UNIVERSITY OF CALGARY

Structural Changes Of Unipennate Skeletal Muscle

During Isometric Contractions

by

Wendy Irene Carvalho

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ABSTRACT

At present there are little available data which can explain what structures deform and how changes take place in unipennate cat medial gastrocnemius (MG) during contractions. Therefore the purposes of this thesis were (1) to quantify using sonomicrometry (MG, n = 8) the contribution of tendon and aponeurosis deformation to changes in fascicle geometry during isometric contractions, (2) to determine how fibre pinnation and series elasticity influence length changes in relaxed and activated fascicles during whole muscle excursion and, (3) to identify the functional range of sarcomere lengths in relaxed and active unipennate muscle. When going from the relaxed to the maximally activated state, fascicle lengths decreased on average by 27 %, angles of pinnation increased on average by 42 %, and muscle height remained constant. Observed changes in fascicle geometry during force production were primarily attributed to deformation of the aponeuroses. A decrease in the pinnation angle and elongation of the series elastic structures were responsible for differences in excursion between relaxed and activated fascicles and the whole muscle. It was concluded that pinnation and series elasticity allow sarcomeres of relaxed and activated fascicles to work on distinctly different limbs of the force-length relation.

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To my parents, Anne and John

Thank-you for your love, support and patience

And to Nicole, and Kevin

Thanks for being there, always!

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DEFINITION OF TERMS

Activated State:

The state of the muscle when peak force is produced during maximal muscle activation.

Aponeurosis:

Tendinous expansion into which the muscle fibres insert, serving mainly to connect muscle and tendon.

Fascicle:

(Fibre bundle), consists of a number of muscle fibres surrounded by a connective tissue sheath called perimysium.

Force-Length Relationship:

The relation between the maximal force a muscle (or fibre, or sarcomere) can exert and its length. Force-length relations are obtained under isometric conditions and for maximal activation of the muscle.

In-Series Elastic Structures:

The passive elastic or viscoelastic tendons and aponeuroses that run in-series with the contractile element (muscle fibres). By their morphological arrangement, the in-series elastic structures transmit the forces from the muscle to the bone (Epstein & Herzog, 1998).

Isometric Contraction:

A contraction of a muscle in which length remains constant. The length referred to may be that of the muscle-tendon complex, the fibre, or the sarcomere, depending on the context.

Muscle Fibre:

An individual muscle cell.

Muscle Height:

The distance between the medial and lateral aponeuroses of the cat medial gastrocnemius. Muscle height was determined as the product of fascicle length and the sine of the corresponding pinnation angle.

Myofibril:	A tiny fibril containing myofilaments that runs parallel to the long axis of the fibre (cell).
Pinnation Angle:	(Denoted as alpha or by the symbol α), The angle between a muscle fascicle (fibre) and the line of action of the muscle. In pennate muscles, fascicles are arranged obliquely with respect to the tendon, and this angulation (pinnation angle) changes by contraction (Kawakami et al., 1998).
Relaxed State:	The passive (non-contracted) state of the muscle prior to muscle activation.
Sarcomere:	The basic contractile (or functional) unit of striated muscle. It contains the region of a myofibril that lies between two adjacent dark lines called the Z-lines (Herzog, 1994).
Skeletal Muscle:	Striated muscle attached to bones responsible for skeletal movements; controlled by the somatic nervous system.
Sonomicrometry:	The measurement of distances using sound.
Tendon:	A fibrous cord-like structure of connective tissue by which a muscle is attached to bone (Herzog & Gal, 1999).
Thick Filament:	Filament predominantly composed of myosin proteins.
Thin Filament:	Filament predominantly composed of actin, tropomyosin and troponin proteins.
Unipennate Muscle:	Fascicles (fibres) run at a distinct angle to the line of action of the entire muscle, and all fibres are approximately parallel to one another (Herzog, 1994).

Every man's condition is a solution in hieroglyphic to those inquiries he would put.

He acts it as life, before he apprehends it as truth. In like manner, nature is

already, in its forms and tendencies, describing its own design.

Ralph Waldo Emerson, Nature, edited by Hershel Parker (New York: W.W. Norton, 1995), p. 440.

CHAPTER 1.

INTRODUCTION

1.1 A Brief Historical Highlight

Research on unipennate muscle is not a new concept. In fact, during the period of the scientific revolution, the Danish scientist Niels Stensen described the structure of unipennate muscle and formulated an early theory of the mechanics of muscular contraction (Kardel, 1990). In 1663, Stensen made a sketch of a unipennate muscle (figure 1.1), and described the structure as having equally long muscle fibres forming a parallelogram between parallel tendons (Kardel, 1990). Extending to his geometric model in 1667 Stensen developed and published the following theory in the book Elementorum: 'during uniform contraction of motor fibres, tendon plates move in parallel planes, the muscle shortens but the distance between the two tendon surfaces remains constant' (Kardel, 1990). Stensen's theory however was received with much resistance from the scientific community for its suggestion that contractions may occur without changes in muscular volume (Herzog, 1994; Kardel, 1990). Consequently, in 1675 Stensen took holy orders and abandoned the study of the natural sciences for the remainder of his life (Kardel, 1990). Interestingly, Niels Stensen's work lay dormant for over 300 years until the 1980's when researchers found structural similarities between various pennate muscles and Stensen's description (Kardel, 1990).

Now moving into the new millenium with many modern advances in technology it is possible to see with ultrasound images (figure 1.2) that Stensen's geometric model (figure 1.1) matches closely to the actual structure of unipennate muscle (human MG) (image from video courtesy of Dr. Y. Kawakami, Tokyo). The ultrasonography further lends support to Stensen's theory of contraction, demonstrating qualitatively that muscle deformation consists of a parallel sliding of the two tendinous plates relative to one another during contraction. From this brief historical highlight it is clear that work on unipennate muscle started long ago, and began with a simple geometric description. The simple planimetric model proves to have a place in the context of today's research and will be used in this thesis to represent qualitatively and quantitatively the deformation of unipennate cat medial gastrocnemius. The following section details what is known at present regarding changes of muscle geometry during isometric contractions, and what information is still missing in this area of research.

1.2 Introduction

There are many studies in which changes in fascicle length and pinnation angle in unipennate skeletal muscle were examined during fixed-end, isometric contractions (Muhl, 1982; Griffiths, 1987 & 1991; Fukunaga et al., 1997; Kawakami et al., 1998; Maganaris et al., 1998). When going from zero to maximal force, fascicles of the medial gastrocnemius have been found to shorten by 18-28 % and 47-49 % in the cat and human, respectively (Griffiths, 1991; Maganaris et al., 1998). Simultaneously, pinnation angles increase by up to 33 % in the cat, and by 90-110 % in humans (Brooks et al., 1994; Maganaris et al., 1998).
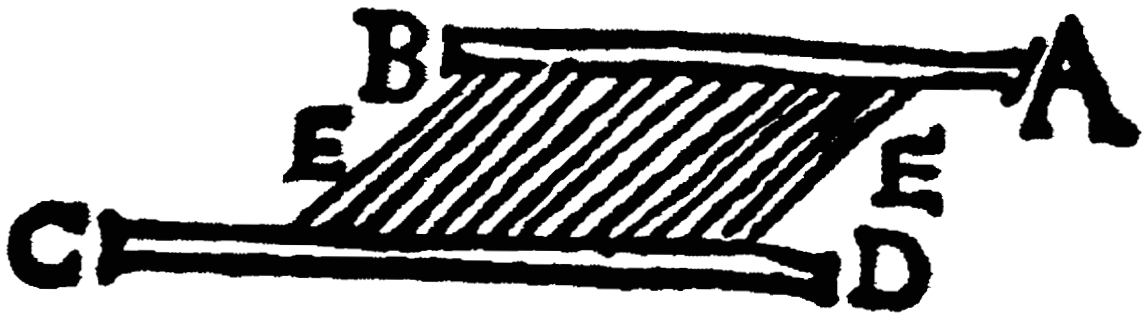
Figure 1.1. Stensen's drawing of a unipennate muscle from a letter in 1663 to T. Bartholin. Printed in 1667 Copenhagen (Kardel, 1990). A,B and D,C represent tendon plates, E represents muscle fibres.

Figure 1.2. Ultrasonographic image of the human medial gastrocnemius; obtained from a video courtesy of Dr. Y. Kawakami in Tokyo. A representation of Stensen's (figure 1.1) geometric sketch is superimposed on the ultrasound image, notice the similarity between the sketch and the actual muscle structure.

Although large changes in fascicle length and pinnation angle have been reported during isometric contractions in unipennate muscles, there has been no systematic attempt to explain how these changes occur. It has been speculated that compliance in the series elastic structures of the muscle enable fascicles to shorten and pinnation angles to increase during force production (Griffiths, 1991; Gans & Gaunt, 1991). However, no direct measurements of tendon or aponeurosis elongation have been made to determine the contribution of these elements to muscle and fascicle deformation.

Although much information has been generated regarding changes of muscle architecture during isometric contractions and whole muscle excursion (Muhl, 1982; Griffiths, 1991; Fukunaga et al., 1997; Kawakami et al., 1998), little is known about the associated functional implications of such changes. Specifically, there exists limited information regarding how series elasticity and pinnation may influence the contractile properties of whole muscle. Recently, Kawakami and Fukunaga (1999) estimated that the functional range of sarcomere excursion for relaxed and activated fascicles of human medial gastrocnemius occurs on different parts of the force-length relationship. Relaxed sarcomeres were found to work on the ascending, plateau and descending regions of the force-length relationship, while activated sarcomeres only worked on the ascending limb. However, Kawakami and Fukunaga (1999) could not explain how sarcomere lengths shifted from one part of the force-length relationship to another during muscle activation. In order to understand how changes in muscle deformation during contraction may influence muscle function, it is imperative to know how muscle deforms and how such deformations affect force production in whole muscle.

1.3 Purpose and Hypotheses

In this investigation, we measured the changes in fascicle lengths, pinnation angles, aponeurosis and tendon lengths, and muscle height in the unipennate cat medial gastrocnemius from the relaxed to the maximally activated state. The purpose of these measurements was to determine the contribution of the tendon, and medial and lateral aponeuroses to fascicle deformation, and how pinnation and series elasticity may influence the functional contractile properties of the muscle. It was hypothesized, (1) that fascicles will shorten, pinnation angles will increase and muscle height will remain approximately constant during maximal muscle activation; (2) that changes in muscle geometry during activation can primarily be accounted for by elongations in the aponeuroses, and (3) that the presence of long compliant series elastic structures and a pennate fibre arrangement will reduce the range of excursion of fascicles and sarcomeres, and shift the sarcomere functional length range to the left on the length axis of the force-length relationship.

1.4 Rationale

At present limited data are available which can explain what structures deform and how structural changes take place in unipennate muscle during contraction. Without such information little progress can be made in understanding the relation between muscle structure and function, specifically how force is produced by whole skeletal muscle. I hope that this thesis will provide valuable data that can fill some of the gaps in our understanding of unipennate muscle, and will be useful in other areas of research which delve into muscle modeling and motor control.

1.5 New and Notable

The new and notable results that emerge from this thesis should be emphasized. This study is the first to:

1. Describe how large deformations of fibre length and pinnation can occur, and give a detailed account of the contribution of the tendon and aponeuroses.

2. Show how changes of muscle architecture during isometric contractions influence the functional working range of sarcomeres on the force-length relationship.

1.6 Overview of Thesis

The thesis continues with a review of literature in Chapter 2, providing background information on relevant issues related to the structure and deformation of unipennate muscle during contraction, and functional considerations. In Chapter 3, the experimental methods are described. Chapter 4, details the results of the thesis, and Chapter 5 follows with a discussion of the results. Chapter 6 contains a summary of the main results and some general conclusions of the thesis. This thesis ends in Chapter 6 with some comments on future directions for related muscle research, and suggestions for the potential clinical applications of this work.

CHAPTER 2.

REVIEW OF LITERATURE

2.1 Pennate Muscle Structure

In an attempt to better understand the mechanics of muscular contraction, and the relation between morphological structure and function, many investigators have chosen to study the deformation of pennate muscles during contractions (Hoffer et al., 1989; Griffiths, 1991; Kawakami et al., 1998 & Maganaris et al., 1998). In contrast to fusiform muscles in which fibres run parallel to the line of action of the muscle (the line connecting proximal and distal tendons), pennate muscles contain fibres which are arranged at a distinct angle to the muscles line of action (Kardel, 1990; Herzog, 1994). Further structural and functional differences that exist between pennate and non-pennate muscles are influenced by fibre length, and the arrangement of fibres within a given volume of muscle (Herzog, 1994). In fact, pennate muscles by design have shorter fibres than fusiform muscles of equal volume (Epstein & Herzog, 1998). Consequently, the absolute length range over which pennate muscles can generate force is smaller compared to fusiform or parallel-fibred muscles of equal volume (Muhl, 1982). However, pennate muscles are afforded the functional advantage of greater strength, or maximal peak force potential than fusiform muscles, because of the larger number of fibres that pennate

muscles can accommodate in parallel in a given muscle volume (Gans, 1982; Herzog, 1994).

With a diversity of organizational structures, pennate muscles are further subdivided into several categories based on the number (n) of distinct fibre directions within a muscle (Epstein & Herzog, 1998). For example, bipennate (n = 2) or multipennate (n ≥ 3) muscles contain two or more bundles of fibres running in different directions at a distinct angle to the line of action of the muscle (Gans, 1982; Herzog, 1994; Epstein & Herzog, 1998). The muscle under investigation in this thesis is the cat medial gastrocnemius, a unipennate (n = 1) muscle. Unipennate muscles contain fibres which run in the same direction and insert at a distinct angle into two virtually parallel tendon sheaths known as aponeuroses (Kardel, 1990; Zajac, 1989). Interestingly, to describe structural and functional properties of pennate muscles, many investigators have created geometric muscle models based on experimental data (Alexander & Vernon, 1975; Huijing & Woittiez, 1984; Maganaris et al., 1998). Some investigators suggested that differences between bipennate and unipennate muscle models are small, and that the unipennate structural arrangement can be used to explain the function of even more complex fibre arrangements (Zajac, 1989; Spoor et al., 1991). Consequently, the unipennate human and animal medial gastrocnemius has been frequently chosen for architectural analysis during muscular contractions, because of its pennate fibre arrangement, superficial location, and bi-articular crossing of two joints; the knee and the ankle (Griffiths, 1991; Zuurbier & Huijing, 1991; Caputi, 1992; Maganaris 1998; Kawakami, 1998).

2.2 Structural Deformation of Pennate Muscle During Isometric Contractions

Investigations of muscle deformation during isometric contractions have focused on fascicle (or fibre) length, pinnation angle, and muscle thickness (Griffiths, 1987, 1991; Fukunaga, 1997; Kawakami, 1998; Huijing, 1985). Various approaches have been used to investigate muscle architecture, including the use of animal models, cadavers and human subjects (Griffiths, 1987, 1991; Zuurbier & Huijing, 1991; Huijing, 1985; Kawakami, 1998). Griffiths (1987 & 1991) used the ultrasound transit time technique, sonomicrometry, to resolve the relative movements of fibres in the cat medial gastrocnemius during isometric contractions. It was found that during supramaximal isometric contractions, fibres shortened by up to 28% of their initial resting length. Using ultrasonography Narici et al., (1996) further demonstrated that in the transition from rest to maximal voluntary isometric contractions, fibre lengths of the human medial gastrocnemius decreased by as much as 35 %. Using the same technique, Maganaris et al., (1998) found that fibre lengths of human medial gastrocnemius were always smaller when comparing maximal voluntary contractions (MVC) with resting conditions at any given ankle angle. Furthermore, Maganaris et al., (1998) demonstrated that fibre lengths decreased by as much as 47-49 % from resting conditions during contractions up to 100 % of MVC. Although these large deformations of fascicle (fibre) length have been found during maximal muscle activation, no complete explanation exists on how such changes occur. Griffiths (1991) and Fukunaga et al., (1997) suggested that fascicle shortening during muscle activation can be attributed to fibres elongating the series elastic components of the muscle. However, neither group made any direct

measurements of tendon deformation during isometric contractions (Griffiths, 1991; Fukunaga et al., 1997). Similarily, Kawakami et al., (1998) and Zuurbier & Huijing, (1991) suggested that the series elastic aponeuroses primarily contribute to changes of fascicle length during muscle activation, however they did not make direct experimental measurements of aponeurosis deformation during muscle contractions.

Using ultrasonography, Narici et al., (1996), Kawakami et al., (1998) and Maganaris et al., (1998) demonstrated that human medial gastrocnemius (MG) fibre lengths did not remain constant with changing muscle length. During resting conditions (Narici et al., 1996), maximal voluntary isometric contractions (Kawakami et al., 1998; Maganaris et al., 1998), and graded isometric contractions (Maganaris et al., 1998), a stepwise increase of whole muscle length (resulting from a change in the MG ankle joint angle) caused muscle fibre length to increase. Interestingly, Hoffer et al., (1989) demonstrated that length changes imposed on fibres did not directly reflect length changes undergone by the parent muscle during MG excursion in the freely walking cat. The difference between muscle and fibre excursion was suggested to be caused by a deformation of the muscles' external tendon (Hoffer et al., 1989). Griffiths, (1991) showed that fibres of the cat MG only increased in length by 0.7 mm for each 1 mm increase of maximally activated whole muscle length. The difference in excursion was attributed to a decrease in the pinnation angle (Griffiths, 1991); these findings are in agreement with those of Muhl, (1982) who found for rabbit digastric muscle that pennate muscle fibres extend less than the whole muscle belly stretches during excursion. Muhl, (1982), noted that such a relationship between muscle and fibre length also exists when

the muscle shortens; that is fibres would shorten less than the parent muscle. Deformation of the muscle's external tendon and changes of fibre geometry have been suggested to influence fibre length changes during whole muscle excursion (Muhl, 1982; Hoffer et al., 1989 & Griffiths, 1991). However, no studies have quantified the contribution of the series elastic tendons and the angle of pinnation to changes of fibre length during muscle excursion. Therefore, it is not known in detail how changes in fibre length occur during length changes of unipennate muscle.

Pennate muscles have received much interest in terms of their structural and functional characteristics. Muscle models (Otten, 1988; Woittez et al., 1984) have generally assumed angles of pinnation to be relatively small (< 20°) and changes in angles of pinnation to be negligible during contraction. However, such small and relatively constant pinnation angles seem to closely resemble only data from cadaveric specimens. For instance, Alexander & Vernon, (1975) made anatomical observations in human cadaveric specimens and reported pinnation angles of the medial gastrocnemius to be about 16°. However, many problems are associated with such measurements on cadavers. For example, there may be changes in the muscle's architecture during the fixation process that could alter the fibre angles from the in vivo values (Rutherford & Jones, 1992). Therefore, many investigators have opted to analyze changes in pinnation angles during isometric contractions in vivo, in humans (Narici et al., 1996; Fukunaga et al., 1997; Maganaris et al., 1998 & Kawakami et al., 1998), or in situ, in animal preparations (Brooks et al., 1994; Zuurbier & Huijing, 1991). In contrast to pinnation angles obtained from cadaveric data, pinnation angles of human MG have been found to

increase on average from 16° to 34° during isometric contractions (Narici et al., 1996).

