990). Relations between identified tendon organs and motor units in the medial gastrocnemius muscle of the cat. *Experimental Brain Research* **81**, 602-608.

GREGORY, J. E., BROCKETT, C. L., MORGAN, D. L., WHITEHEAD, N. P. & PROSKE, U. (2002). Effect of eccentric muscle contractions on Golgi tendon organ responses to passive and active tension in the cat. *Journal of Physiology* **538**, 209-218.

GRILLNER, S. (1972). The role of muscle stiffness in meeting the changing postural and locomotor requirements for force development by the ankle extensors. *Acta Physiologica Scandinavica* **86**, 92-108.

HAUGEN, P. & STEN-KNUDSEN, O. (1981). The dependence of the short-range elasticity of sarcomere length in resting isolated frog muscle fibres. *Acta Physiologica Scandinavica* **112**, 113-120.

HERBERT, R. D. & GANDEVIA, S. C. (1995). Changes in pennation with joint angle and muscle torque: in vivo measurements in human brachialis muscle. *Journal of Physiology* **484**, 523-532.

HERBERT, R. D. & CROSBIE, J. (1997). Rest length and compliance of non-immobilised and immobilised rabbit soleus muscle and tendon. *European Journal of Applied Physiology and Occupational Physiology* **76**, 472-479.

HERBERT, R. D., MOSELEY, A. M., BUTLER, J. E. & GANDEVIA, S. C. (2002). Change in length of relaxed muscle fascicles and tendons with knee and ankle movement in humans. *Journal of Physiology* **539**, 637-645.

HERBST, M. (1976). Studies on the relation between latency relaxation and resting cross- bridges of frog skeletal muscle. *Pflugers Archiv- European Journal of Physiology* **364**, 71-76.

HERZOG, W., GUIMARAES, A. C. S. & ZHANG, Y. T. (1999). EMG signal recording. In *Biomechanics of the musculo-skeletal system* ed. B. M. Nigg and W. Herzog, pp 351-355. West Sussex: Wiley.

HILL, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proceedings of the Royal Society of London B* **126**, 135-195.

HILL, D. K. (1968). Tension due to interaction between the sliding filaments in resting striated muscle. The effect of stimulation. *Journal of Physiology* **199**, 637-684.

HOROWITS, R., KEMPNER, E. S., BISHER, M. E. & PODOLSKY, R. J. (1986). A physiological role for titin and nebulin in skeletal muscle. *Nature* **323**, 160-164.

HOROWITS, R. (1999). The physiological role of titin in striated muscle. *Reviews of Physiology, Biochemistry and Pharmacology* **138**, 57-96.

HOUGH, T. (1902). Ergographic studies in muscular soreness. *American Journal of Physiology* **7**, 76-92.

HOWELL, J. N., CHILA, A. G., FORD, G., DAVID, D. & GATES, T. (1985). An electromyographic study of elbow motion during postexercise muscle soreness. *Journal of Applied Physiology* **58**, 1713-1718.

HOWELL, J. N., CHLEBOUN, G. & CONATSER, R. (1993). Muscle stiffness, strength loss, swelling and soreness following exercise-induced injury in humans. *Journal of Physiology* **464**, 183-196.

HUFSCHMIDT, A. & SCHWALLER, I. (1987). Short-range elasticity and resting tension of relaxed human lower leg muscles. *Journal of Physiology* **391**, 451-465.

HUIJING, P. A. (1999). Muscle as a collagen fiber reinforced composite: a review of force transmission in muscle and whole limb. *Journal of Biomechanics* **32**, 329-345.

HUNT, C. C. (1954). Relation of function to diameter in afferent fibers of muscle nerves. *Journal of General Physiology* **38**, 117-131.

HUXLEY, A. F. & NIEDERGERKE, R. (1954). Structural changes in muscle during contraction. *Nature* **173**, 971-973.

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

HUXLEY, A. F. & SIMMONS, R. M. (1970). Rapid 'give' and the tension 'shoulder' in the relaxation of frog muscle fibres. *Journal of Physiology* **210**, 32P-33P.

HUXLEY, A. F. (2000). Cross-bridge action: present views, prospects, and unknowns. *Journal of Biomechanics* **33**, 1189-1195.

HUXLEY, H. E. & HANSON, J. (1954). Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. *Nature* **173**, 973-976.

HUXLEY, H. E. (1957b). The double array of filaments in cross-striated muscle. *Journal of Biophysical and Biochemical Cytology* **3**, 631-648.