In fact, Maganaris et al., (1998) found that when comparing MVC with resting conditions

at any given ankle angle, the pinnation angle increased by 19° - 28° (90 – 110%). Such

large deformations of the pinnation angle during force production were also reported by

Brooks et al., (1994) who found that pinnation angles of the cat MG increased in excess

of ten degrees or on average from 30° to 44° during maximal isometric contractions. In

addition, fibre angles also appear to be directly influenced by changes of whole muscle

length. Specifically, the angle of pinnation has been found to decrease (Brooks et al.,

1994; Narici et al., 1996; Fukunaga et al., 1997; Kawakami et al., 1998; Maganaris et al.,

1998) with increasing muscle length, at rest and during contraction. For instance, in

humans Kawakami et al., (1998) found that pinnation angles decreased from 45° to 22°

during relaxed conditions and from 67° to 33° during maximally activated conditions as

MG was moved from its shortest to its longest length. Similarly, Maganaris et al., (1998)

demonstrated that when human MG was moved from its shortest to longest length the

angle of pinnation decreased on average from 25° to 17°, and from 53° to 37°, during

resting and maximal voluntary contraction conditions, respectively. Overall, these results

demonstrate that changes in pinnation angle during maximal force production and muscle

excursion are large, and far greater than the static values reported for cadaveric

specimens.

Interestingly, many investigators have attempted to predict the functional

consequences of the pinnation angle on the mechanics of force transmission from fibres

to tendons and bones (Huijing & Woittiez, 1984; Otten et al., 1988; Fukunaga et al.,

1997; Kawakami et al.,1998; Maganaris et al., 1998). Using muscle models, some investigators suggested that because pennate fibres run at an oblique angle to the line of action of the muscle the force transmitted from the fibre to the tendon would be proportional to the cos α (where α = angle of pinnation) (Otten et al., 1988; Rutherford & Jones, 1992). Such predictions however have been based on free body diagrams of muscle where it is assumed that the resultant force (vector) acts in the direction of the muscle fibres (Epstein & Herzog, 1998). Consequently, such models of unipennate muscle suggest that the force in the tendon would be less than the sum of the forces produced by individual fibres (Rutherford & Jones et al., 1992). Interestingly, it has been argued that such theoretical models either fail to account for the fact that muscle volume remains nearly constant during contraction or have not accounted for the forces of constraints that arise because of volume preservation (Epstein & Herzog, 1998). In fact, Herzog et al., (1999) demonstrated, theoretically and experimentally, that the resultant force exerted by entire unipennate muscle (cat MG) does not occur along the fibre direction but occurs along the collagen fibril direction of the aponeuroses and tendons. The transmission of the resultant force along the tensile axis of the aponeurosis was assumed to occur because of the forces of constraints imposed (by isovolumetricity) on the muscle during contraction (Herzog et al., 1999). Exact mechanisms for why the resultant muscle force acts along the aponeurosis rather than along the muscle fibres have not been determined to date (Herzog et al., 1999). However, as a consequence of the above results, the force acting along the aponeuroses and tendon must be larger than the force acting along the muscle fibre direction for physiologically meaningful geometries.

They further predicted that if their results were correct, unipennate muscle should deform during contraction by a parallel sliding of the two tendon plates relative to one another and that muscle height should remain about constant (Herzog et al., 1999). In agreement with the findings of Herzog et al., (1999), many investigations on pennate muscle have theoretically and/or experimentally demonstrated that muscle height remains about constant during muscular contractions (Alexander & Vernon; 1975; Narici et al., 1996; Kawakami et al., 1998; Maganaris et al., 1998). In addition, ultrasonography of unipennate muscle (human MG) shows qualitatively that muscle height remains constant during muscle contraction and deformation of MG consists primarily of a parallel sliding of the two aponeuroses relative to one another (video courtesy from Dr. Y Kawakami, Tokyo). Overall, these results suggest that the long-held notion that the angle of pinnation results in a loss of force that is transmitted from the fibres to the muscle tendon may be incorrect (Herzog et al., 1999) despite its firm embedding in the biomechanics literature and textbooks (Winters & Woo, 1990; Lieber, 1992).

2.3 Functional Considerations

Although deformations of unipennate muscle have been quantified in a series of studies, few investigations have attempted to determine the functional consequences of such deformations with respect to the force-length relationship. Force-length relationships describe the relation between the maximal force a muscle (or fibre, or sarcomere) can exert and its length. Force-length relationships are obtained under isometric conditions and for maximal activation of a muscle. Isometric may refer to the length of the entire muscle, the length of a fibre, or the length of a sarcomere, depending

on the system that is studied (Herzog, 1994). Interestingly, while attempts have been made to investigate mechanical properties of activated skeletal muscles, some studies have failed to take active shortening of muscles into account while determining length-tension (or force-length) relationships (Rack & Westbury, 1969). In contrast, Muhl, (1982) suggested that the contractile force in active muscles is better described by length-tension curves based on active muscle lengths. To support this statement, Muhl, (1982) derived an isometric length-tension relation using activated muscle lengths for rabbit digastric muscle and clearly showed that distinct differences exist between length-tension curves where relaxed muscle lengths are used to plot active muscle forces (hereafter referred to as relaxed length-tension curves) and activated length-tension curves. Specifically, each point on the active length-tension curve was found to be shifted to the left of the relaxed length-tension curve (Muhl, 1982). Muhl, (1982), attributed the differences between the length-tension curves to internal shortening of the muscle and compliance of the tendon (Muhl, 1982). Although, these differences were found by Muhl (1982), little is still known regarding how pinnation and series elasticity actually influence the range of excursion of fascicles and sarcomeres during relaxed and activated conditions. At present, there is only one investigation in which the functional range of sarcomere lengths for relaxed and activated fascicles was estimated (Kawakami & Fukunaga, 1999). Kawakami and Fukunaga, (1999) demonstrated for human MG that functional sarcomere lengths were on the ascending, plateau and descending portions of the force-length relation in the relaxed condition, but were only on the ascending limb for the activated condition. Unfortunatley, Kawakami and Fukunaga, (1999) could not

quantitatively explain the differences in excursion between relaxed and activated sarcomeres, and they failed to provide any information on how the series elastic structures or the angle of pinnation may have contributed to such differences. Future research is warranted in order to understand how structural changes of unipennate muscle influence functional or force-length properties of unipennate skeletal muscle.

CHAPTER 3.

METHODOLOGY

3.1 General

Fixed-end isometric contractions were performed maximally via tibial nerve stimulation of the cat medial gastrocnemius. Muscle-tendon length was controlled with a muscle puller. Muscle deformation was studied as the muscle went from a passive state to a maximally activated state during isometric contractions. Fascicle lengths, pinnation angles, muscle height, aponeuroses and tendon lengths were determined using sonomicrometry. Functional sarcomere lengths were determined using laser diffraction.

Eight medial gastrocnemii (MG) from four adult, outbred cats (3.0-4.5 kg) were used. Animals were sedated with a subcutaneous injection of Atravet (2.0 mg). Approximately one half hour after sedation, animals were deeply anaesthetized with a 4-5% halothane/$0.7L/minO_2$/$0.6L/min$ NO_2 mixture administered through an anesthetic mask. Animals were then intubated (using a laryngoscope blade with a 3-4 mm cuffed endotracheal tube) and maintained throughout the experiment with a 0.5-1.0% halothane oxygen/nitrous oxide mixture. Once anesthetized, the animal's hindlimbs were shaved in preparation for surgery. During all surgical and experimental procedures, the animal's vital signs, temperature, and anesthesia were regularly checked. All exposed areas were continuously moistened with 37° C saline throughout the surgical procedure. Muscle

temperature was monitored, and maintained at 34-35° C throughout the experiment using

a heat lamp, warming pad, and saline (0.9 %) bath that completely submerged the MG.

Cats were given 120 ml of 5 % Dextrose in a 0.9 % saline subcutaneously to avoid

dehydration and 0.2 mg of subcutaneous atropine for ease of breathing and to prevent

tracheal secretions from clogging the intubation tube. The study was approved by the

University of Calgary Animal Ethics Committee.

3.2 Mechanical Arrangement

Cats were mounted in a stereotaxic frame. The experimental hind limb was firmly

fixed to the frame with sharp, bilateral bone pins at the pelvis, femoral condyles, and the

malleoli. The MG tendon was cut from its insertion leaving a remnant piece of bone for

attachment to a hydraulic muscle puller (Servovalue 252.22C-01 with a 100 mm actuator

242-02 MTS, Eden Prairie, MN) via sutures. The muscle puller (natural frequency > 1

kHz) was equipped with a 100 N load cell (10V) and was set for a functional length range

of 20 mm (10V).

3.3 Experimental Protocol, Force and Muscle length

The experimental protocol consisted of isometric contractions at nine muscle

lengths. Whole muscle-tendon unit length was increased in 2 mm increments, for a total

muscle length change of 16 mm covering the physiological range of the cat MG. At a

length change of 10 mm (i.e. hereafter referred to as the reference length), passive muscle

force was 1 N. This reference length corresponds to approximately the longest muscle

length found in a walking cat (Goslow et al., 1973). During isometric contractions, MG

was maximally stimulated (i.e. three times the α motoneuron threshold) via an implanted

tibial nerve cuff electrode and a Grass Instruments (Astro-Med, Quebec, S88-00) stimulator. An incision along the postero-lateral aspect of the thigh was done to allow for placement of a nerve cuff (5 mm) electrode on the tibial nerve just distal to the tibial nerve-peroneal nerve junction (Herzog et al., 1995). Once implanted, the α-motoneuron threshold and the fusion frequency of MG were determined. The incision was closed with surgical staples and protected with gauze throughout the surgery to avoid movement of the cuff or trauma to the tissues surrounding the incision. The stimulation parameters were: frequency = 60 Hz, pulse duration = 0.1 ms, and stimulation duration = 3 s. A two-minute rest was given between contractions, and every trial was performed twice. Data were sampled at a rate of 200 Hz, and force and muscle lengths were stored on line to a personal computer for later analysis.

3.4 Fascicle Length, Pinnation Angle, Muscle Height, Aponeurosis and Tendon Length

Piezoelectric crystals were implanted on the MG so that three fascicle lengths (proximal, central and distal), three angles of pinnation, muscle height, two aponeurosis segment lengths on the medial and lateral aponeuroses, and the tendon lengths could be determined during isometric contractions (figure 3.1). Measurements of the distance between piezoelectric crystals were made with a sonomicrometer, (Sonometric Corporation, London, ON, Canada) using the ultrasound transit time technique described by Griffiths, (1987), Hoffer et al., (1989) and Caputi et al., (1992). The average speed of sound through muscle was taken as 1540 m/s. Caputi et al., (1992) investigated the ultrasound transit time technique (sonomicrometry) in active and passive cat medial

gastrocnemius muscle and found that the velocity of ultrasound was independent of the state of activation of the muscle, and independent of the direction of the measurement with respect to fibre orientation. For example, Caputi et al., (1992) concluded that in the worst-case situation, the largest change in the velocity of ultrasound observed in their study (0.4 %) would require a correction of only 80 μm (for ~ 20 mm long fibres).

In order to implant the cylindrical, 2 mm diameter, piezoelectric crystals, the ankle plantar flexor muscle group was surgically exposed, and soleus, plantaris and lateral gastrocnemius were removed. Both lateral and medial surfaces of the MG were exposed with its tendon left intact and six piezoelectric crystals were sutured to the aponeurotic insertions at each end of three separate fascicles. A seventh crystal was sutured to the most distal part of the MG tendon. Aponeurotic insertion sites were determined by microstimulating (5 V, 10 Hz, continuous stimulation) one end of a fascicle resulting in a tiny dimple on the opposite surface of the muscle where the fascicle ended. The origin and insertion of the fascicle were marked with Methylene Blue to indicate the placement of the piezoelectric crystals. Piezoelectric crystals were tightly embedded in a small piece of silicone tubing and fixed to the fascia of MG using three separate sutures. This fixation minimized movements of the crystals relative to the fascicle insertion sites.

Sonomicrometry is the measurement of distances using sound. Crystals made from piezo-electric ceramic material transmit and receive sound energy. Typically, these transducers operate at ultra-sound frequencies (1 MHz and higher). To perform a single distance measurement, one crystal will transmit a burst of ultrasound, and a second

crystal will receive this ultrasound signal. The elapsed time from transmission to reception is a direct and linear representation of the physical separation of the crystals. The digital sonomicrometer performs a transmit-cycle during which an array of user-defined crystals is sequentially energized (pinged). The cycle begins with the first user-defined transmitter and ends with the last user-defined crystal. Therefore transit time measurements are made only for one user-defined crystal pair at a time. Transit time is measured digitally in steps of 15 nano-seconds, the digital delay between crystal firing is 562.5 nano-seconds, and the inhibit delay between the firing of different crystal pairs is 3.25 microseconds. Based on literature provided by Sonometrics Corporation (ON, Canada), each distance measurement has a relative error (dynamic resolution) of 0.015 mm, and an absolute (static) error of 1 mm. From an investigation on sonomicrometry in cat skeletal muscle (MG), Caputi et al., (1992) suggested that distance measurements may have a measurement uncertainty of ($\pm$ 1 mm) because of uncertainties in the placement of crystals with respect to the ends of muscle fascicles.

3.5 Geometric Muscle Model

A geometric model was used to describe the contribution of the various series elastic elements to fascicle shortening and to changes in the angle of pinnation when going from a relaxed to an activated state (figure 3.1). The model is a mid-sagittal section of the muscle where fascicle, aponeurosis and tendon length measurements were made. It was assumed that the aponeurosis, tendon, and fascicles were straight. Aponeurosis segment lengths, and fascicle lengths were obtained from direct measurements (figure 3.1). Relaxed and activated lengths were also measured directly
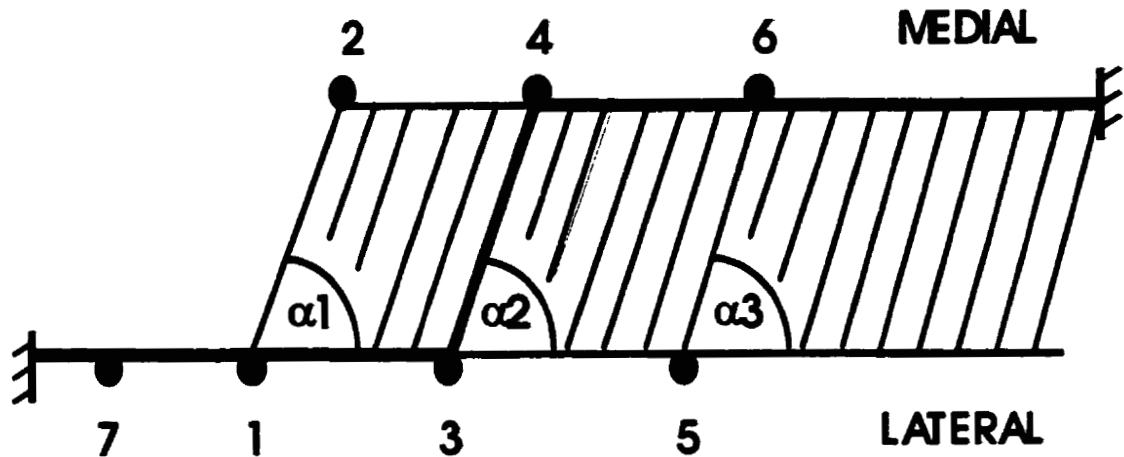
Figure 3.1. Geometric model of the piezoelectric crystal setup on the cat medial gastrocnemius. The angles of pinnation are denoted by α 1, 2 and 3. The medial and lateral aponeurotic sheaths are defined by (transmitter crystal, receiver crystal) (2,4 and 4,6) and (1,3 and 3,5), respectively. Three separate fascicles are shown by crystals: (1,2), (3,4), (5,6). Crystal 1 was placed at the myotendinous junction; Crystal 7 was placed at the most distal portion of the tendon that was possible. This is a schematic representation. Linear and angular scaling are not correct but were drawn like this for clarity.

for a segment spanning the proximal half of the external tendon. Assuming the proximal and distal halves of the tendon deformed in a like manner, total tendon deformation was derived from the known total length of the external tendon (relaxed length at the muscles shortest length) and the measured strain deformations of the proximal segment of the external tendon. Direct measurements were not made for the most proximal segment length of the medial aponeurosis which was calculated by subtracting the appropriate, known series elastic element lengths from the known total deformation of the series elastic elements. The known total deformations of the series elastic structures correspond to the difference of the projections of the muscle fascicles onto the lateral aponeurosis in the active and passive state. Here, we show the results for the middle fascicle (crystals 3 to 4, figure 3.1).

3.6 Functional Sarcomere Lengths

Functional mean sarcomere lengths were obtained by dividing fascicle lengths by the number of sarcomeres arranged in-series in a fascicle. In order to plot the functional sarcomere force-length relationship, it was assumed that 84 % of the maximal force occurred at a sarcomere length of 1.7 µm (Gordon et al., 1966; Herzog et al., 1992).

Following data collection, animals were euthanized with an intravenous injection of euthanol. The complete MG was excised, and removed from the bony attachments. The length of the external tendon and internal aponeuroses were measured with a steel ruler. The muscle was fixed in a 10 % formalin solution (>1 month), the central third of MG was extracted and digested in 30 % nitric acid until connective tissue had softened sufficiently to allow for teasing of small fascicles (Loeb & Gans, 1986; Koh, 1997). The

MG was then rinsed in buffer solution (PBS), and placed in glycerol. Three small fascicles in the vicinity of crystals 3 and 4 (figure 3.1) were teased out, mounted in glycerol jelly on microscope slides, and fascicle length was measured using a video analysis system (Media Cybernetic, Image Pro Plus, Silver Springs, MD, USA). Mean fascicle length for all muscle preparations was determined. Sarcomere lengths were then measured at 2 mm from the proximal and distal ends of each fascicle, and at the centre using a laser diffraction system (Allinger, 1995; Spoor et al., 1991). Mean sarcomere length was determined for all muscle preparations. Dividing mean fascicle length by mean sarcomere length gave an estimate of the in-series sarcomere number in a fascicle (Simpson et al., 1995; Tabary et al.,1976).

When a laser beam is projected through a muscle, the different refractive indices of the A and I bands of the sarcomeres produce a diffraction pattern. Measurement of the deviation of the first-order diffraction peak from the zero-order peak gives a direct measure of sarcomere length (Allinger, 1995). Thus, the laser diffraction system measured the sarcomere length within the region illuminated by the beam (10 mW He-Ne laser, wavelength 632.8 nm, beam diameter 0.8 mm; Model 1125, Uniphase, Manteca, CA). The laser source was fixed to a retort stand with two clamps so that it stood perpendicular to the base of a table. Below the laser source a small ring clamp was attached to the retort stand so that a microscope slide containing the fibre bundles could be shifted under the laser beam. For sarcomere measurements, the laser beam was directed directly through microscope slide containing a fibre bundle so that the beam was perpendicular to the long axes of the muscle fibres. This caused a diffraction pattern to

appear on the table, and the distance between the zero-order and first-order diffraction peaks were measured directly with a steel ruler. The precision of the sarcomere length measurements was determined using a 12.5 um known grating which was placed on a microscope slide, and illuminated by the laser beam. From repeated measurements using the grating the precision was found to be ± 0.03 um (n = 16).

3.7 Data Analysis and Statistics

All data are given as means and standard errors (SE). The data presented are for the middle fascicle of the MG, denoted by crystals 3 and 4 in figure 3.1. However, brief mention will be given to the general trends of all three measured fascicles. Pinnation angles were calculated using the distance measurements between three crystals (i.e. crystals 3, 4, and 5 for $\alpha 2$, figure 3.1) and the law of cosines. Muscle height was determined as the product of fascicle length and the sine of the corresponding pinnation angle. Differences in fascicle length, pinnation angle, and muscle height between relaxed and activated isometric conditions at different muscle lengths were tested using a non-parametric Sign test. Differences in strains along the two measured segments of the medial and lateral aponeuroses were investigated using a Mann-Whitney test. A Kruskal-Wallis test was used to assess differences in strains along the two segments of the lateral aponeurosis and the tendon and differences of fascicle length, pinnation angle and muscle height at the three regions (proximal, central and distal) along the mid-saggital axis of MG for both relaxed and activated isometric conditions. A level of significance of $\alpha = 0.05$ was chosen for statistical significance.

CHAPTER 4.

RESULTS

4.1 General Architectural Trends

Muscle reference length (10 mm) from one representative animal was chosen to illustrate the general changes in geometry of the middle fascicle, the corresponding angle of pinnation, two segments from both the medial and lateral aponeuroses, and the tendon during isometric contractions (figure 4.1). Upon activation of MG, the fascicles shortened and the pinnation angles, lateral aponeurosis segment lengths and tendon length increased until maximal tetanic force was achieved. Distal and middle segment lengths for the medial aponeurosis decreased and increased, respectively during muscle activation. When activation stopped, force declined, and fascicles, pinnation angles, aponeurosis lengths and tendon length returned to their approximate initial resting values.

4.2 Structural Heterogeneity

Proximal, central and distal fascicle lengths along the midsaggital plane of MG were the same in the relaxed state but were found to be significantly different in the activated isometric state. Pinnation angles and muscle heights between proximal, central, and distal sites were significantly different for both the relaxed and the activated
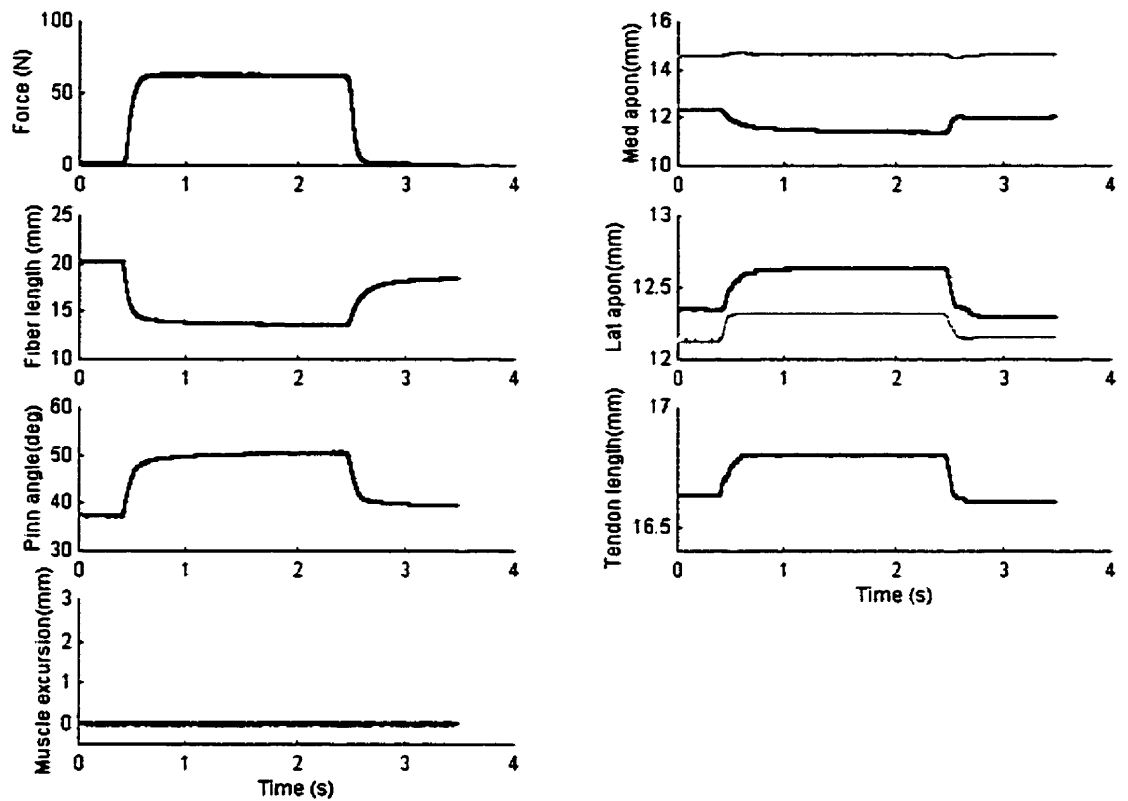
Figure 4.1. General architectural changes from one representative animal during an isometric contraction at the muscle reference length (10 mm). Left column from top to bottom: force, fibre length for middle fascicle (3-4), pinnation angle for middle fascicle, and muscle length. Right column from top to bottom: distal (dark) and middle (light) segment lengths for medial aponeurosis, distal (dark) and middle (light) segment lengths for lateral aponeurosis, and external tendon segment length.

isometric conditions. These results suggest that muscle architecture is heterogeneous in the midsaggital plane of cat MG.

4.3 Fascicle Lengths

Mean lengths for the distal, central and proximal fascicles increased for the relaxed and activated conditions as muscle length was increased (figure 4.2). However, while muscle length was increased by 16 mm, the corresponding relaxed and activated central fascicle lengths only increased by 7.4 mm and 4.8 mm, respectively. Fascicle lengths were significantly smaller in the activated compared to the relaxed isometric state at all muscle lengths for all fascicles. For instance, mean relaxed length for the central fascicle was 15.5 mm (±0.7) at the shortest muscle length, and 22.9 mm (±0.9) at the longest muscle length. Mean activated length for the central fascicle was 12.0 mm (±0.4) and 16.8 mm (±1.1) at the shortest and longest muscle length, respectively. The mean percentage change in central fascicle length from the relaxed to the maximally activated state was 22.5 % (±1.5) and 27.1 % (±2.1) at the shortest and longest muscle length, respectively.

4.4 Pinnation Angles

Mean relaxed and activated pinnation angles decreased for all fascicles as muscle length was increased (figure 4.3). For instance, mean relaxed pinnation angles of the central fascicle were 42.4° (±2.3) at the muscles shortest length and 28.2° (±2.1) at the longest muscle length. Mean activated pinnation angles of the central fascicle were 59.3° (±2.6) and 38.9° (±2.4) at the shortest and longest muscle length, respectively.
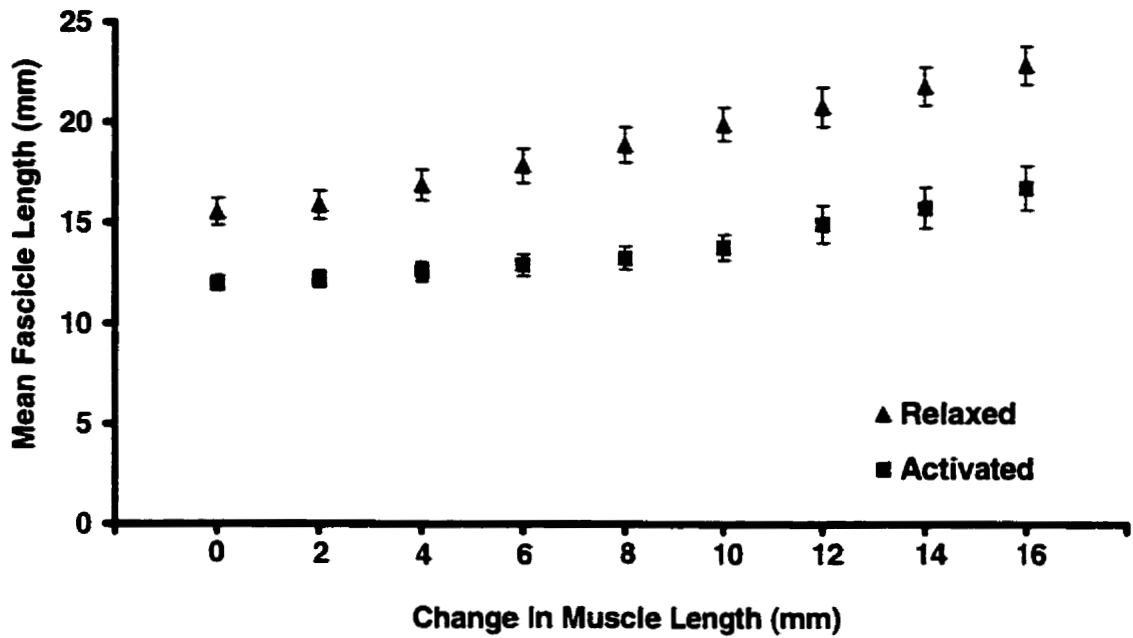
Figure 4.2. Mean (±SE) fascicle lengths as a function of muscle length and active state. The data presented are for the central fascicle, 3-4.
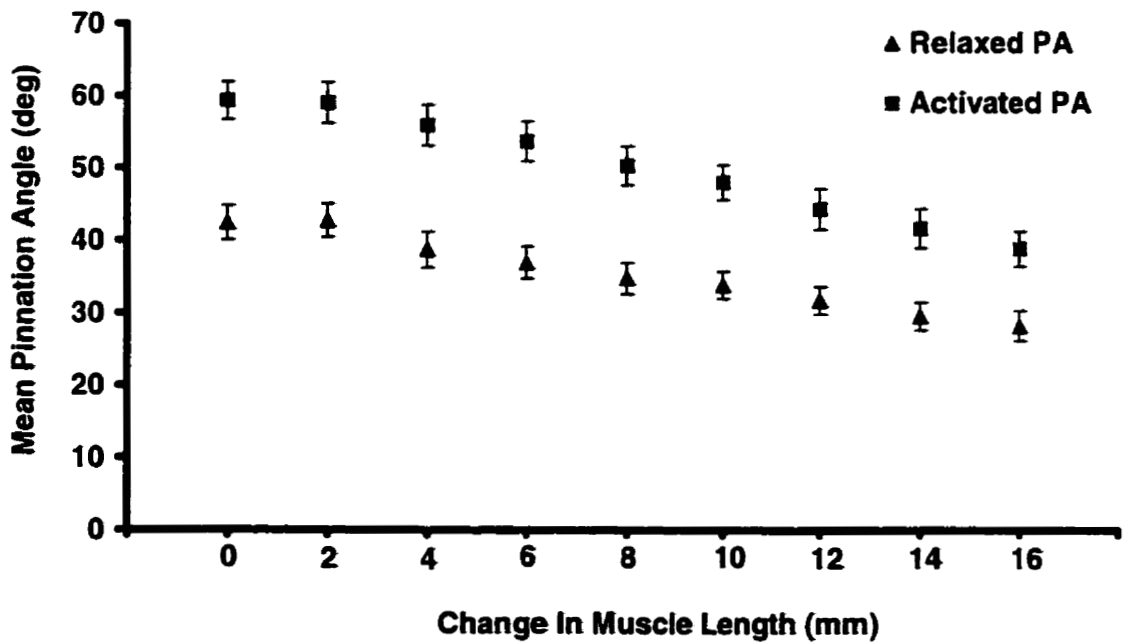

Figure 4.3. Mean (±SE) pinnation angles as a function of muscle length and active state. The data presented are for the central pinnation angle, $\alpha2$.

Pinnation angles in the relaxed state were significantly smaller than in the activated state for all fascicles. The mean percentage change of the pinnation angles for the central fascicle was similar at all muscle lengths (figure 4.3). For instance, at the shortest and longest muscle length, the pinnation angle changed by 40.8 % (±3.9) and 39.5 % (±5.3), respectively from the relaxed to the maximally activated state.

4.5 Muscle Height

Mean height in the central part of the relaxed muscle was 10.3 mm (±0.4) at the shortest length and 10.6 mm (±0.5) at the longest length (figure 4.4). The corresponding height of the activated muscle was 10.2 mm (±0.1) and 10.3 mm (±0.3) at the shortest and longest length, respectively. Height was similar at all lengths for the relaxed and activated conditions, but was slightly smaller (i.e. on average by about 0.41 mm or 3.1 % of the resting muscle height) in the activated compared to the relaxed state.

4.6 Aponeurosis and Tendon Deformation

Strains in the distal and middle segments of the medial aponeurosis were significantly different (table 4.1). No significant differences were found between strains along the lateral aponeurosis and the tendon.

4.7 Geometric Muscle Model

In calculating the contributions of the series elastic elements on the medial and lateral side of MG to fascicle deformation and realignment, it is obvious that the absolute contributions depend on the location of the fascicle. For example, for the most proximal fascicle of MG, the contribution of the medially located series elastic elements would be zero (or nearly so), since the most proximal fascicle inserts almost directly into the bone

with no visible series elasticity (figure 3.1). However, considering the middle fascicle (3-4, figure 3.1) that had, on average, the same amount of series elasticity on either side of MG (that is: lateral = tendon + aponeurosis 1-3, relaxed length at shortest muscle length = 54 mm; medial = aponeurosis 4-6 + aponeurosis 6 to the bone, relaxed length at shortest muscle length = 57 mm), it became apparent that medial aponeurosis elongations upon activation were about 2-4 times those of the lateral aponeurosis and tendon (table 4.2).
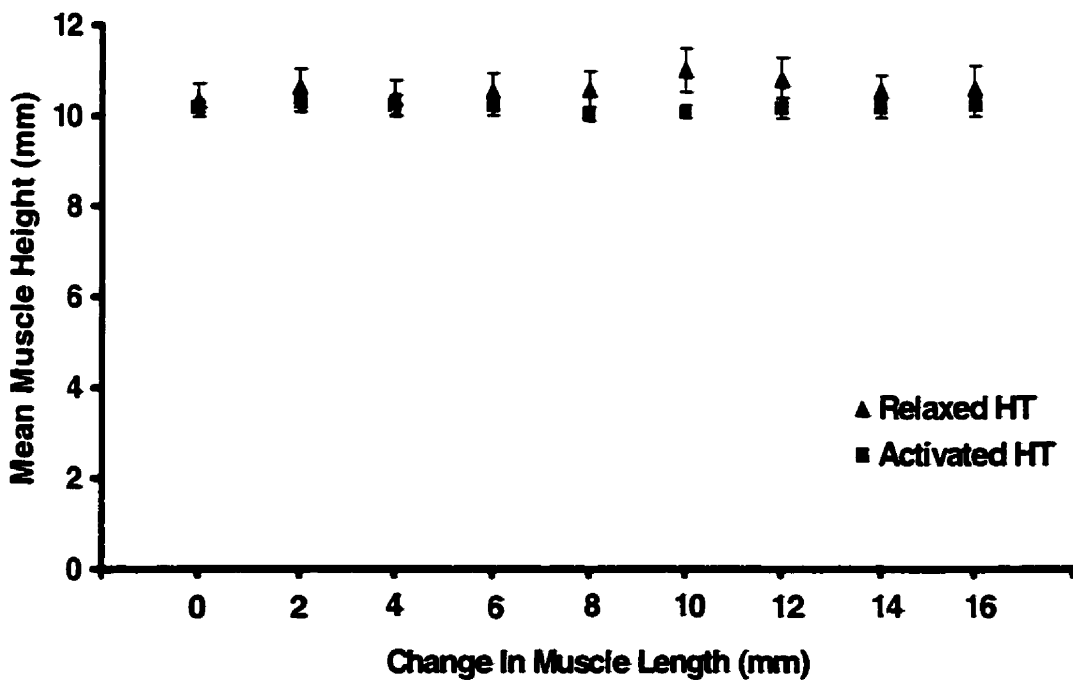
Figure 4.4. Mean (±SE) muscle height at the location of the central fascicle as a function of muscle length and active state.

Table 4.1. Mean (±SE) strains for the external tendon and distal and middle segments of the medial and lateral aponeuroses.

Muscle Length (mm)	Strain Medial Aponeurosis Distal Segment (2-4) (%)	Strain Medial Aponeurosis Middle Segment (4-6) (%)	Strain Lateral Aponeurosis Distal Segment (1-3) (%)	Strain Lateral Aponeurosis Middle Segment (3-5) (%)	Strain External Tendon (%)
0	-3.8(3.5)	0.8(1.4)	2.9(2.4)	3.6(0.57)	3.8(1.2)
2	-2.1(3.5)	1.4(1.3)	2.1(2.1)	4.3(2.2)	2.3(0.6)
4	-2.6(3.6)	1.0(1.2)	3.4(1.2)	4.3(1.8)	3.0(1.3)
6	-2.7(4.0)	1.2(1.2)	3.0(1.0)	2.7(1.2)	1.7(0.60)
8	-4.0(4.8)	1.7(1.1)	2.7(0.75)	3.6(1.3)	3.6(1.2)
10	-3.9(5.0)	1.6(1.2)	2.6(0.66)	3.6(1.1)	4.5(1.3)
12	-3.1(4.6)	-0.88(2.1)	2.2(0.74)	2.8(0.63)	2.4(1.1)
14	-1.6(4.2)	-1.0(2.1)	1.6(1.0)	3.4(0.64)	6.3(3.9)
16	-0.68(3.9)	-1.0(2.1)	2.2(0.70)	5.4(2.4)	3.2(0.91)

4.8 Force-length Relationship

Functional sarcomere lengths for the activated fascicles were on the ascending limb of the force-length relationship (figure 4.5). Average changes in sarcomere lengths were 0.7 μm, and 0.5 μm for the relaxed and activated fascicles, respectively, when the muscle underwent a 16 mm change in length (figure 4.6). The sarcomere functional length range (SFLR) for the relaxed fascicles was on the top part of the ascending limb and the plateau region of the force-length relationship and overlapped only slightly with the sarcomere lengths for the activated fascicles at the top of the ascending limb (figure 4.6). If fascicle lengths had changed by the same amount as the length of the entire muscle, the range of the sarcomere excursion would be far greater (1.7 μm) than the actual change in sarcomere length observed experimentally; that is sarcomere lengths would extend from the top of the ascending limb, across the plateau, to the lower part of the descending limb for this theoretical situation (figure 4.6).

Table 4.2. Elongations of the medial and lateral in-series elastic structures when going from the relaxed to the activated state. Values shown refer to the middle fascicle and all muscle lengths tested. Note, that the total elongations of the medial and lateral aponeuroses depend on the fascicle that is considered.