INGALLS, C. P., WARREN, G. L., WILLIAMS, J. H., WARD, C. W. & ARMSTRONG, R. B. (1998). E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions. *Journal of Applied Physiology* **85**, 58-67.

JONES, C., ALLEN, T., TALBOT, J., MORGAN, D. L. & PROSKE, U. (1997). Changes in the mechanical properties of human and amphibian muscle after eccentric exercise. *European Journal of Applied Physiology and Occupational Physiology* **76**, 21-31.

JONES, D. A., NEWHAM, J. M., ROUND, J. M. & TOLFREE, S. E. J. (1986). Experimental human muscle damage: morphological changes in relation to other indices of damage. *Journal of Physiology* **375**, 435-448.

JONES, D. A., NEWHAM, D. J. & CLARKSON, P. M. (1987). Skeletal muscle stiffness and pain following eccentric exercise of the elbow flexors. *Pain* **30**, 233-242.

JONES, D. A., NEWHAM, D. J. & TORGAN, C. (1989). Mechanical influences on long-lasting human muscle fatigue and delayed-onset pain. *Journal of Physiology* **412**, 415-427.

KATZ, B. (1939). The relation between force and speed in muscular contraction. *Journal of Physiology* **96**, 45-64.

KELLERMAYER, M. S., SMITH, S. B., BUSTAMANTE, C. & GRANZIER, H. L. (1998). Complete unfolding of the titin molecule under external force. *Journal of Structural Biology* **122**, 197-205.

KELLERMAYER, M. S., SMITH, S. B., BUSTAMANTE, C. & GRANZIER. H. L. (2001). Mechanical fatigue in repetitively stretched single molecules of titin. *Biophysical Journal* **80**, 852-863.

KOMULAINEN, J., TAKALA, T. E., KUIPERS, H. & HESSELINK, M. K. (1998). The disruption of myofibre structures in rat skeletal muscle after forced lengthening contractions. *Pflugers Archiv- European Journal of Physiology* **436**, 735-741.

LABEIT, S. & KOLMERER, B. (1995). Titins: giant proteins in charge of muscle ultrastructure and elasticity. *Science* **270**, 293-296.

LAKIE, M., WALSH, E. G. & WRIGHT, G. W. (1984). Resonance at the wrist demonstrated by the use of a torque motor: an instrumental analysis of muscle tone in man. *Journal of Physiology* **353**, 265-285.

LAKIE, M. & ROBSON, L. G. (1988a). Thixotropy: the effect of stimulation in frog muscle. *Quarterly Journal of Experimental Physiology* **73**, 627-630.

LAKIE, M. & ROBSON, L. G. (1988b). Thixotropic changes in human muscle stiffness and the effects of fatigue. *Quarterly Journal of Experimental Physiology* **73**, 487-500.

LAKIE, M. & ROBSON, L. G. (1988c). Thixotropy: stiffness recovery rate in relaxed frog muscle. *Quarterly Journal of Experimental Physiology* **73**, 237-239.

LAKIE, M. & ROBSON, L. G. (1988d). Thixotropy: the effect of stretch size in relaxed frog muscle. *Quarterly Journal of Experimental Physiology* **73**, 127-129.

LANNERGREN, J. (1971). The effect of low-level activation on the mechanical properties of isolated frog muscle fibres. *The Journal of General Physiology* **58**, 145-162.

LAZARIDES, E. (1980). Intermediate filaments as mechanical integrators of cellular space. *Nature* **283**, 249-256.

LIEBER, R. L., THORNELL, L.-E. & FRIDÉN, J. (1996). Muscle cytoskeletal disruption occurs within the first 15 min of cyclic eccentric contraction. *Journal of Applied Physiology* **80**, 278-284.

LINKE, W. A., IVEMIBYER, M., OLIVIERI, N., KOLMERER, B., RUEGG, J. C. & LABEIT, S. (1996). Towards a molecular understanding of the elasticity of titin. *Journal of Molecular Biology* **261**, 62-71.

LOWE, D. A., WARREN, G. L., INGALLS, C. P., BOORSTEIN, D. B. & ARMSTRONG, R. B. (1995). Muscle function and protein metabolism after initiation of eccentric contraction-induced injury. *Journal of Applied Physiology* **79**, 1260-1270.

LYNCH, G. S., FARY, C. J. & WILLIAMS, D. A. (1997). Quantitative measurement of resting skeletal muscle [Ca2+]i following acute and long-term downhill running exercise in mice. *Cell Calcium* **22**, 373-383.