Muscle Length	Elongation of Medial Aponeurosis	Elongation of Lateral Aponeurosis and External Tendon	Contribution of Medial Aponeurosis to Total Elongation of S.E.S.	Contribution of Lateral Aponeurosis and External Tendon to Total Elongation of S.E.S.
(mm)	(mm)	(mm)	(%)	(%)
0	3.5	1.8	66	34
2	4.2	1.2	78	22
4	4.4	1.7	72	28
6	5.5	1.1	83	17
8	5.2	1.9	73	27
10	5.0	2.3	68	32
12	5.6	1.4	80	20
14	4.3	2.9	60	40
16	5.4	1.7	76	24
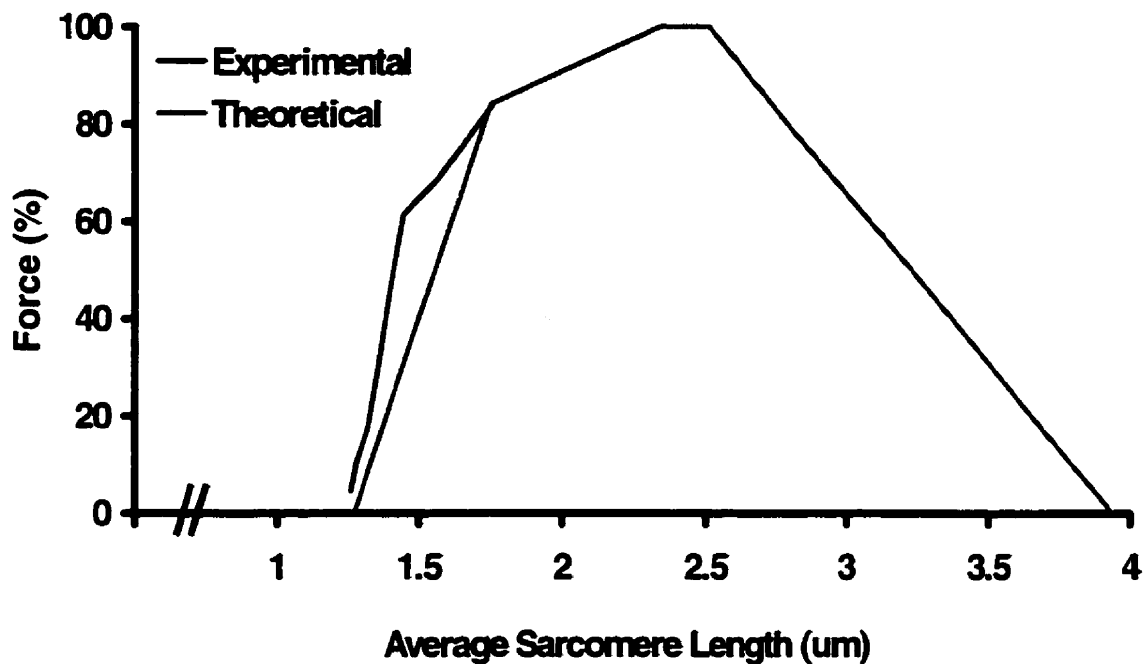
Figure 4.5. Mean (n = 8) force-sarcomere length relationship for the activated central fascicle (experimental) and the corresponding theoretical force-length relationship for cat skeletal muscle as described by Herzog et al. (1992).
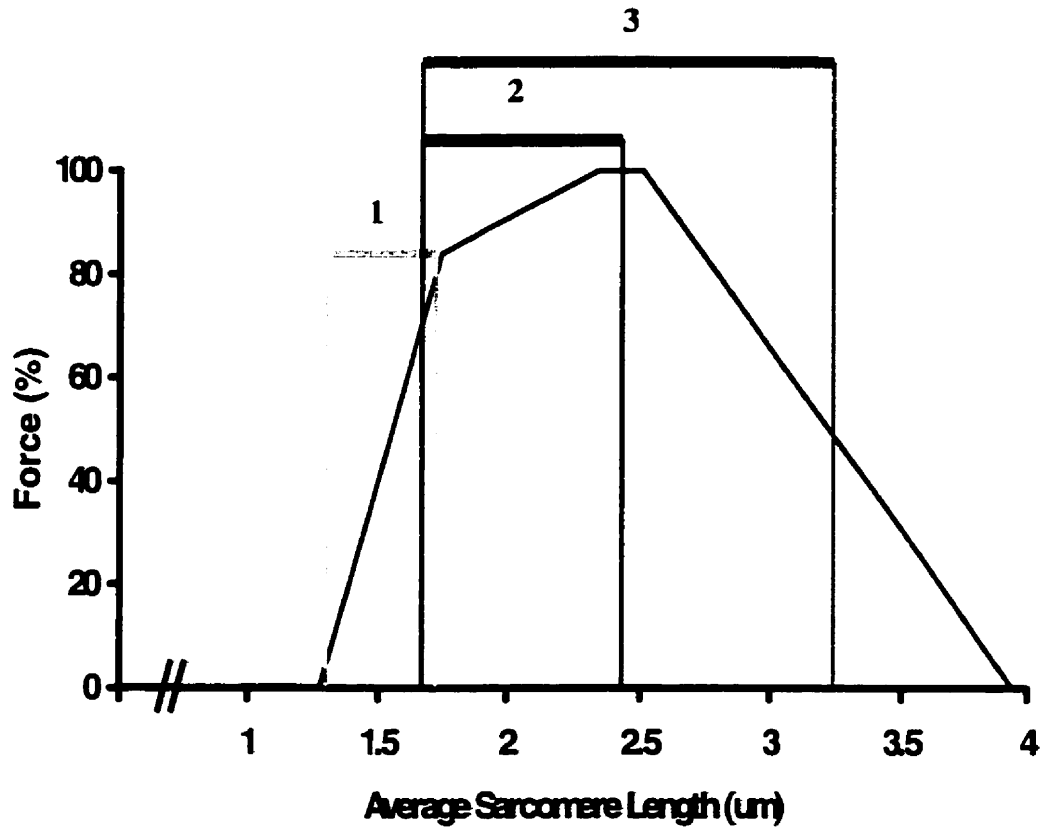
Figure 4.6. A comparison of functional sarcomere length range for activated muscle fascicles (1, light), relaxed muscle fascicles (2, dark) and theoretical fascicles assumed to undergo identical length changes as the whole muscle (3, medium).

CHAPTER 5.

DISCUSSION

5.1 Discussion

In this investigation, we demonstrated that fascicles shorten, pinnation angles increase and muscle height remains about constant during fixed-end isometric contractions of the cat MG. Specifically, results from the middle fascicle showed that fascicle lengths decreased on average by 27 %, the angle of pinnation increased on average by 42 %, and muscle height decreased on average by 3 % during maximal isometric contractions. Similar changes in muscle geometry have been observed in previous studies (Griffiths, 1991; Brooks et al., 1994; Narici et al.,1996; Maganaris et al., 1998). For instance, Griffiths, (1991) showed that fascicle lengths decreased on average by 28 %, and Brooks et al., (1994) demonstrated that pinnation angles increased on average by 46 % during maximal isometric contractions of cat MG. Narici et al., (1996) and Maganaris et al., (1998) reported that muscle height remained constant during isometric contractions of human MG. However the results of Maganaris et al., (1998) did show a slight, albeit non-significant decrease in muscle height in the central portion of the muscle. A non-significant but systematic decrease in muscle height was also found by Ichinose et al., (1995) who investigated the deformation of human vastus lateralis

muscle during submaximal isometric contractions (10 % MVC). Although our findings agree with previous studies, the role of specific elastic elements in fascicle deformation and realignment was not determined in these past investigations, and the effect of fascicle deformation on contractile properties of the muscle were ignored. For example, Hoffer et al., (1989) associated the entire difference between fascicle and whole muscle excursion with elongations of the tendon; the possible influences of pinnation angles and aponeurosis elongations were not considered. Others have suggested that the aponeuroses may contribute to fascicle deformation (Griffiths, 1991; Kawakami et al., 1998), however, they did not quantify aponeurosis strains or elongations.

It has been assumed previously that elongation of the external tendon accounts for most of the observed changes in fascicle geometry (Griffiths, 1991; Hoffer et al., 1989). However, our results suggest that tendon elongation only accounts, on average, for about 22 % of the entire elastic element elongation (table 5.1). Analysis of the elongations and strains in the different parts of the series elastic element revealed that: (a) total series elastic elongation found on the medial aspect of the muscle (medial aponeurosis) was greater than that on the lateral aspect of the muscle (lateral aponeurosis and tendon); (b) lateral aponeurosis and tendon had similar strains; and (c) medial aponeurosis strains increased from distal to proximal. Variable aponeurosis strains have been reported before by Zuurbier et al. (1994) who showed greater strains in the end regions than the mid portion of the medial aponeurosis of rat MG during muscle activation. Based on these results, we speculate that either compliance or stress at the proximal end of the

Table 5.1. Elongations of the external tendon and the aponeuroses (medial and lateral) when going from the relaxed to the activated state, and percent contributions of the external tendon and aponeuroses to total series elastic elongation. Values shown refer to the elastic structures in series with the middle fascicle at all muscle lengths tested.

Muscle Length	Elongation of External Tendon	Percent Contribution of Tendon to Total Elongation of S.E.S	Elongation of Aponeuroses	Percent Contribution of Aponeuroses to Total Elongation of S.E.S
(mm)	(mm)	(%)	(mm)	(%)
0	1.5	28	3.8	72
2	1.0	18	4.4	82
4	1.3	21	4.8	79
6	0.7	11	5.9	89
8	1.5	21	5.6	79
10	2.0	27	5.3	73
12	1.1	16	5.9	84
14	2.7	37	4.5	63
16	1.4	20	5.7	80

medial aponeurosis is larger than the corresponding values at the medial and distal regions. One observation that might be of particular importance, and likely has functional implications for the contractile properties of MG, is that despite the fact that the medial and lateral elastic elements in series with the middle fascicle are about equal in length, the total series elastic elongation was greater on the medial side than the lateral side of the muscle.

There is much information on the deformation of muscles during isometric contractions (Fukunaga et al., 1997; Kawakami et al., 1998; Maganaris et al., 1998). However, little information is known regarding how pinnation and series elasticity may affect changes in fascicle length during whole muscle excursion. Muhl (1982) and Griffiths (1991) demonstrated in rabbit digastric and cat medial gastrocnemius, respectively, that activated fibres only extended by about 0.7 mm for every 1 mm increase in whole muscle-tendon unit length. Muhl (1982) and Griffiths (1991) suggested that activated fibre excursion was less than whole muscle excursion because of changes in pinnation angle. However neither of them mentioned the possible influence of series elasticity on fascicle (fibre) excursion. Our results indicate that the mean activated and relaxed fascicle lengths in cat MG increase by 30 % and 46 %, respectively, of the increase in whole muscle-tendon length. We determined that the change in the pinnation angle accounted for about 19 % and the elongation of the series elastic structures accounted for 81 % of the difference between activated fascicle excursion and whole muscle excursion. This result suggests that the series elastic components of cat MG play an important role in reducing fascicle excursion during muscular length changes.

Initially, we assumed, like others (Muhl, 1982; Griffiths, 1991), that differences between *relaxed* fascicle excursion and whole muscle excursion were primarily accounted for by changes of the pinnation angle; however this assumption was not correct. We found that only 15 % of the excursion difference between relaxed fascicles and the whole muscle could be attributed to a change in the angle of pinnation, while 85 % of the excursion difference was explained by strains in the series elastic structures. These large strains in the series elastic elements occurred with little external force, as passive forces at the shortest muscle length were always zero and averaged about 2.0 N at the longest muscle length. Presumably, the strains in the series elastic element during passive muscle stretch corresponded to an unfolding of the crimp patterns in the tendon and aponeuroses. This unfolding is known to give large tissue elongations (up to about 3 % strain) for relatively small forces (Herzog & Gal, 1999).

The deformation of the muscle during activation, and the small excursion of fascicles for large muscle length changes, have direct functional consequences for muscle contractility and force production. One way to arrive at these functional consequences is by determining the sarcomere force-length properties of cat MG in the relaxed and activated state. The functional range of sarcomeres for the fully activated muscle was about 0.5 μm and occupied the lower and mid-portion of the ascending limb of the force-length relationship (figure 4.6). The functional sarcomere range for the relaxed muscle was about 0.7 μm and covered the top part of the ascending limb and the plateau region. Finally, a theoretical, relaxed fascicle that undergoes the same length change as the muscle would undergo sarcomere length changes of 1.7 μm and would occupy the top

part of the ascending limb, the plateau and much of the descending limb of the force-length relationship (figure 4.6). One of the open questions regarding the sarcomere working range is: why would sarcomere length decrease from a mean optimal length to a suboptimal length during activation (figure 4.6)? This study was not aimed at answering this question but several possibilities exist. From direct force and length measurements in cat MG during normal locomotion, it is known that MG exerts its largest forces at the longest (in vivo) muscle lengths (Walmsley et al., 1978; Hodgson, 1983). Therefore, peak forces may be produced near the sarcomere optimal length during voluntary contractions. Also, it is well accepted that MG fascicle (and thus mean sarcomere) lengths do not change appreciably during normal cat locomotion (Griffiths, 1991; Hoffer et al., 1989) and that forces are submaximal, therefore the in vivo force requirements of MG during normal locomotion can be accommodated easily. No in vivo force and muscle/fascicle length records are available for cats galloping at full speed or climbing trees. It is quite perceivable that during these high intensity activities the knee is more extended and the ankle more flexed than during normal locomotion at the instant of peak force requirement; therefore sarcomere lengths for these movements might be close to optimal when high forces are required. Future research is needed to fully explain why sarcomere length during normal (slow) locomotion is not optimal for force production in the cat MG.

5.2 Speculative Extension of Discussion

Muscle height of the cat MG remained relatively constant during muscle activation and whole muscle excursion. This result is in agreement with previous

investigations of the medial gastrocnemius in human subjects (Narici et al., 1996; Maganaris et al., 1998). Interestingly, it is not known exactly why, or how, muscle height remains constant during muscular contractions, and an answer can only be speculated at this point. One explanation given by Maganaris et al., (1998) was that as intramuscular pressure builds up during muscle force production the muscle may become less compliant in the direction of its thickness. Maganaris et al., (1998) further theorized that "the line of action of the effective muscle fibre force transmitted to the tendon lies on the longitudinal axis of the tendon"; therefore the spatial orientation of the distal tendon plate may not change during contraction. Interestingly, Herzog et al., (1999) demonstrated experimentally that forces were directed along the longitudinal axis of the distal aponeurosis of the cat MG, independent of the magnitude of the force, the length of the muscle, or the state of muscle deformation. Forces in the plane of the muscle fibres (x, z plane) were found to be substantial, whereas forces perpendicular to the fibre plane (y plane) were negligible (Herzog et al., 1999). In addition, Herzog et al., (1999) found that the forces along the distal aponeurosis and tendon (that is forces normally referred to as the muscle force) were, on average, 10-20 % larger than the forces in the direction of the fibres. Herzog et al., (1999) speculated that forces of constraints imposed by isovolumetricity may redirect the forces parallel to the muscle fibres in such a way that the resultant force always acts in the direction of the aponeuroses. The results of this thesis and of Herzog et al., (1999) suggest that unipennate muscle should deform by a parallel sliding of the two tendon plates relative to one another. Such deformation of the tendon plates may explain the constancy of muscle height during muscular contraction

and excursion. The results presented in this thesis for the deformation of muscle height provide important and relevant information needed for understanding the mechanics of force production and transmission in unipennate skeletal muscle.

During force production of an isometric contraction series elastic structures were found to undergo large deformations in length as fascicle length decreased on average by 27 %. Interestingly, it is not known exactly why fascicles shorten by up to a third of their length during force production or how this may be important for normal locomotion? Although the results demonstrated in this thesis are only for isometric contractions, the information generated regarding fascicle and tendon deformation may be relevant for understanding dynamic animal locomotion. For instance, in the freely hopping wallaby (Griffiths et al., 1989) and in the walking cat (Hoffer et al., 1989; Griffiths, 1991), muscle fibres were found to shorten in the early stages of the stance phase during whole muscle stretch because of tendon compliance. It has been suggested that the relationship between fascicle, and tendon deformation during force production may play an important role for the storage and release of elastic energy during animal locomotion (Hoffer et al., 1989; Roberts et al., 1997). During the loading phase of the cat step-cycle fascicles may shorten by up to a third of their length in order to generate enough force to stretch the series elastic structures. Interestingly, tendons are well suited to store potential energy during loading of the muscle-tendon complex, and can release much of this energy as mechanical work when the muscle is unloaded (Hoffer et al., 1989). Roberts et al., (1997) suggested from an investigation on the gastrocnemius of turkeys that stretch and recoil of tendon and muscle springs may supply mechanical work to maintain cyclic

movements of the body and limbs during running (Roberts et al., 1997). However, in addition to maintaining tension in the tendon springs, Roberts et al., (1997) argued that active muscle fibres must also provide forces necessary to support the weight of the body during running. It can be speculated from all of these findings that shortening of fascicles plays an important role in producing forces necessary to support the body during locomotion, and to deform tendons for elastic energy storage.

Another result presented in this thesis that requires further discussion is the fact that length changes imposed on the whole muscle were not directly transferred to the fascicles of the cat MG. For a 16 mm increase of whole muscle length across the physiological range of MG, activated and relaxed fascicle lengths were found to only increase on average by 30 % and 46 %, respectively. Differences in fascicle and whole muscle excursion were clearly attributed to elongation of the series elastic structures, and a decrease in the angle of pinnation. Interestingly, Griffiths (1991) demonstrated that activated fibres of the cat MG extended by about 0.7 mm for every 1 mm increase in whole muscle-tendon unit length. The large difference in fascicle (fibre) excursion between our study and that of Griffiths (1991) may be explained by differences in our experimental methods. For instance, Griffiths (1991) used disc crystals whereas our investigation used cylindrical piezoelectric crystals. Cylindrical crystals have been reported to perform far better than disc crystals as disc crystals must be critically aligned on fascicles and must remain well aligned through a movement in order to obtain a useable signal (Hoffer et al., 1989). The strength of the ultrasound signal detected with cylindrical crystals has been found to be independent of rotational changes (Caputi et al.,

1992; Hoffer et al., 1989). In addition, Griffiths (1991) reported difficulties in measuring muscle fibre length because of problems with the alignment of piezoelectric (disc) crystals. Differences may also be attributed to the fact that Griffiths (1991) examined MG at much longer lengths than our investigation. For instance, Griffiths (1991) reported activated fibre lengths that extended to the descending limb of a muscle fibre length-tension curve (activated fibre lengths ranged from about 16 mm to 24 mm). In contrast, activated fibre lengths of MG were only about 16 mm at the longest muscle length examined in our investigation. These experimental variations may explain the difference in fascicle excursion found between our investigation and that of Griffiths (1991). Nevertheless, it may still be speculated from all of these findings that changes of the pinnation angle, and deformation of series elastic structures may provide a protective mechanism for ankle extensor muscles like the MG, which undergo eccentric or stretch contractions. By limiting fascicle excursion during whole muscle excursion, the long compliant aponeuroses and tendons may serve as mechanical buffers for fibres in order to protect them from sudden impulses that may occur during dynamic locomotion (Griffiths, 1991). Griffiths, (1991) suggested that muscle fibres of MG would become rapidly injured if they underwent identical length changes as the whole muscle, and that a major role of tendon compliance is to protect fibres from injury associated with stretch. The results of this thesis clearly demonstrate that muscle architecture has a great influence on the relationship between fascicles and the parent muscle, especially during whole muscle excursion.

Another surprising finding of this thesis was that the functional working range of sarcomere lengths shifted from the plateau region to the ascending limb of the force-length relationship during muscle activation. It is not known exactly why sarcomere length would decrease from a mean optimal length to a suboptimal length during muscle activation, and an explanation can only be speculated upon at this point in the discussion. It may be suggested that there is a connection between the functional demands of a muscle and the region of the force-length relationship that it occupies during muscle activation. Rassier et al., (1999) suggested that in vivo force-length properties of a muscle adapt to changes in the muscle's daily requirements (i.e. chronic training). Rassier et al., (1999) further reported that during everyday movement tasks, muscles can undergo either a stretch-shortening or a shortening-stretch cycle. In a stretch-shortening cycle, a muscle is stretched initially as it increases its force, and then shortens as it decreases its force. Such a function follows a force-length curve with a positive slope, similar to that of the ascending limb of the force-length relationship (Rassier et al., 1999). The cat medial gastrocnemius has been reported to undergo stretch-shortening cycles during locomotion (Hoffer et al., 1989; Griffiths, 1991), which may explain why the muscle operates on the ascending limb during muscle activation. In addition, Rassier et al., (1999) reported that skeletal muscle is able to adapt sarcomere number and therefore sarcomere length to movement demands imposed upon the muscle. More specifically, the average sarcomere length of a muscle undergoing stretch-shortening cycles has been speculated to be smaller than a muscle undergoing shortening-stretch cycles (Rassier et al., 1999). In conclusion, it seems probable that the stretch-shortening requirements of

the cat MG during normal locomotion may have caused sarcomeres to adapt to working

on the ascending limb during muscle activation. These findings may suggest that the

force-length properties of MG are adapted to the requirements imposed on the muscle

during daily movement tasks.

CHAPTER 6.

SUMMARY AND CONCLUSIONS

6.1 Summary and Conclusions

- Fascicles shortened, pinnation angles increased, and muscle height remained approximately constant during maximally activated isometric contractions. Such deformations imply that fascicle length and the angle of pinnation are uniquely related to one another and an equation for predicting fibre length or angle of pinnation becomes:

$$FL_r \times \sin(\alpha)_r = FL_a \times \sin(\alpha)_a$$

Where FL and α represent fascicle lengths and pinnation angles, respectively during relaxed (r) conditions prior to muscle activation and activated (a) conditions during maximal force production. Such an equation may be useful for predicting changes of muscle geometry and may have important implications for muscle modeling.