LYNN, R. & MORGAN, D. L. (1994). Decline running produces more sarcomeres in rat vastus intermedius muscle fibres than incline running. *Journal of Applied Physiology* **79**, 831-838.

LYNN, R., TALBOT, J. A. & MORGAN, D. L. (1998). Differences in rat skeletal muscles after incline and decline running. *Journal of Applied Physiology* **85**, 98-104.

MACPHERSON, P. C., DENNIS, R. G. & FAULKNER, J. A. (1997). Sarcomere dynamics and contraction-induced injury to maximally activated single muscle fibres from soleus muscles of rats. *Journal of Physiology* **500**, 523-533.

MAGANARIS, C. N., BALTZOPOULOS, V. & SARGEANT, A. J. (1998). Changes in Achilles tendon moment arm from rest to maximum isometric plantarflexion: in vivo observations in man. *Journal of Physiology* **510**, 977-985.

MAGID, A. & LAW, D. J. (1985). Myofibrils bear most of the resting tension in frog skeletal muscle. *Science* **230**, 1280-1282.

MALACHY, P., MCHUGH, S., MAGNUSSON, P., GLEIM, G. W. & NICHOLAS, J. A. (1992). Viscoelastic stress relaxation in human skeletal muscle. *Medicine and Science in Sports and Exercise.* **24**, 1375-1382.

MARUYAMA, K., KIMURA, M., KIMURA, S., OHASHI, K., SUZUKI, K. & KATUNUMA, N. (1981). Connectin, an elastic protein of muscle. Effects of proteolytic enzymes in situ. *Journal of Biochemistry (Tokyo)* **89**, 711-715.

McBRIDE, T. A., STOCKERT, B. W., GORIN, F. A. & CARLSEN, R. C. (2000). Stretch-activated ion channels contribute to membrane depolarization after eccentric contractions. *Journal of Applied Physiology* **88**, 91-101.

McCALLISTER, L. P. & HADEK, R. (1970). Transmission electron microscopy and stereo ultrastructure of the T system in frog skeletal muscle. *Journal of Ultrastructure Research* **33**, 360-368.

McCULLY, K. K. & FAULKNER, J. A. (1985). Injury to skeletal muscle fibers of mice following lengthening contractions. *Journal of Applied Physiology* **59**, 119-126.

McCULLY, K. K. & FAULKNER, J. A. (1986). Characteristics of lengthening contractions associated with injury to skeletal muscle fibers. *Journal of Applied Physiology* **61**, 293-299.

McHUGH, M. P., CONNOLLY, D. A., ESTON, R. G. & GLEIM, G. W. (1999). Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. *Sports Medicine* **27**, 157-170.

MELZER, W., HERRMANN-FRANK, A. & LUTTGAU, H. C. (1995). The role of Ca2+ ions in excitation-contraction coupling of skeletal muscle fibres. *Biochimica et Biophysica Acta* **1241**, 59-116.

MENSE, S. (1977). Muscular nociceptors. *Journal of Physiology (Paris)* **73**, 233-240.

MINAJEVA, A., KULKE, M., FERNANDEZ, J. M. & LINKE, W. A. (2001). Unfolding of titin domains explains the viscoelastic behavior of skeletal myofibrils. *Biophysical Journal* **80**, 1442-1451.

MORGAN, D. L., PROCHAZKA, A. & PROSKE, U. (1984). The after-effects of stretch and fusimotor stimulation on the responses of primary endings of cat muscle spindles. *Journal of Physiology* **356**, 465-477.

MORGAN, D. L. (1990). New insights into the behaviour of muscle during active lengthening. *Biophysical Journal* **57**, 209-221.

MORGAN, D. L., CLAFLIN, D. R. & JULAIN, F. J. (1996). The effects of repeated active stretches on tension generation and myoplasmic calcium in frog single muscle fibres. *Journal of Physiology* **497**, 665-674.

MORGAN, D. L. & ALLEN, D. G. (1999). Early events in stretch-induced muscle damage. *Journal of Applied Physiology* **87**, 2007-2015.

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 contracting muscle. *Journal of Physiology* **522**, 503-513.

MOSS, R. L., SOLLINS, M. R. & JULIAN, F. J. (1976). Calcium activation produces a characteristic response to stretch in both skeletal and cardiac muscle. *Nature* **260**, 619-621.

MOSS, R. L. & HALPERN, W. (1977). Elastic and viscous properties of resting frog skeletal muscle. *Biophysical Journal* **17**, 213-228.