- Deformation of the aponeuroses contributed, on average, to 78 % of the change in fascicle geometry during isometric contractions, while the external tendon only contributed, on average, to 22 % of the change in fascicle geometry. This finding is contrary to the suggestions of Hoffer et al., (1989) who theorized that all or most of the series elastic deformation would take place in the tendon during force production,

and that the tendon would primarily contribute to changes in fibre geometry during contractions.

- For a 16 mm increase in MG length, activated and relaxed fascicle lengths only increased by 30 % and 46 %, respectively. The difference in excursion range between activated and relaxed fascicles and the whole muscle were attributed to:

  1. **Pinnate fibre arrangement** – A decrease in the angle of pinnation across the physiological length range of MG contributed 15 % during relaxed conditions, and 19 % during activated conditions to the excursion difference between fascicles and the whole muscle.

  2. **Compliance in the tendon and aponeuroses** – Deformation of the series elastic structures across the physiological length range of MG contributed 85 % during relaxed conditions, and 81 % during activated conditions to the excursion difference between fascicles and the whole muscle.

- The sarcomere functional length range (SFLR) is shifted to a different part of the force-length relationship for active compared to relaxed fascicles. Activated sarcomeres were found to work on the ascending limb of the force-length relationship, while relaxed sarcomere excursion occurred at the top of the ascending limb and the plateau region of the force-length relation. The difference between the force-length behavior of relaxed and activated sarcomeres suggests that relaxed sarcomere lengths should not be used to predict the in vivo sarcomere lengths in active muscle.

6.2 Limitations

1. Direct measurements along the proximal segment of the medial aponeurosis were not made. Deformations of this segment were calculated by subtracting the appropriate, known series elastic segment lengths from the known total deformation of the series elastic structures. Future investigations, could confirm the calculated results of this study directly.

2. Data were recorded for fixed-end isometric contractions. Future investigations may be aimed at determining deformations of muscle structures during dynamic contractions.

6.3 Future Directions

Some authors (Otten, 1988; Winters & Woo, 1990; Lieber, 1992; Rutherford & Jones, 1992; Kawakami et al., 1998) suggested that the resultant force (vector) acts along the muscle fibre direction, and that muscular force (F_m) is related to the fibre force (F_f) by the equation $F_m = F_f \cos (\alpha)$, where α is the angle between the line of action of the muscle and the muscle fibre. Herzog et al., (1999) argued on theoretical grounds that the resultant force (vector) could not occur in the direction of the fibres because the corresponding muscle deformation would not be consistent with the preservation of volume. Using three-dimensional force measurement on the distal aponeurosis of the cat MG, Herzog et al., (1999) demonstrated that the resultant force is directed along the aponeurosis and tendon and not along the fibres. They showed that the forces of constraints imposed by isovolumetricity must redirect the active fibre forces in such a

way that the resultant force becomes aligned with the tensile axis of the aponeurosis and corresponding tendon.

The results of this thesis demonstrate that large deformations of the aponeuroses and the series elastic tendons take place during muscle activation, and that muscle height remains about constant during contraction. These findings and those by Herzog et al., (1999) suggest that unipennate muscle should deform by a parallel sliding of the two tendon plates relative to one another. Future research should be aimed at determining what forces of constraints are imposed on the muscle during activation and contraction, and how these force constraints influence the mechanics of force production in unipennate muscle?

6.4 Potential Clinical Applications

Muscle models have been developed to analyze characteristics of force production, and to determine how skeletal muscle deforms based on muscle architecture (Lemos et al., 1999). Experimental data reported in this thesis may provide a framework of information to study muscle dysfunction via muscle modeling. In fact some experimental results demonstrated in this thesis have already been implemented into a three-dimensional geometric model of skeletal muscle (Lemos et al., 1999). Lemos et al., (1999) found that modifying various input parameters of a three-dimensional model, such as stiffness of the series-elastic elements, improved the agreement between the model and the experimental results demonstrated in this thesis. Changing architectural parameters of the three-dimensional model such as fibre, aponeurosis, or tendon stiffness or length may allow one to study muscle deformation from the perspective of an injured or dysfunctional

system. It is conceivable that if a muscle model could be generated to fit closely to actual in vivo characteristics of the MG then modifications could be made to the model in order to study how normal muscle deformation is influenced by dysfunction.

Muscle modeling using the data reported in this thesis may also be used to study the influence of various clinical interventions on muscle deformation and muscle function (i.e. force producing capabilities of the muscle). For example Jaspers et al., (1999) reported that little is known about the surgical intervention of intramuscular aponeurotomy commonly used in orthopaedics to length overly short muscles. The technique consists of transsecting the aponeurosis perpendicular to its length at one or more locations (Jaspers et al., 1999). Such surgical operations have been reported to be successful in restoring more normal gait patterns in spastic children (Jaspers et al., 1999). Muscle models incorporating data presented in this thesis may clearly be useful for studying how the aponeurotomy actually alters mechanics of the "dysfunctional" muscle. With the use of muscle models it appears hopeful that surgeons and researchers may have the ability to study many clinical interventions and their outcomes in a precise and a non-invasive manner.

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APPENDIX A. NUMERICAL RAW DATA

A.1 Summary of Relaxed and Activated Fibre Length and Percent Change of Fibre Length for Middle Fascicle (3-4).

FL (3-4) cat	Relaxed -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	16.35	16.59	17.56	18.28	19.25	20.31	21.25	22.70	23.89
1R(2)	14.60	14.80	15.46	15.97	16.66	17.68	18.65	19.80	20.88
2L(3)	14.85	15.51	16.48	17.63	19.10	20.45	21.98	23.13	24.12
2R(4)	15.34	15.45	16.08	17.08	18.31	20.11	20.47	21.08	22.47
3L(5)	18.94	19.57	20.74	22.33	23.17	24.00	24.82	25.00	25.62
3R(6)	16.91	17.41	19.17	20.29	21.65	21.96	23.67	25.28	26.62
4L(7)	12.57	12.83	13.83	14.85	15.72	16.62	16.49	17.56	18.53
4R(8)	14.85	15.08	15.78	16.48	17.32	18.37	19.05	20.00	21.01
Mean (mm)	15.55	15.90	16.89	17.86	18.90	19.94	20.80	21.82	22.89
SD (mm)	1.88	2.00	2.20	2.43	2.50	2.38	2.73	2.69	2.69
SE (mm)	0.67	0.71	0.78	0.86	0.89	0.84	0.97	0.95	0.95

FL (3-4) cat	Active -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	12.57	12.81	13.51	13.92	14.37	15.08	15.88	17.22	18.36
1R(2)	11.40	11.46	11.54	11.63	11.81	11.98	12.72	13.38	14.18
2L(3)	11.53	11.80	12.09	12.37	12.72	13.23	14.11	14.89	15.78
2R(4)	11.43	11.66	11.98	12.18	12.77	13.47	14.04	14.51	15.78
3L(5)	13.07	13.15	13.96	14.44	14.75	15.57	18.32	19.62	20.79
3R(6)	13.53	14.03	14.53	15.21	15.80	16.49	19.12	20.17	21.49
4L(7)	10.33	10.35	10.49	10.70	10.93	11.27	11.80	12.41	13.00
4R(8)	12.10	12.32	12.61	12.84	13.03	13.26	13.63	14.11	14.77
Mean (mm)	12.00	12.20	12.59	12.91	13.27	13.79	14.95	15.79	16.77
SD (mm)	1.04	1.14	1.34	1.51	1.60	1.79	2.61	2.89	3.11
SE (mm)	0.37	0.40	0.48	0.53	0.57	0.63	0.92	1.02	1.10

FL (3-4) cat	Percent change from relaxed to activated isometric conditions								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	23.16	22.75	23.07	23.84	25.36	25.78	25.29	24.15	23.15
1R(2)	21.95	22.58	25.34	27.20	29.11	32.23	31.79	32.43	32.08
2L(3)	22.30	23.93	26.64	29.87	33.39	35.32	35.81	35.62	34.55
2R(4)	25.51	24.53	25.48	28.67	30.28	33.01	31.38	31.19	29.75
3L(5)	30.98	32.82	32.67	35.33	36.35	35.14	26.18	21.54	18.88
3R(6)	19.95	19.46	24.18	25.04	27.03	24.90	19.20	20.22	19.26
4L(7)	17.77	19.33	24.17	27.91	30.46	32.16	28.48	29.30	29.84
4R(8)	18.46	18.29	20.10	22.05	24.80	27.83	28.46	29.48	29.69
Mean (%)	22.51	22.96	25.21	27.49	29.60	30.80	28.32	27.99	27.15
SD (%)	4.26	4.60	3.60	4.10	3.95	4.09	4.99	5.46	5.93
SE (%)	1.51	1.63	1.27	1.45	1.40	1.44	1.77	1.93	2.10

A2. Summary of Relaxed and Activated Pinnation Angles and Percent Change for Middle Angle ($\alpha 2$).

PA ($\alpha 2$) cat	Relaxed								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	36.14	45.80	36.26	37.07	34.31	31.06	26.43	22.80	21.96
1R(2)	52.43	51.79	49.83	47.19	44.42	43.21	38.24	36.59	34.89
2L(3)	48.62	46.88	43.97	40.76	37.53	36.89	33.26	25.83	22.99
2R(4)	47.68	47.00	44.58	41.49	39.59	37.20	36.39	32.90	33.65
3L(5)	34.77	33.31	29.91	28.45	26.04	28.45	26.71	25.14	21.00
3R(6)	36.94	35.64	32.18	30.76	29.29	31.33	29.19	26.28	26.83
4L(7)	44.84	44.03	40.30	38.16	36.83	35.53	37.95	36.42	35.22
4R(8)	38.19	37.36	33.17	31.63	30.03	27.48	25.71	30.43	29.17
Mean (deg)	42.45	42.73	38.78	36.94	34.76	33.89	31.74	29.55	28.21
SD (deg)	6.74	6.51	7.02	6.33	6.06	5.27	5.36	5.32	5.91
SE (deg)	2.38	2.30	2.48	2.24	2.14	1.86	1.89	1.88	2.09

PA (α2) cat	Active								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	56.58	64.08	57.56	55.08	47.06	43.51	36.39	33.37	33.62
1R(2)	62.96	64.42	62.68	61.04	59.00	55.59	53.85	50.81	48.02
2L(3)	67.81	63.49	61.42	58.87	56.28	53.40	48.01	43.50	36.73
2R(4)	64.15	64.52	61.34	58.59	53.31	50.37	47.64	46.66	42.21
3L(5)	49.39	47.12	44.55	43.13	41.18	41.58	36.88	34.29	32.25
3R(6)	51.24	48.55	44.48	41.87	39.26	37.57	34.57	32.94	31.41
4L(7)	67.75	66.50	63.74	61.46	58.41	55.82	54.46	51.25	48.81
4R(8)	54.95	53.42	52.05	49.69	48.67	47.16	43.42	40.89	38.26
Mean (deg)	59.35	59.01	55.98	53.72	50.40	48.12	44.40	41.72	38.91
SD (deg)	7.27	7.96	7.97	7.88	7.60	6.81	7.86	7.59	6.82
SE (deg)	2.57	2.81	2.82	2.78	2.69	2.41	2.78	2.69	2.41

PA (α2) cat	Percent change from relaxed to activated isometric conditions								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	56.54	39.91	58.74	48.58	37.14	40.11	37.67	46.38	53.07
1R(2)	20.09	24.37	25.78	29.34	32.83	28.67	40.83	38.87	37.64
2L(3)	39.47	35.45	39.68	44.44	49.94	44.77	44.34	68.42	59.79
2R(4)	34.55	37.29	37.61	41.21	34.66	35.39	30.92	41.82	25.43
3L(5)	42.05	41.49	48.91	51.61	58.12	46.14	38.07	36.44	53.59
3R(6)	38.70	36.20	38.19	36.11	34.05	19.90	18.42	25.34	17.06
4L(7)	51.12	51.03	58.14	61.03	58.60	57.08	43.50	40.74	38.57
4R(8)	43.91	42.99	56.92	57.10	62.04	71.57	68.87	34.38	31.18
Mean (%)	40.80	38.59	45.50	46.18	45.92	42.96	40.33	41.55	39.54
SD (%)	10.95	7.59	12.04	10.61	12.54	16.18	14.25	12.50	14.97
SE (%)	3.87	2.68	4.26	3.75	4.44	5.72	5.04	4.42	5.29

A3. Summary of Relaxed and Activated Muscle Height and Percent Change of Height in the Vicinity of the Middle Fascicle (3-4).

Ht (3-4) cat	Relaxed -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	9.65	11.89	10.38	11.02	10.85	10.48	9.46	8.80	8.93
1R(2)	11.57	11.63	11.81	11.72	11.66	12.10	11.54	11.80	11.94
2L(3)	11.14	11.32	11.44	11.51	11.63	12.28	12.05	10.08	9.42
2R(4)	11.34	11.30	11.28	11.31	11.67	12.16	12.14	11.45	12.45
3L(5)	10.80	10.75	10.34	10.64	10.17	11.43	11.16	10.62	9.18
3R(6)	10.16	10.15	10.21	10.38	10.59	11.42	11.54	11.19	12.02
4L(7)	8.86	8.92	8.95	9.17	9.43	9.66	10.14	10.42	10.68
4R(8)	9.18	9.15	8.64	8.64	8.66	8.48	8.26	10.13	10.24
Mean (mm)	10.34	10.64	10.38	10.55	10.58	11.00	10.79	10.56	10.61
SD (mm)	1.03	1.13	1.14	1.11	1.12	1.36	1.38	0.95	1.39
SE (mm)	0.36	0.40	0.40	0.39	0.39	0.48	0.49	0.34	0.49

Ht (3-4) cat	Active -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	10.49	11.52	11.40	11.41	10.52	10.38	9.42	9.47	10.16
1R(2)	10.15	10.33	10.26	10.17	10.12	9.89	10.27	10.37	10.54
2L(3)	10.68	10.56	10.62	10.59	10.58	10.62	10.48	10.25	9.44
2R(4)	10.28	10.52	10.51	10.40	10.24	10.38	10.38	10.55	10.60
3L(5)	9.92	9.64	9.80	9.87	9.71	10.33	11.00	11.05	11.09
3R(6)	10.55	10.51	10.18	10.15	10.00	10.05	10.85	10.97	11.20
4L(7)	9.56	9.49	9.40	9.40	9.31	9.32	9.60	9.68	9.78
4R(8)	9.91	9.89	9.74	9.79	9.78	9.72	9.37	9.23	9.15
Mean (mm)	10.19	10.31	10.24	10.22	10.03	10.09	10.17	10.20	10.25
SD (mm)	0.38	0.65	0.62	0.61	0.43	0.43	0.64	0.68	0.75
SE (mm)	0.13	0.23	0.22	0.21	0.15	0.15	0.23	0.24	0.26

Ht (3-4) cat	Percent change from relaxed to activated isometric conditions								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	8.73	-3.09	9.78	3.59	-3.07	-0.94	-0.42	7.69	13.77
1R(2)	-12.29	-11.14	-13.20	-13.17	-13.19	-18.33	-11.01	-12.14	-11.73
2L(3)	-4.12	-6.73	-7.21	-8.04	-9.06	-13.49	-13.01	1.72	0.23
2R(4)	-9.33	-6.84	-6.83	-8.12	-12.27	-14.66	-14.53	-7.87	-14.83
3L(5)	-8.12	-10.34	-5.29	-7.19	-4.55	-9.65	-1.44	4.08	20.80
3R(6)	3.86	3.59	-0.27	-2.18	-5.61	-11.95	-6.00	-2.03	-6.78
4L(7)	7.95	6.44	5.13	2.49	-1.19	-3.44	-5.37	-7.11	-8.47
4R(8)	7.98	8.13	12.77	13.34	12.92	14.66	13.34	-8.86	-10.66
Mean (%)	-0.67	-2.50	-0.64	-2.41	-4.50	-7.22	-4.80	-3.07	-2.21
SD (%)	8.74	7.59	9.12	8.54	8.23	10.55	8.96	7.03	12.94
SE (%)	3.09	2.68	3.23	3.02	2.91	3.73	3.17	2.49	4.57

mean across all lengths (%) =	-3.11
SD across all muscle lengths (%) =	2.11
SE across all muscle lengths (%) =	0.70

A4. Summary of Relaxed and Activated Medial Aponeurosis Length and Percent Strains for Segments (2-4) and (4-6).

MA (2-4) cat	Relaxed								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	9.67	9.77	10.27	10.23	12.39	12.81	13.09	12.96	13.07
1R(2)	10.58	10.61	10.79	11.05	11.41	11.61	11.55	11.90	12.33
2L(3)	12.35	13.07	13.16	13.56	13.87	14.26	14.47	14.44	14.67
2R(4)	10.43	10.59	11.02	11.52	11.88	12.29	12.36	12.39	12.96
3L(5)	10.48	10.81	11.13	11.64	12.02	12.56	12.98	13.47	13.86
3R(6)	14.78	14.86	15.26	15.27	15.50	15.63	15.75	16.01	16.15
4L(7)	7.42	7.40	7.23	7.10	7.02	6.97	7.31	7.20	7.20
4R(8)	10.07	10.16	10.16	10.31	10.39	10.45	10.51	10.58	10.65
Mean (mm)	10.72	10.91	11.13	11.34	11.81	12.07	12.25	12.37	12.61
SD (mm)	2.13	2.22	2.33	2.42	2.49	2.59	2.57	2.65	2.72
SE (mm)	0.75	0.79	0.83	0.85	0.88	0.92	0.91	0.94	0.96

MA (2-4) cat	Active -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	8.31	8.47	8.96	8.83	9.39	9.64	9.70	10.44	11.12
1R(2)	9.88	9.96	10.18	10.45	10.79	10.93	11.10	11.41	11.68
2L(3)	11.82	12.61	12.76	13.09	13.39	13.73	14.21	14.61	14.98
2R(4)	9.45	9.82	10.21	10.51	10.90	11.35	11.79	11.95	12.79
3L(5)	9.88	10.19	10.53	10.79	11.02	11.28	11.73	12.15	12.58
3R(6)	13.69	14.03	14.27	14.63	14.89	15.17	16.01	16.30	16.47
4L(7)	8.82	8.84	8.79	8.77	8.74	8.81	8.90	8.80	8.80
4R(8)	9.85	9.98	9.98	10.02	10.10	10.18	10.27	10.44	10.55
Mean (mm)	10.21	10.49	10.71	10.89	11.15	11.38	11.71	12.01	12.37
SD (mm)	1.74	1.89	1.88	2.02	2.04	2.11	2.37	2.41	2.45
SE (mm)	0.61	0.67	0.67	0.71	0.72	0.75	0.84	0.85	0.87

MA (2-4) cat	% strains -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	-14.02	-13.38	-12.75	-13.69	-24.23	-24.77	-25.89	-19.48	-14.96
1R(2)	-6.55	-6.10	-5.71	-5.43	-5.40	-5.84	-3.87	-4.14	-5.25
2L(3)	-4.24	3.53	-3.04	-3.52	-3.44	-3.67	-1.81	1.17	2.10
2R(4)	-9.35	-7.27	-7.40	-8.69	-8.29	-7.65	-4.61	-3.60	-1.31
3L(5)	-5.73	-5.70	-5.40	-7.28	-8.33	-10.17	-9.61	-9.83	-9.22
3R(6)	-7.40	-5.60	-6.46	-4.23	-3.97	-2.96	1.66	1.83	2.00
4L(7)	18.89	19.34	21.50	23.66	24.58	26.33	21.70	22.23	22.23
4R(8)	-2.14	-1.82	-1.82	-2.84	-2.82	-2.65	-2.34	-1.31	-1.01
Mean (%)	-3.82	-2.12	-2.63	-2.75	-3.99	-3.92	-3.10	-1.64	-0.68
SD (%)	9.84	9.91	10.28	11.23	13.45	14.19	13.12	11.87	10.94
SE (%)	3.48	3.50	3.64	3.97	4.76	5.02	4.64	4.20	3.87

MA (4-6) cat	Relaxed								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	14.79	14.20	14.81	15.00	15.09	15.13	14.86	15.67	15.72
1R(2)	13.12	13.15	13.29	13.49	13.55	13.52	15.27	15.29	14.98
2L(3)	12.19	12.86	12.72	12.82	12.89	12.90	13.27	13.29	12.96
2R(4)	13.75	14.12	14.40	14.49	14.52	14.55	14.55	14.26	14.56
3L(5)	10.59	10.56	10.58	10.51	10.50	10.78	10.21	10.53	10.47
3R(6)	13.38	13.39	13.36	13.38	13.39	13.41	13.56	13.46	13.46
4L(7)	14.88	14.95	15.13	15.21	15.26	15.03	15.27	15.41	15.50
4R(8)	13.81	13.90	14.40	14.58	14.73	14.78	14.84	14.61	14.95
Mean (mm)	13.31	13.39	13.58	13.68	13.74	13.76	13.98	14.06	14.08
SD (mm)	1.40	1.32	1.47	1.53	1.56	1.46	1.69	1.68	1.74
SE (mm)	0.50	0.47	0.52	0.54	0.55	0.52	0.60	0.59	0.62

MA (4-6) cat	Active								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	15.55	15.09	15.50	15.60	15.66	15.70	15.53	16.26	16.29
1R(2)	12.49	12.81	13.24	13.83	13.89	13.95	13.04	13.07	12.75
2L(3)	12.50	12.99	12.55	12.59	12.64	12.70	13.04	13.07	12.75
2R(4)	14.23	14.43	14.32	14.40	14.47	14.63	14.63	14.35	14.70
3L(5)	9.94	9.99	10.01	10.01	10.02	10.36	10.04	10.33	10.34
3R(6)	13.66	13.72	13.76	13.78	13.83	13.87	13.70	13.66	13.64
4L(7)	15.26	15.63	15.90	16.10	16.24	16.03	16.03	16.07	16.14
4R(8)	14.06	14.30	14.76	14.81	14.86	14.89	14.83	14.60	14.92
Mean (mm)	13.46	13.62	13.76	13.89	13.95	14.02	13.85	13.93	13.94
SD (mm)	1.81	1.75	1.88	1.92	1.94	1.82	1.89	1.90	1.99
SE (mm)	0.64	0.62	0.67	0.68	0.69	0.64	0.67	0.67	0.70

MA (4-6) cat	% strains -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	5.14	6.29	4.68	4.01	3.78	3.77	4.56	3.73	3.62
1R(2)	-4.81	-2.58	-0.35	2.51	2.50	3.20	-14.62	-14.51	-14.91
2L(3)	2.53	1.08	-1.33	-1.80	1.91	-1.57	-1.74	-1.62	-1.66
2R(4)	3.47	2.18	-0.54	-0.64	-0.32	0.53	0.53	0.65	0.95
3L(5)	-6.11	-5.39	-5.39	-4.83	-4.55	-3.86	-1.66	-1.90	-1.18
3R(6)	2.07	2.42	3.00	2.99	3.22	3.45	1.02	1.49	1.37
4L(7)	2.56	4.53	5.09	5.87	6.46	6.66	4.94	4.30	4.07
4R(8)	1.78	2.88	2.57	1.58	0.84	0.73	-0.10	-0.11	-0.21
Mean (%)	0.83	1.43	0.97	1.21	1.73	1.61	-0.88	-1.00	-0.99
SD (%)	4.03	3.77	3.53	3.46	3.25	3.34	6.09	5.90	5.99
SE (%)	1.43	1.33	1.25	1.22	1.15	1.18	2.15	2.09	2.12

A5. Summary of Relaxed and Activated Lateral Aponeurosis Length and Percent Strains for Segments (1-3) and (3-5).

LA (1-3) cat	Relaxed -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	5.88	5.56	5.91	5.89	5.89	5.89	5.91	5.91	5.96
1R(2)	12.67	12.73	12.53	12.56	12.56	12.58	12.59	12.61	12.59
2L(3)	15.06	14.98	15.10	15.24	15.33	15.43	15.49	15.50	15.50
2R(4)	12.02	12.22	12.29	12.32	12.33	12.35	12.36	12.36	12.38
3L(5)	17.71	17.97	17.94	18.57	18.68	19.14	19.15	18.98	19.09
3R(6)	15.77	16.27	16.14	16.40	16.49	16.83	16.58	16.55	16.57
4L(7)	10.07	10.53	10.58	10.87	11.32	11.58	11.93	12.21	12.45
4R(8)	15.55	15.49	13.90	13.84	13.83	13.74	13.86	13.93	13.96
Mean (mm)	13.09	13.22	13.05	13.21	13.30	13.44	13.48	13.51	13.56
SD (mm)	3.80	3.92	3.71	3.85	3.85	3.97	3.92	3.87	3.87
SE (mm)	1.34	1.38	1.31	1.36	1.36	1.40	1.39	1.37	1.37

LA (1-3) cat	Active -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	6.05	5.79	6.14	6.17	6.17	6.17	6.22	6.26	6.28
1R(2)	12.53	12.50	12.45	12.44	12.45	12.47	12.49	12.52	12.55
2L(3)	16.27	16.06	16.09	16.17	16.20	16.21	16.26	16.27	16.23
2R(4)	12.58	12.55	12.56	12.59	12.64	12.62	12.69	12.01	12.72
3L(5)	18.01	18.43	18.23	18.86	19.00	19.43	19.23	19.15	19.29
3R(6)	16.54	16.64	16.67	16.76	16.86	17.28	16.81	16.80	16.81
4L(7)	11.35	11.58	11.65	11.68	11.82	11.92	12.25	12.42	12.67
4R(8)	13.87	13.92	13.95	13.98	14.03	14.06	14.06	14.07	14.09
Mean (mm)	13.40	13.43	13.47	13.58	13.65	13.77	13.75	13.69	13.83
SD (mm)	3.77	3.89	3.76	3.88	3.91	4.05	3.92	3.91	3.89
SE (mm)	1.33	1.38	1.33	1.37	1.38	1.43	1.39	1.38	1.37

LA (1-3) cat	% strains -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	2.88	4.16	3.91	4.69	4.70	4.70	5.21	5.99	5.43
1R(2)	-1.09	-1.81	-0.61	-0.98	-0.86	-0.86	-0.86	-0.73	-0.37
2L(3)	8.08	7.20	6.53	6.06	5.62	5.09	4.98	4.97	4.67
2R(4)	4.61	2.64	2.26	2.25	2.50	2.25	2.62	-2.84	2.74
3L(5)	1.74	2.57	1.63	1.58	1.73	1.53	0.40	0.89	1.05
3R(6)	4.88	2.27	3.34	2.22	2.24	2.65	1.39	1.49	1.49
4L(7)	12.70	9.95	10.19	7.51	4.49	2.93	2.71	1.77	1.73
4R(8)	-10.79	-10.14	0.33	1.00	1.45	2.27	1.44	0.99	0.88
Mean (%)	2.88	2.10	3.45	3.04	2.73	2.57	2.24	1.57	2.20
SD (%)	6.91	6.07	3.51	2.82	2.11	1.85	2.11	2.85	1.97
SE (%)	2.44	2.14	1.24	1.00	0.75	0.66	0.74	1.01	0.70

LA (3-5) cat	Relaxed -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	9.13	8.92	9.35	9.50	9.62	9.69	9.76	9.62	9.86
1R(2)	7.99	9.09	9.36	9.58	9.69	9.70	9.10	9.44	9.56
2L(3)	10.70	10.81	10.84	10.87	10.87	10.89	10.83	10.83	10.84
2R(4)	11.27	11.24	11.14	11.09	12.09	12.14	12.10	10.72	12.07
3L(5)	11.82	11.95	11.87	11.71	11.86	11.62	11.59	11.79	11.75
3R(6)	14.63	14.90	14.80	15.01	15.08	15.13	15.29	15.32	15.37
4L(7)	15.45	15.52	15.40	15.35	14.94	14.69	14.51	14.21	14.00
4R(8)	7.04	7.11	7.21	7.28	7.36	7.41	7.55	7.47	7.48
Mean (mm)	11.00	11.19	11.25	11.30	11.44	11.41	11.34	11.17	11.37
SD (mm)	2.98	2.92	2.78	2.75	2.66	2.60	2.63	2.57	2.52
SE (mm)	1.05	1.03	0.98	0.97	0.94	0.92	0.93	0.91	0.89

LA (3-5) cat	Active -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	10.01	9.83	10.29	10.33	10.35	10.39	10.33	10.12	11.98
1R(2)	8.59	10.39	10.58	9.81	10.69	10.56	9.55	9.89	9.90
2L(3)	10.66	10.72	10.78	10.81	10.83	10.86	10.89	10.92	10.89
2R(4)	12.15	11.12	11.16	11.16	12.27	12.32	12.32	10.93	12.35
3L(5)	12.20	12.18	12.11	12.01	12.10	11.93	11.95	12.49	12.52
3R(6)	15.29	15.37	15.34	15.39	15.44	15.50	15.53	15.55	15.55
4L(7)	15.20	15.18	15.22	15.17	15.01	14.95	14.78	14.66	14.51
4R(8)	7.78	7.75	7.72	7.73	7.75	7.76	7.76	7.75	7.76
Mean (mm)	11.48	11.57	11.65	11.55	11.80	11.78	11.64	11.54	11.93
SD (mm)	2.78	2.61	2.56	2.61	2.52	2.53	2.60	2.58	2.47
SE (mm)	0.98	0.92	0.91	0.92	0.89	0.89	0.92	0.91	0.87

LA (3-5) cat	% strains -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	9.60	10.20	10.06	8.76	7.52	7.31	5.84	5.12	21.57
1R(2)	7.52	14.41	12.99	2.41	10.33	8.90	4.90	4.72	3.54
2L(3)	-0.43	-0.85	-0.57	-0.57	-0.42	-0.28	0.56	0.85	0.42
2R(4)	7.79	-1.09	0.18	0.69	1.53	1.52	1.78	2.01	2.29
3L(5)	3.25	1.93	2.04	2.54	2.08	2.64	3.06	5.88	6.55
3R(6)	4.53	3.13	3.64	2.50	2.38	2.44	1.61	1.55	1.19
4L(7)	-1.60	-2.18	-1.19	-1.20	0.52	1.78	1.91	3.14	3.63
4R(8)	10.50	8.88	7.05	6.12	5.23	4.78	2.86	3.72	3.70
Mean (%)	3.56	4.30	4.27	2.66	3.65	3.63	2.81	3.37	5.36
SD (%)	1.60	6.12	5.25	3.35	3.73	3.12	1.77	1.81	6.81
SE (%)	0.57	2.17	1.85	1.18	1.32	1.10	0.63	0.64	2.41

A6. Summary of Relaxed and Activated External Tendon Length and Percent Strains for Segment (1-7).

Tendon (1-7) cat	Relaxed -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	18.91	19.49	20.86	21.33	20.20	20.02	21.09	21.14	21.51
1R(2)	13.35	14.16	14.43	14.61	15.04	14.98	15.04	15.06	15.07
2L(3)	17.71	17.88	17.91	17.92	17.86	19.66	19.74	19.77	17.83
2R(4)	16.77	16.64	16.61	16.58	16.58	16.63	16.71	16.37	16.69
3L(5)	15.66	16.06	16.09	16.32	16.41	16.60	16.50	16.52	16.55
3R(6)	15.57	15.94	15.83	15.78	15.75	15.74	15.87	15.83	15.87
4L(7)	20.37	20.48	20.57	20.65	19.97	20.28	20.39	20.34	20.34
4R(8)	28.36	30.18	28.52	31.44	28.73	31.55	28.99	24.01	31.74
Mean (mm)	18.34	18.85	18.85	19.33	18.82	19.43	19.29	18.63	19.45
SD (mm)	4.59	5.01	4.51	5.42	4.42	5.31	4.52	3.15	5.43
SE (mm)	1.62	1.77	1.60	1.92	1.56	1.88	1.60	1.12	1.92

Tendon (1-7) cat	Active								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	19.49	19.58	20.64	21.09	21.14	21.25	21.17	22.33	22.63
1R(2)	14.63	14.67	14.75	14.76	15.28	15.23	15.30	15.33	15.33
2L(3)	18.25	18.29	18.26	18.20	18.17	21.82	20.22	20.42	18.41
2R(4)	16.67	16.69	16.72	16.74	16.75	16.80	16.86	16.58	16.95
3L(5)	16.95	16.97	16.94	16.95	16.97	16.98	16.64	16.66	16.67
3R(6)	15.89	16.23	16.26	16.30	16.38	16.47	16.38	16.46	16.49
4L(7)	20.76	20.76	20.84	20.82	20.15	20.52	20.46	20.46	20.54
4R(8)	29.23	31.00	31.64	32.57	32.00	34.14	31.80	32.00	34.38
Mean (mm)	18.98	19.27	19.51	19.68	19.60	20.40	19.85	20.03	20.18
SD (mm)	4.58	5.11	5.34	5.65	5.38	6.07	5.30	5.44	6.22
SE (mm)	1.62	1.81	1.89	2.00	1.90	2.15	1.88	1.92	2.20

Tendon (1-7) cat	% strains								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	3.09	0.47	-1.06	-1.08	4.65	6.16	0.37	5.61	5.23
1R(2)	9.58	3.59	2.24	1.05	1.56	1.65	1.74	1.84	1.74
2L(3)	3.04	2.33	1.98	1.55	1.72	10.97	2.42	3.27	3.28
2R(4)	-0.55	0.28	0.65	0.93	1.02	1.02	0.92	1.32	1.57
3L(5)	8.29	5.66	5.26	3.87	3.38	2.32	0.84	0.84	0.74
3R(6)	2.08	1.84	2.73	3.32	4.01	4.70	3.20	3.99	3.88
4L(7)	1.89	1.35	1.33	0.82	0.93	1.21	0.38	0.61	0.98
4R(8)	3.04	2.70	10.96	3.58	11.36	8.20	9.67	33.27	8.35
Mean (%)	3.81	2.28	3.01	1.75	3.58	4.53	2.44	6.34	3.22
SD (%)	3.40	1.76	3.68	1.71	3.44	3.67	3.09	11.01	2.59
SE (%)	1.20	0.62	1.30	0.60	1.22	1.30	1.09	3.89	0.91

A7. Summary of Relaxed and Activated Fibre Length and Percent Change of Fibre Length for Distal (1-2) and Proximal (5-6) Fascicles.

FL (1-2) cat	Relaxed -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	12.44	12.44	13.01	13.10	13.99	14.63	15.13	16.21	17.04
1R(2)	16.26	17.54	18.01	18.37	19.12	21.59	22.62	23.57	25.90
2L(3)	16.66	16.98	17.63	18.54	21.33	22.79	22.66	22.06	26.13
2R(4)	18.66	18.88	19.45	20.28	22.59	24.05	24.54	24.94	24.73
3L(5)	26.81	25.98	28.44	29.56	30.64	31.55	31.57	32.61	33.48
3R(6)	18.40	19.11	20.37	21.66	22.79	24.24	24.82	26.05	27.36
4L(7)	18.46	19.15	20.25	21.31	22.42	23.48	23.65	25.01	26.27
4R(8)	16.98	17.37	18.09	18.85	19.79	20.88	21.85	22.90	24.08
Mean (mm)	18.08	18.43	19.41	20.21	21.58	22.90	23.36	24.17	25.62
SD (mm)	4.06	3.74	4.33	4.62	4.66	4.67	4.50	4.57	4.51
SE (mm)	1.43	1.32	1.53	1.63	1.65	1.65	1.59	1.61	1.59

FL (1-2) cat	Active -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	9.91	10.16	10.76	10.73	11.33	11.76	12.22	13.18	13.99
1R(2)	11.70	12.02	12.05	12.12	12.19	12.50	13.30	13.92	14.73
2L(3)	12.70	12.84	13.06	13.26	13.56	14.06	14.67	15.32	15.74
2R(4)	13.70	13.84	13.90	14.06	14.35	16.41	17.12	17.15	17.95
3L(5)	20.92	21.19	23.43	23.94	24.13	24.91	25.64	26.61	27.70
3R(6)	15.47	15.84	16.50	16.94	17.37	17.98	19.74	20.62	21.89
4L(7)	15.33	15.52	15.69	15.90	16.15	16.60	17.37	18.31	19.20
4R(8)	13.46	13.75	13.99	14.38	14.72	14.87	15.49	16.03	16.72
Mean (mm)	14.15	14.40	14.92	15.17	15.47	16.14	16.94	17.64	18.49
SD (mm)	3.29	3.30	3.90	4.06	4.01	4.12	4.25	4.34	4.51
SE (mm)	1.16	1.17	1.38	1.43	1.42	1.46	1.50	1.53	1.59

FL (1-2) cat	Percent change -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	20.33	18.32	17.28	18.10	19.04	19.58	19.23	18.71	17.89
1R(2)	28.04	31.46	33.08	34.04	36.25	42.09	41.19	40.96	43.11
2L(3)	23.76	24.39	25.94	28.49	36.40	38.32	35.26	30.54	39.78
2R(4)	26.57	26.68	28.51	30.68	36.47	31.76	30.24	31.24	27.40
3L(5)	21.94	18.44	17.60	19.01	21.26	21.04	18.78	18.42	17.25
3R(6)	15.90	17.09	18.98	21.82	23.79	25.80	20.47	20.87	19.98
4L(7)	16.93	18.97	22.51	25.37	27.96	29.32	26.57	26.79	26.91
4R(8)	20.77	20.84	22.64	23.70	25.61	28.77	29.11	30.00	30.57
Mean (%)	21.78	22.02	23.32	25.15	28.35	29.58	27.61	27.19	27.86
SD (%)	4.26	5.04	5.58	5.62	7.16	7.80	8.04	7.69	9.69
SE (%)	1.51	1.78	1.97	1.99	2.53	2.76	2.84	2.72	3.43

FL (5-6) cat	Relaxed -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	18.40	18.20	19.15	24.81	26.11	25.95	26.85	34.57	39.68
1R(2)	17.69	17.95	18.91	19.43	20.17	21.33	22.42	23.71	24.90
2L(3)	16.57	17.18	18.38	19.69	21.20	23.05	22.70	23.85	24.93
2R(4)	16.89	17.32	18.28	19.46	20.79	22.51	22.97	23.99	29.44
3L(5)	16.83	17.18	18.31	19.25	20.43	21.82	22.54	24.04	25.28
3R(6)	16.77	17.15	19.23	19.92	21.20	22.60	22.90	24.34	25.61
4L(7)	13.75	13.95	15.13	15.95	16.97	18.15	18.71	20.06	21.31
4R(8)	20.40	20.71	21.57	22.31	23.16	24.07	26.78	28.90	29.97
Mean (mm)	17.16	17.46	18.62	20.10	21.25	22.43	23.23	25.43	27.64
SD (mm)	1.88	1.85	1.77	2.56	2.61	2.25	2.61	4.39	5.58
SE (mm)	0.66	0.65	0.62	0.91	0.92	0.79	0.92	1.55	1.97

FL (5-6)	Active								
cat	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	14.18	14.10	15.03	15.60	16.21	17.07	18.17	19.74	23.14
1R(2)	13.64	13.87	14.23	14.61	15.00	15.35	16.20	16.95	17.94
2L(3)	12.33	12.73	13.26	13.76	14.30	15.07	15.95	16.84	17.80
2R(4)	13.42	13.84	14.24	14.75	15.30	16.18	16.75	17.58	18.51
3L(5)	12.29	12.53	13.29	13.87	14.32	14.96	16.04	17.14	18.23
3R(6)	13.36	12.76	14.23	14.95	15.55	16.61	18.43	19.52	20.82
4L(7)	11.52	11.58	11.93	12.44	12.92	13.42	14.18	14.93	15.80
4R(8)	15.84	16.21	16.77	17.17	17.48	18.08	18.63	18.92	22.28
Mean (mm)	13.32	13.45	14.12	14.64	15.13	15.84	16.79	17.70	19.31
SD (mm)	1.34	1.40	1.42	1.39	1.37	1.45	1.53	1.61	2.51
SE (mm)	0.47	0.50	0.50	0.49	0.48	0.51	0.54	0.57	0.89

FL (5-6)	Percent change								
cat	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	22.93	22.51	21.55	37.13	37.92	34.19	32.34	42.90	41.68
1R(2)	22.89	22.73	24.76	24.81	25.66	28.02	27.75	28.51	27.96
2L(3)	25.56	25.90	27.89	30.11	32.54	34.61	29.72	29.38	28.60
2R(4)	20.52	20.09	22.08	24.21	26.37	28.12	27.08	26.70	37.14
3L(5)	27.00	27.07	27.42	27.93	29.92	31.41	28.83	28.70	27.90
3R(6)	20.30	25.59	26.03	24.97	26.66	26.50	19.51	19.80	18.70
4L(7)	16.24	17.00	21.17	22.01	23.87	26.05	24.20	25.56	25.87
4R(8)	22.34	21.71	22.27	23.05	24.54	24.89	30.42	34.53	25.67
Mean (%)	22.22	22.83	24.15	26.78	28.44	29.22	27.48	29.51	29.19
SD (%)	3.32	3.33	2.73	4.93	4.78	3.73	4.03	6.80	7.13
SE (%)	1.17	1.18	0.96	1.74	1.69	1.32	1.43	2.40	2.52

A8. Summary of Relaxed and Activated Pinnation Angles and Percent Change of Pinnation Angles for Distal (α1) and Proximal (α2) Fascicles.