MUTUNGI, G. & RANATUNGA, K. W. (1996a). The viscous, viscoelastic and elastic characteristics of resting fast and slow mammalian (rat) muscle fibres. *Journal of Physiology* **496**, 827-836.

MUTUNGI, G. & RANATUNGA, K. W. (1996b). The visco-elasticity of resting intact mammalian (rat) fast muscle fibres. *Journal of Muscle Research and Cell Motility* **17**, 357-364.

MUTUNGI, G. & RANATUNGA, K. W. (1998). Temperature-dependent changes in the viscoelasticity of intact resting mammalian (rat) fast- and slow-twitch muscle fibres. *Journal of Physiology* **508**, 253-265.

MUTUNGI, G. & RANATUNGA, K. W. (2000). Do cross-bridges contribute to the tension during stretch of passive muscle? a response. *Journal of Muscle Research and Cell Motility* **21**, 301-302.

NEWHAM, D. J., MCPHAIL, G., MILLS, K. R. & EDWARDS, R. H. (1983a). Ultrastructural changes after concentric and eccentric contractions of human muscle. *Journal of the Neurological Sciences* **61**, 109-122.

NEWHAM, D. J., MILLS, K. R., QUIGLEY, B. M. & EDWARDS, R. H. T. (1983b). Pain and fatigue after concentric and eccentric muscle contractions. *Clinical Science* **64**, 55-62.

NEWHAM, D. J., JONES, D. A. & EDWARDS, R. H. (1986). Plasma creatine kinase changes after eccentric and concentric contractions. *Muscle and Nerve* **9**, 59-63.

NEWHAM, D. J., JONES, D. A. & CLARKSON, P. M. (1987). Repeated high-force eccentric exercise: effects on muscle pain and damage. *Journal of Applied Physiology* **63**, 1381-1386.

NEWHAM, D. J. (1988). The consequences of eccentric contractions and their relationship to delayed onset muscle pain. *European Journal of Applied Physiology and Occupational Physiology* **57**, 353-359.

NEWHAM, D. J., JONES, D. A., GHOSH, G. & AURORA, P. (1988). Muscle fatigue and pain after eccentric contractions at long and short length. *Clinical Science* **74**, 553-557.

NOSAKA, K., CLARKSON, P. M., MCGUIGGIN, M. E. & BYRNE, J. M. (1991). Time course of muscle adaptation after high force eccentric exercise. *European Journal of Applied Physiology and Occupational Physiology* **63**, 70-76.

OGILVIE, R. W., ARMSTRONG, R. B., BAIRD, K. E. & BOTTOMS, C. L. (1988). Lesions in the rat soleus muscle following eccentrically biased exercise. *American Journal of Anatomy* **182**, 335-346.

PAGE, S. & HUXLEY, H. E. (1963). Filament length in striated muscle. *Journal of Cell Biology* **19**, 369-390.

PATEL, T. J., CUIZON, D., MATHIEU-COSTELLO, O., FRIDÉN, J. & LIEBER, R. L. (1998). Increased oxidative capacity does not protect skeletal muscle fibers from eccentric contraction-induced injury. *American Journal of Physiology* **274**, R1300-1308.

PIZZA, F. X., DAVIS, B. H., HENRICKSON, S. D., MITCHELL, J. B., PACE, J. F., BIGELOW, N., DILAURO, P. & NAGLIERI, T. (1996). Adaptation to eccentric exercise: effect on CD64 and CD11b/CD18 expression. *Journal of Applied Physiology* **80**, 47-55.

PLOUTZ-SNYDER, L. L., TESCH, P. A. & DUDLEY, G. A. (1998). Increased vulnerability to eccentric exercise-induced dysfunction and muscle injury after concentric training. *Archives of Physical Medicine and Rehabilitation* 79, 58-61.

PODOLSKY, R. J. (1964). The maximum sarcomere length for contraction of isolated myofibrils. *Journal of Physiology* 170, 110-123.

PROSKE, U. (1981). The Golgi tendon organ. Properties of the receptor and reflex action of impulses arising from tendon organs. *International Reviews in Physiology* 25, 127-171.

PROSKE, U. & STUART, G. J. (1985). The initial burst of impulses in responses of toad muscle spindles during stretch. *Journal of Physiology* 368, 1-17.

PROSKE, U., MORGAN, D. L. & GREGORY, J. E. (1993). Thixotropy in skeletal muscle and in muscle spindles: a review. *Progress in Neurobiology* 41, 705-721.

PROSKE, U. & MORGAN, D. L. (1999). Do cross-bridges contribute to the tension during stretch of passive muscle? *Journal of Muscle Research and Cell Motility* 20, 433-442.

PROSKE, U. & MORGAN, D. L. (2001). Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *Journal of Physiology* 537, 333-345.

PURSLOW, P. P. (1989). Strain-induced reorientation of an intramuscular connective tissue network: implications for passive muscle elasticity. *Journal of Biomechanics* 22, 21-31.

PURSLOW, P. P. & TROTTER, J. A. (1994). The morphology and mechanical properties of endomysium in series-fibred muscles: variations with muscle length. *Journal of Muscle Research and Cell Motility* 15, 299-308.

RACK, P. M. & WESTBURY, D. R. (1969). The effects of length and stimulus rate on tension in the isometric cat soleus muscle. *Journal of Physiology* 204, 443-460.

RACK, P. M. & WESTBURY, D. R. (1974). The short range stiffness of active mammalian muscle and its effect on mechanical properties. *Journal of Physiology* 240, 331-350.

RAMSEY, R. W. & STREET, S. F. (1940). The isometric length-tension diagram of isolated skeletal muscle fibres of the frog. *Journal of Cellular and Comparative Physiology* **15**, 11-34.

RANATUNGA, K. W. (2001). Sarcomere visco-elasticity of chemically skinned skeletal muscle fibres of the rabbit at rest. *Journal of Muscle Research and Cell Motility* **22**, 399-414.

RAPOPORT, S. I. (1972). Mechanical properties of the sarcolemma and myoplasm in frog muscle as a function of sarcomere length. *Journal of General Physiology* **59**, 559-585.

RUGG, S. G., GREGOR, R. J., MANDELBAUM, B. R. & CHIU, L. (1990). In vivo moment arm calculations at the ankle using magnetic resonance imaging (MRI). *Journal of Biomechanics* **23**, 495-501.

SAM, M., SHAH, S., FRIDÉN, J., MILNER, D. J., CAPETANAKI, Y. & LIEBER, R. L. (2000). Desmin knockout muscles generate lower stress and are less vulnerable to injury compared with wild-type muscles. *American Journal of Physiology* **279**, C1116-1122.

SANDERCOCK, T. G. (2000). Nonlinear summation of force in cat soleus muscle results primarily from stretch of the common-elastic elements. *Journal of Applied Physiology* **89**, 2206-2214.

SAXTON, J. M., CLARKSON, P. M., JAMES, R., MILES, M., WESTERFER, M., CLARK, S. & DONNELLY, A. E. (1995). Neuromuscular dysfunction following eccentric exercise. *Medicine and Science in Sports and Exercise* **27**, 1185-1193.

SAXTON, J. M. & DONNELLY, A. E. (1996). Length-specific impairment of skeletal muscle contractile function after eccentric muscle actions in man. *Clinical Science* **90**, 119-125.

SMITH, L. L. (1991). Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Medicine and Science in Sports and Exercise* **23**, 542-551.

SPECTOR, S. A., GARDINER, P. F., ZERNICKE, R. F., ROY, R. R. & EDGERTON, V. R. (1980). Muscle architecture and force-velocity characteristics of cat soleus and

medial gastrocnemius: implications for motor control. *Journal of Neurophysiology* **44**, 951-960.

STACEY, M. J. (1969). Free nerve endings in skeletal muscle of the cat. *Journal of Anatomy* **105**, 231-254.

STAUBER, W. T., FRITZ, V. K., VOGELBACH, D. W. & DAHLMANN, B. (1988). Characterization of muscles injured by forced lengthening. 1. Cellular infiltrates. *Medicine and Science in Sports and Exercise* **20**, 345-353.

STREET, S. F. (1983). Lateral transmission of tension in frog myofibers: A myofibrillar network and transverse cytoskeletal connections are possible transmitters. *Journal of Cellular Physiology* **114**, 346-364.

TAKEKURA, H., FUJINAMI, N., NISHIZAWA, T., OGASAWARA, H. & KASUGA, N. (2001). Eccentric exercise-induced morphological changes in the membrane systems involved in excitation-contraction coupling in rat skeletal muscle. *Journal of Physiology* **533**, 571-583.

TALAG, T. S. (1972). Residual muscular soreness as influenced by concentric, eccentric and static contractions. *Research Quarterly* **44**, 458-469.

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.