PA (1-2) cat	Relaxed								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	34.72	29.08	33.48	37.00	43.31	30.31	38.98	37.65	36.56
1R(2)	27.66	23.50	21.97	20.38	15.65	#	#	#	#
2L(3)	37.65	37.46	36.33	34.79	28.68	23.10	19.69	21.50	16.26
2R(4)	25.28	22.69	19.38	19.78	15.29	10.82	#	#	15.10
3L(5)	10.35	16.57	#	#	#	#	#	#	#
3R(6)	33.09	32.01	28.35	27.10	26.41	26.38	25.78	24.13	22.45
4L(7)	42.85	40.20	37.28	35.20	33.07	31.83	33.55	31.18	29.19
4R(8)	32.90	32.29	29.76	28.55	26.33	22.36	23.12	26.46	24.89
Mean (deg)	30.56	29.23	29.51	28.97	26.96	24.13	28.22	28.18	24.07
SD (deg)	9.82	7.94	6.88	7.06	9.77	7.54	7.89	6.38	8.09
SE (deg)	3.47	2.81	2.60	2.67	3.69	3.08	3.53	2.85	3.30

PA (1-2) cat	Active								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	48.00	43.68	43.76	45.08	41.59	47.09	35.97	34.25	32.13
1R(2)	39.16	38.49	38.31	38.02	37.51	36.72	35.63	34.24	32.69
2L(3)	46.92	47.16	46.30	45.14	43.85	42.21	40.92	39.33	34.70
2R(4)	41.81	39.81	39.23	38.44	36.92	31.42	28.40	27.42	25.74
3L(5)	26.54	26.16	21.32	20.86	20.82	19.85	19.09	17.18	15.31
3R(6)	41.65	40.44	37.96	36.25	34.66	33.73	31.08	29.46	27.43
4L(7)	51.80	50.60	49.52	48.02	46.77	45.12	43.87	41.19	39.19
4R(8)	44.60	43.29	41.97	41.03	40.19	39.44	36.40	34.92	33.40
Mean (deg)	42.56	41.21	39.80	39.10	37.79	36.95	33.92	32.25	30.07
SD (deg)	7.63	7.27	8.49	8.44	7.89	8.76	7.75	7.59	7.27
SE (deg)	2.70	2.57	3.00	2.98	2.79	3.10	2.74	2.68	2.57

PA (1-2) cat	percent change								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	38.22	50.22	30.72	21.83	-3.98	55.35	-7.72	-9.02	-12.11
1R(2)	41.60	63.83	74.38	86.55	139.63	#	#	#	#
2L(3)	24.65	25.90	27.44	29.77	52.88	82.77	107.86	82.92	113.47
2R(4)	65.41	75.44	102.50	94.34	141.43	190.37	#	#	70.48
3L(5)	156.39	57.84	#	#	#	#	#	#	#
3R(6)	25.85	26.33	33.90	33.75	31.22	27.89	20.58	22.10	22.22
4L(7)	20.88	25.88	32.82	36.43	41.45	41.73	30.75	32.10	34.23
4R(8)	35.57	34.06	41.01	43.72	52.66	76.36	57.47	31.96	34.16
Mean (%)	51.07	44.94	48.96	49.49	65.04	79.08	41.78	32.01	43.74
SD (%)	44.78	19.54	28.46	28.84	55.02	58.29	43.68	33.07	43.22
SE (%)	15.83	6.91	10.76	10.90	20.79	23.80	19.54	14.78	17.64

PA (5-6) cat	Relaxed								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	13.63	26.30	14.58	38.51	39.02	28.94	27.42	#	#
1R(2)	42.59	39.22	39.57	37.15	35.24	38.67	23.17	22.91	23.79
2L(3)	48.96	44.25	41.85	39.12	36.10	37.65	17.79	37.55	46.34
2R(4)	40.73	39.28	36.47	33.74	37.42	35.13	34.69	27.72	#
3L(5)	25.97	24.31	17.90	#	#	#	#	#	#
3R(6)	43.33	42.96	40.69	38.16	36.50	43.62	34.55	31.68	31.79
4L(7)	53.47	52.46	48.11	44.36	40.95	41.14	43.72	41.32	39.87
4R(8)	49.17	48.25	44.06	41.50	38.82	35.24	#	#	#
Mean (deg)	39.73	39.63	35.40	38.93	37.72	37.19	30.22	32.24	35.44
SD (deg)	13.39	9.88	12.33	3.34	1.99	4.75	9.31	7.38	9.78
SE (deg)	4.73	3.49	4.36	1.26	0.75	1.79	3.79	3.30	4.89

PA (5-6) cat	Active								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	31.12	40.74	35.52	33.65	25.17	22.55	16.68	9.73	32.99
1R(2)	56.70	52.02	50.23	47.35	46.55	48.91	48.11	45.99	46.54
2L(3)	58.92	55.53	55.28	53.59	51.63	49.86	45.34	59.53	59.66
2R(4)	58.53	53.09	51.39	49.62	50.46	48.10	45.80	40.64	41.02
3L(5)	59.62	58.35	56.13	48.30	48.22	45.47	29.73	23.79	20.17
3R(6)	59.38	53.29	52.01	49.74	47.30	47.28	41.27	40.09	38.33
4L(7)	70.01	67.05	63.73	60.91	58.17	57.01	56.17	53.53	51.11
4R(8)	81.22	78.26	60.60	63.94	61.87	60.45	56.23	54.10	58.31
Mean (deg)	59.43	57.29	53.11	50.89	48.67	47.45	42.42	40.92	43.52
SD (deg)	14.12	11.17	8.49	9.24	10.92	11.29	13.40	16.78	13.28
SE (deg)	4.99	3.95	3.00	3.27	3.86	3.99	4.74	5.93	4.70

PA (5-6) cat	percent change								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	128.23	54.94	143.53	-12.62	-35.49	-22.07	-39.16	#	#
1R(2)	33.11	32.64	26.96	27.45	32.11	26.50	107.62	100.78	95.63
2L(3)	20.35	25.50	32.09	37.00	43.01	32.43	154.91	58.57	28.76
2R(4)	43.70	35.16	40.91	47.08	34.87	36.95	32.05	46.58	#
3L(5)	129.57	140.02	213.50	#	#	#	#	#	#
3R(6)	37.03	24.06	27.83	30.33	29.60	8.40	19.47	26.53	20.60
4L(7)	30.94	27.81	32.47	37.31	42.04	38.57	28.47	29.54	28.19
4R(8)	65.19	62.21	37.52	54.06	59.39	71.51	#	#	#
Mean (%)	61.01	50.29	69.35	31.51	29.36	27.47	50.56	52.40	43.29
SD (%)	43.83	38.84	70.07	21.52	30.25	28.85	69.26	30.01	35.08
SE (%)	15.49	13.73	24.77	8.13	11.43	10.40	28.27	13.42	17.54

A9. Summary of Relaxed and Activated Muscle Height and Percent Change of Muscle Height in the Vicinity of the Distal (1-2) and Proximal (5-6) Fascicle (5-6).

HT (1-2) cat	Relaxed -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	7.09	6.05	7.18	7.89	9.60	7.38	9.52	9.90	10.15
1R(2)	7.55	6.99	6.74	6.40	5.16	#	#	#	#
2L(3)	10.18	10.33	10.44	10.58	10.23	8.94	7.64	8.08	7.31
2R(4)	7.97	7.28	6.45	6.86	5.96	#	#	#	6.44
3L(5)	4.82	7.41	#	#	#	#	#	#	#
3R(6)	10.05	10.13	9.67	9.87	10.14	10.77	10.79	10.65	10.45
4L(7)	12.55	12.36	12.27	12.28	12.23	12.39	13.07	12.95	12.81
4R(8)	9.22	9.28	8.98	9.01	8.78	7.94	8.58	10.20	10.14
Mean (mm)	8.68	8.73	8.82	8.98	8.87	8.66	9.92	10.36	10.17
SD (mm)	2.35	2.14	2.16	2.11	2.50	2.75	2.11	1.75	1.95
SE (mm)	0.83	0.76	0.82	0.80	0.95	1.12	0.95	0.78	0.87

HT (1-2) cat	Active -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	7.36	7.02	7.44	7.60	7.52	8.61	7.18	7.42	7.44
1R(2)	7.39	7.48	7.47	7.46	7.42	7.47	7.75	7.83	7.96
2L(3)	9.28	9.42	9.44	9.40	9.40	9.44	9.61	9.71	8.96
2R(4)	9.14	8.86	8.79	8.74	8.62	8.56	8.14	7.90	7.80
3L(5)	9.35	9.34	8.52	8.53	8.58	8.46	8.38	7.86	7.31
3R(6)	10.28	10.28	10.15	10.01	9.88	9.99	10.19	10.14	10.09
4L(7)	12.05	11.99	11.93	11.82	11.77	11.76	12.04	12.06	12.13
4R(8)	9.45	9.43	9.36	9.44	9.50	9.45	9.19	9.17	9.20
Mean (mm)	9.29	9.23	9.14	9.12	9.08	9.22	9.06	9.01	8.86
SD (mm)	1.51	1.55	1.47	1.41	1.40	1.29	1.56	1.58	1.63
SE (mm)	0.53	0.55	0.52	0.50	0.50	0.45	0.55	0.56	0.58

HT (1-2) cat	Percent change -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	3.94	16.07	3.72	-3.64	-21.66	16.70	-24.58	-25.10	-26.68
1R(2)	-2.10	7.00	10.88	16.66	43.86	#	#	#	#
2L(3)	-8.82	-8.85	-9.63	-11.16	-8.19	5.65	25.87	20.11	22.47
2R(4)	14.64	21.69	36.30	27.35	44.68	#	#	#	21.04
3L(5)	94.10	26.06	#	#	#	#	#	#	#
3R(6)	2.36	1.46	4.96	1.47	-2.57	-7.25	-5.59	-4.79	-3.44
4L(7)	-4.02	-2.99	-2.70	-3.74	-3.79	-5.05	-7.93	-6.88	-5.32
4R(8)	2.43	1.61	4.21	4.81	8.25	18.94	7.15	-10.08	-9.20
Mean (%)	12.82	7.75	6.82	4.54	8.66	5.80	-1.01	-5.35	-0.19
SD (%)	33.55	12.35	14.53	13.28	25.89	12.03	18.80	16.30	18.89
SE (%)	11.86	4.37	5.49	5.02	9.79	5.38	8.41	7.29	7.71

mean across all lengths (%) =	4.42
SD across all muscle lengths (%) =	5.63
SE across all muscle lengths (%) =	1.88

HT (5-6) cat	Relaxed -10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	4.34	8.06	4.82	15.44	16.44	12.55	12.37	#	#
1R(2)	11.97	11.35	12.04	11.74	11.64	13.32	8.82	9.23	10.04
2L(3)	12.50	11.99	12.26	12.42	12.49	14.08	#	14.53	18.03
2R(4)	11.02	10.97	10.86	10.81	12.63	12.95	13.07	11.16	#
3L(5)	7.37	7.07	5.63	#	#	#	#	#	#
3R(6)	11.51	11.69	12.54	12.31	12.61	15.59	12.98	12.79	13.49
4L(7)	11.05	11.06	11.27	11.15	11.12	11.94	12.93	13.25	13.66
4R(8)	15.44	15.45	15.00	14.78	14.52	13.89	#	#	#
Mean (mm)	10.65	10.95	10.55	12.67	13.06	13.47	12.03	12.19	13.80
SD (mm)	3.37	2.54	3.51	1.78	1.83	1.19	1.81	2.04	3.27
SE (mm)	1.19	0.90	1.24	0.67	0.69	0.45	0.81	0.91	1.63

HT (5-6) cat	Active								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	7.33	9.20	8.73	8.64	6.89	6.55	5.22	3.34	12.60
1R(2)	11.40	10.93	10.93	10.75	10.89	11.57	12.06	12.19	13.02
2L(3)	10.56	10.50	10.90	11.08	11.21	11.52	11.35	14.52	15.36
2R(4)	11.45	11.07	11.13	11.24	11.80	12.04	12.01	11.45	12.15
3L(5)	10.60	10.66	11.03	10.36	10.68	10.67	7.96	6.91	6.29
3R(6)	11.50	10.23	11.21	11.41	11.43	12.21	12.16	12.57	12.91
4L(7)	10.82	10.66	10.70	10.87	10.97	11.26	11.78	12.01	12.29
4R(8)	15.66	15.87	14.61	15.42	15.41	15.72	15.49	15.33	18.96
Mean (mm)	11.16	11.14	11.15	11.22	11.16	11.44	11.00	11.04	12.95
SD (mm)	2.26	1.99	1.61	1.91	2.30	2.50	3.10	3.99	3.54
SE (mm)	0.80	0.70	0.57	0.67	0.81	0.89	1.09	1.41	1.25

HT (5-6) cat	Percent change								
	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	68.96	14.16	81.00	-44.05	-58.06	-47.84	-57.82	#	#
1R(2)	-4.78	-3.67	-9.21	-8.42	-6.45	-13.17	36.68	32.10	29.64
2L(3)	-15.47	-12.45	-11.17	-10.84	-10.24	-18.16	#	-0.11	-14.82
2R(4)	3.90	0.92	2.44	3.95	-6.55	-7.01	-8.14	2.61	#
3L(5)	43.82	50.77	95.01	#	#	#	#	#	#
3R(6)	-0.05	-12.46	-10.57	-7.33	-9.37	-21.73	-6.37	-1.67	-4.28
4L(7)	-2.04	-3.62	-5.04	-2.53	-1.32	-5.72	-8.90	-9.34	-10.00
4R(8)	1.43	2.74	-2.64	4.31	6.17	13.22	#	#	#
Mean (%)	11.97	4.54	17.47	-9.27	-12.26	-14.34	-8.91	4.71	0.135
SD (%)	28.81	20.55	43.92	16.44	20.96	18.62	33.45	15.93	20.14
SE (%)	10.18	7.26	15.52	6.21	7.92	7.04	14.96	7.12	10.06

mean across all lengths (%) =	-0.66
SD across all muscle lengths (%) =	11.22
SE across all muscle lengths (%) =	3.74

A10. Total Muscle Force During Muscle Activation

Force cat	-10mm	-8mm	-6mm	-4mm	-2mm	0mm	2mm	4mm	6mm
1L(1)	2.14	5.99	15.56	28.94	45.20	57.85	55.85	62.54	68.35
1R(2)	1.60	4.72	9.17	17.91	31.09	49.89	56.63	68.25	75.43
2L(3)	6.30	13.12	22.64	35.49	50.18	65.71	70.40	79.28	84.02
2R(4)	7.17	14.44	22.50	35.05	48.13	61.46	70.79	76.06	84.17
3L(5)	7.51	15.17	22.06	33.00	47.16	62.68	68.54	71.08	79.24
3R(6)	5.70	14.73	20.79	31.14	45.45	61.76	59.31	62.98	68.64
4L(7)	2.28	6.04	10.88	19.47	31.77	48.43	69.81	76.26	89.10
4R(8)	1.60	4.77	9.56	17.81	32.36	52.28	66.00	71.37	80.46
Mean (N)	4.29	9.88	16.64	27.35	41.42	57.51	64.67	70.98	78.67
SD (N)	2.61	4.86	6.06	7.71	8.17	6.50	6.38	6.16	7.45
SE (N)	0.92	1.72	2.14	2.73	2.89	2.30	2.25	2.18	2.63

APPENDIX B. RAW DATA TRACES

The raw data traces for all animals preparations (n =8) are organized in the following manner:

Left column from top to bottom

Force, Fibre length (distal (1-2) – medium color line; middle (3-4) – light dashed

line, proximal (5-6) – dark line), Pinnation angle (distal (α1) – medium color line;

middle (α2) – light dashed line; proximal (α3) – dark line), Muscle length.

Right column from top to bottom

Medial aponeurosis (distal segment length (2-4) – dark line; middle segment length

(4-6) – light line), Lateral aponeurosis (distal segment length (1-3) – dark line;

middle segment length (3-5) – light line), External tendon segment length.