TALBOT, J. A. & MORGAN, D. L. (1998). The effects of stretch parameters on eccentric exercise-induced damage to toad skeletal muscle. *Journal of Muscle Research and Cell Motility* **19**, 237-245.

TSKHOVREBOVA, L., TRINICK, J., SLEEP, J. A. & SIMMONS, R. M. (1997). Elasticity and unfolding of single molecules of the giant muscle protein titin. *Nature* **387**, 308-312.

WALMSLEY, B. & PROSKE, U. (1981). Comparison of stiffness of soleus and medial gastrocnemius muscles in cats. *Journal of Neurophysiology* **46**, 250-259.

WANG, K., McCLURE, J. & TU, A. (1979). Titin: major myofibrillar components of striated muscle. *Proceedings of the National Academy of Science* **76**, 3698-3702.

WANG, K., MCCARTER, R., WRIGHT, J., BEVERLY, J. & RAMIREZ-MITCHELL, R. (1991). Regulation of skeletal muscle stiffness and elasticity by titin isoforms: a test of the segmental extension model of resting tension. *Proceedings of the National Academy of Science* **88**, 7101-7105.

WANG, K., MCCARTER, R., WRIGHT, J., BEVERLY, J. & RAMIREZ-MITCHELL, R. (1993). Viscoelasticity of the sarcomere matrix of skeletal muscles. The titin- myosin composite filament is a dual-stage molecular spring. *Biophysical Journal* **64**, 1161-1177.

WANG, K., FORBES, J. G. & JIN, A. J. (2001). Single molecule measurements of titin elasticity. *Progress in Biophysics and Molecular Biology* **77**, 1-44.

WARREN, G. L., LOWE, D. A., HAYES, D. A., KARWOSKI, C. J., PRIOR, B. M. & ARMSTRONG, R. B. (1993). Excitation failure in eccentric contraction-induced injury of mouse soleus muscle. *Journal of Physiology* **468**, 487-499.

WARREN, G. L., LOWE, D. A., HAYES, D. A., FARMER, M. A. & ARMSTRONG, R. B. (1995). Redistribution of cell membrane probes following contraction-induced injury of mouse soleus muscle. *Cell and Tissue Research* **282**, 311-320.

WARREN, G. L., INGALLS, C. P., SHAH, S. J. & ARMSTRONG, R. B. (1999a). Uncoupling of in vivo torque production from EMG in mouse muscles injured by eccentric contractions. *Journal of Physiology* **515**, 609-619.

WARREN, G. L., LOWE, D. A. & ARMSTRONG, R. B. (1999b). Measurement tools used in the study of eccentric contraction-induced injury. *Sports Medicine* **27**, 43-59.

WARREN, G. L., INGALLS, C. P., LOWE, D. A. & ARMSTRONG, R. B. (2001). Excitation-contraction uncoupling: major role in contraction-induced muscle injury. *Exercise and Sports Science Reviews* **29**, 82-87.

WEERAKKODY, N. S., WHITEHEAD, N. P., CANNY, B. J., GREGORY, J. E. & PROSKE, U. (2001). Large-fiber mechanoreceptors contribute to muscle soreness after eccentric exercise. *The Journal of Pain* **2**, 209-219.

WHITEHEAD, N. P., ALLEN, T. J., MORGAN, D. L. & PROSKE, U. (1998). Damage to human muscle from eccentric exercise after training with concentric exercise. *Journal of Physiology* **512**, 615-620.

WISE, A. K., MORGAN, D. L., GREGORY, J. E. & PROSKE, U. (2001). Fatigue in mammalian skeletal muscle stimulated under computer control. *Journal of Applied Physiology* **90**, 189-197.

WOOD, S. A., MORGAN, D. L. & PROSKE, U. (1993). Effects of repeated eccentric contractions on structure and mechanical properties of toad sartorius muscle. *American Journal of Physiology* **265**, C792-800.

YEUNG, E. W., BALNAVE, C. D , BALLARD, H. J., BOURREAU, J. P. & ALLEN, D. G. (2002a). Development of T-tubular vacuoles in eccentrically damaged mouse muscle fibres. *Journal of Physiology* **540**, 581-592.

YEUNG, E. W., BOURREAU, J. P., ALLEN, D. G. & BALLARD, H. J. (2002b). Effect of eccentric contraction-induced injury on force and intracellular pH in rat skeletal muscles. *Journal of Applied Physiology* **92**, 93-99.

ZELENA, J. (1994). *Nerves and Mechanoreceptors*. London: Chapman and Hall.