BI. Raw Data Traces For Cat I Left Leg

Catt leftleg,(ISOMETRIC: 0 mm)

Catt leftleg,(ISOMETRIC: 2 mm)

Cat1 leftleg,(ISOMETRIC: 4 mm)

Cat1 leftleg,(ISOMETRIC: 6 mm)

Cat1 leftleg,(ISOMETRIC: 8 mm)

Cat1 leftleg,(ISOMETRIC: 10 mm)

Cat1 leftleg,(ISOMETRIC: 12 mm)
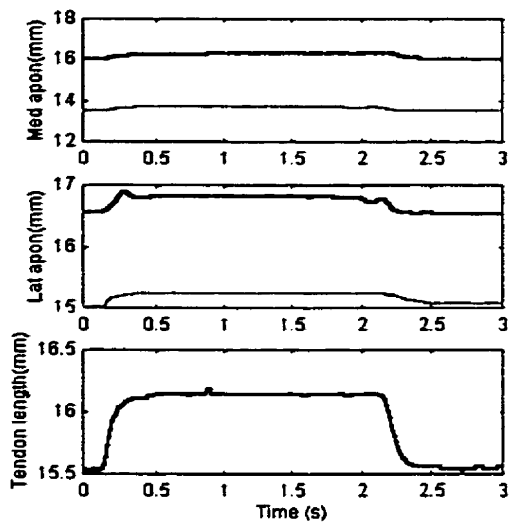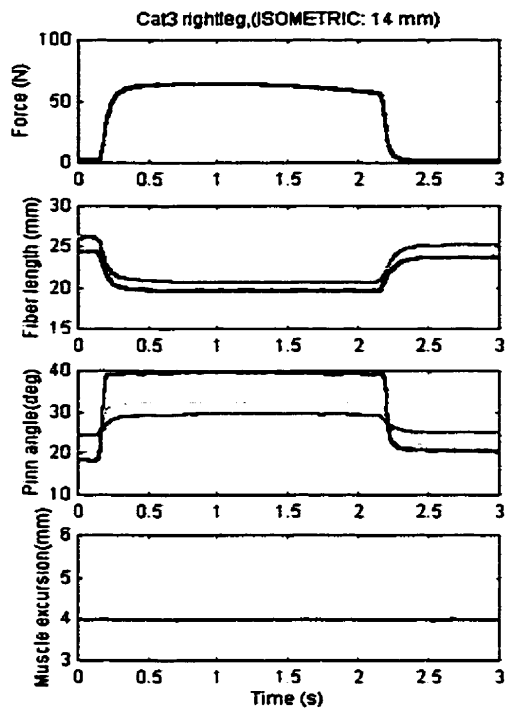

Cat1 leftleg,(ISOMETRIC: 14 mm)

Cat1 leftleg,(ISOMETRIC: 16 mm)
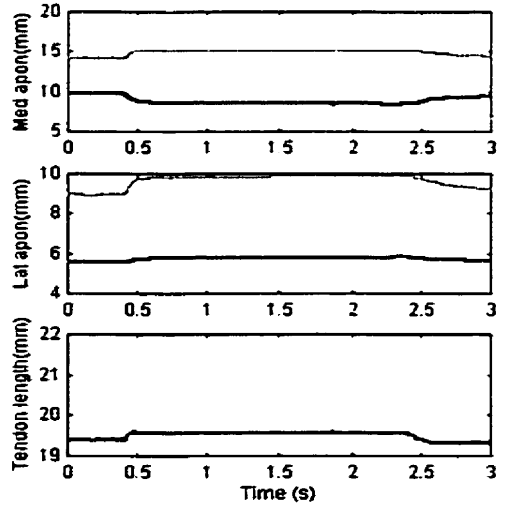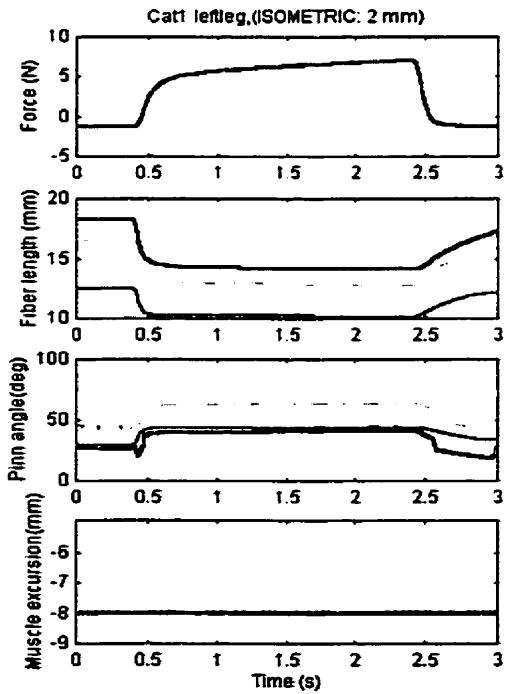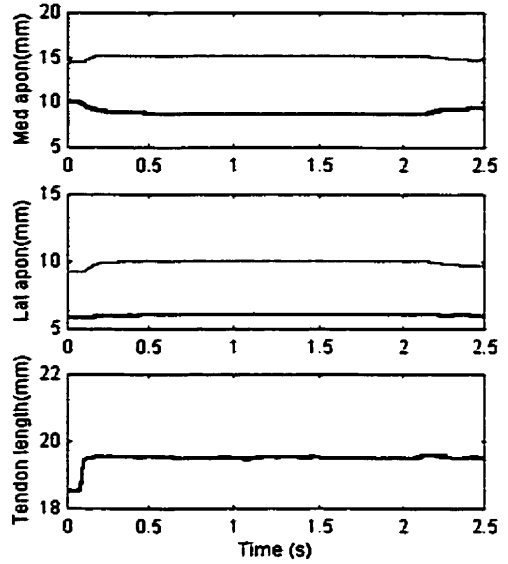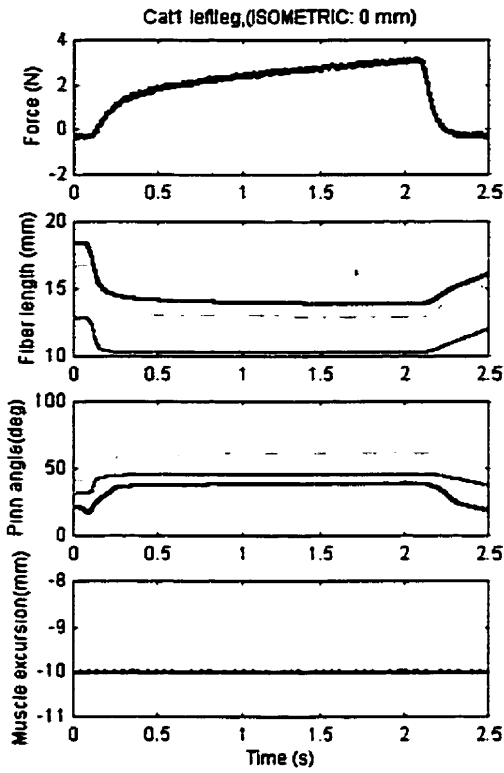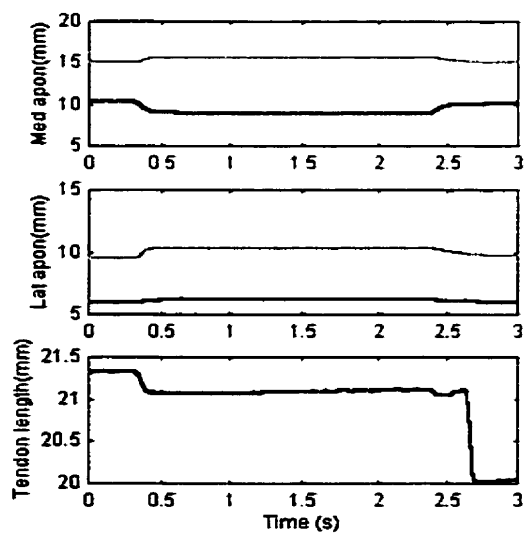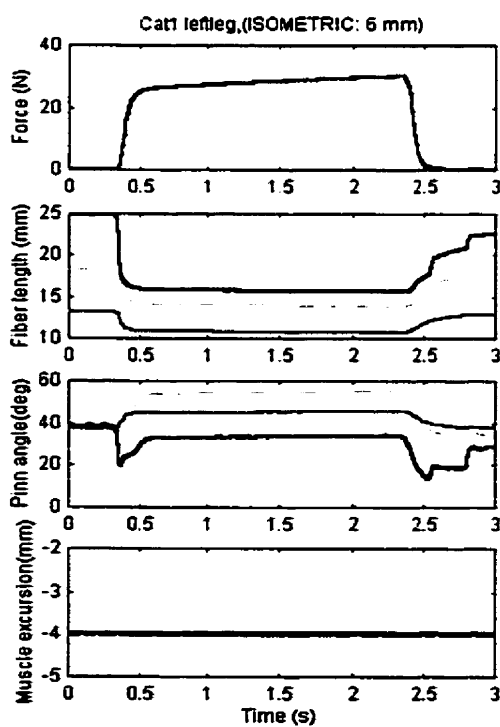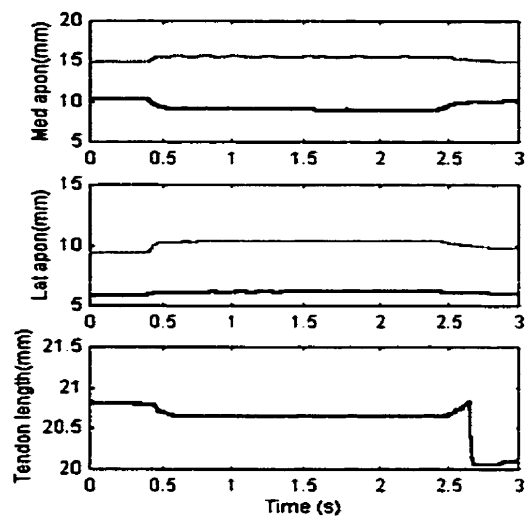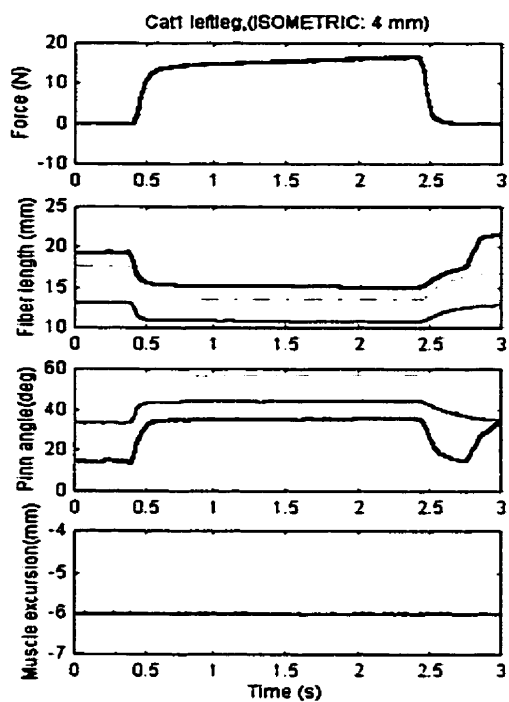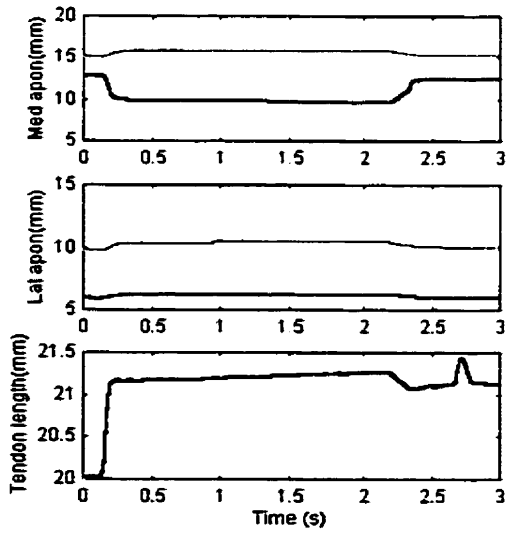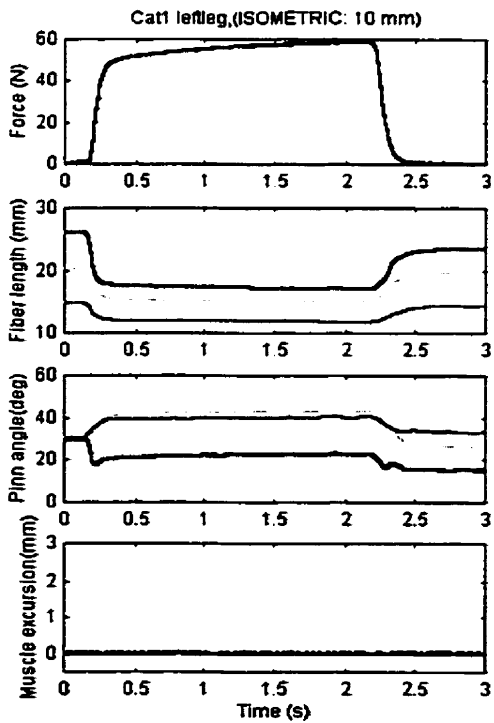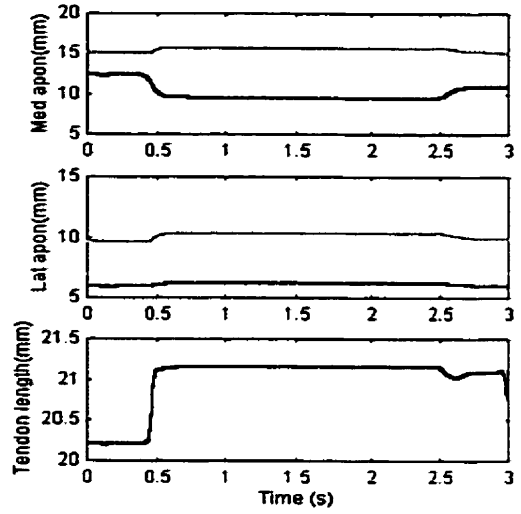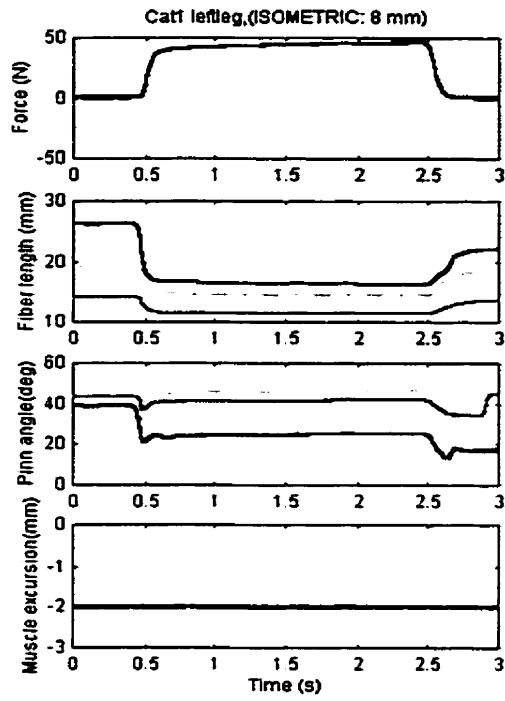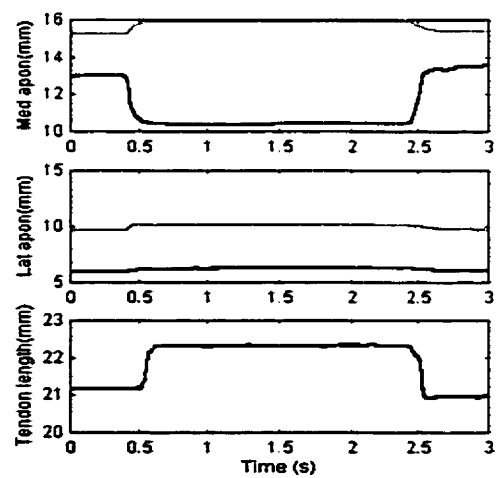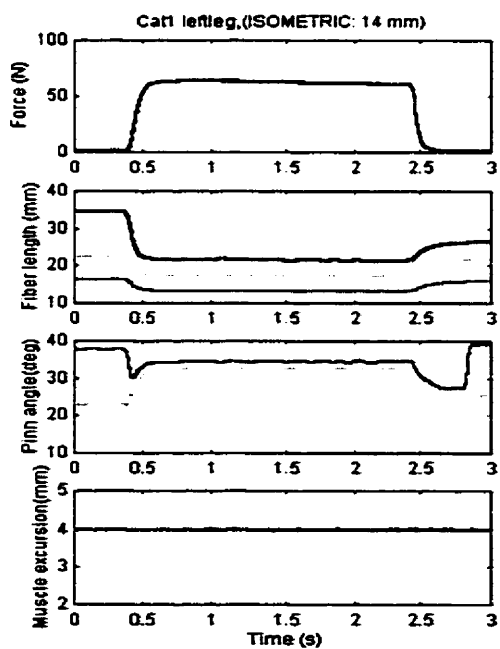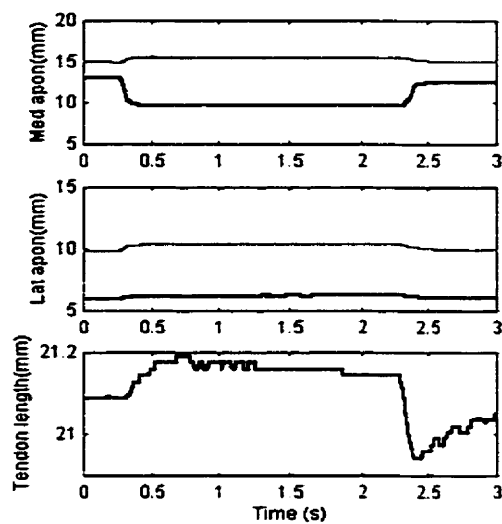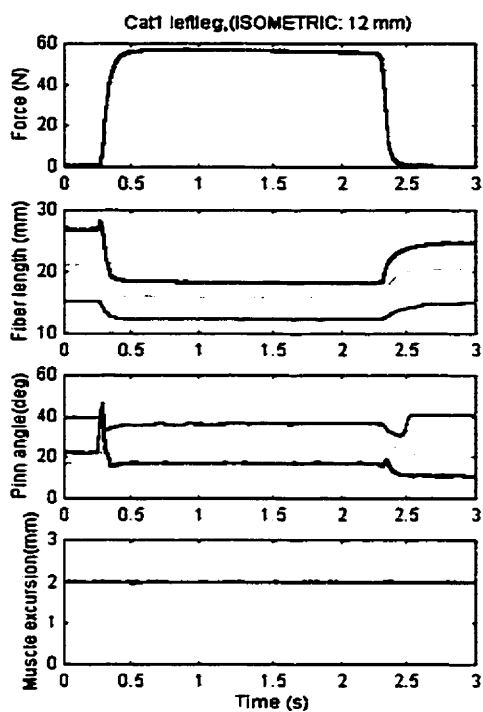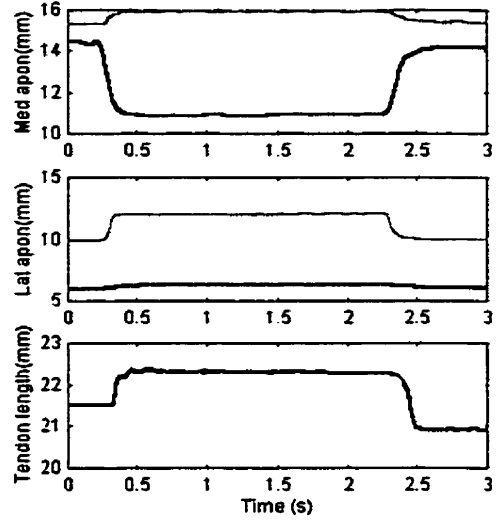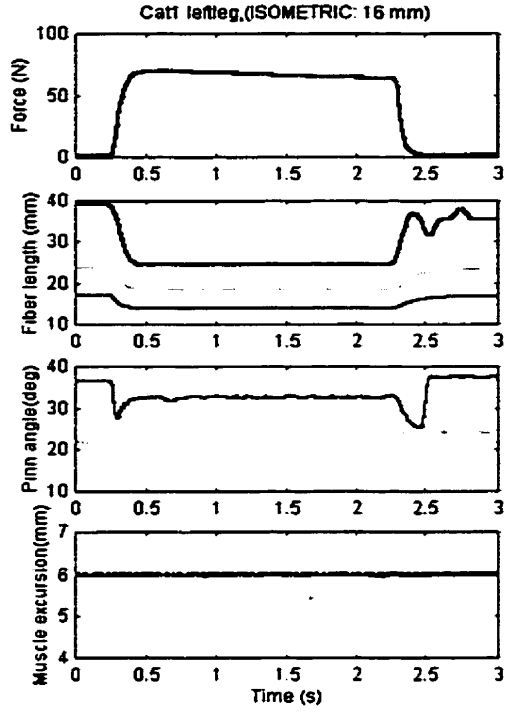
B2. Raw Data Traces For Cat 1 Right Leg

Cat1 rightleg,(ISOMETRIC: 0 mm)

Cat1 rightleg,(ISOMETRIC: 2 mm)

Cat1 rightleg,(ISOMETRIC: 4 mm)

Cat1 rightleg,(ISOMETRIC: 6 mm)

Cat1 rightleg,(ISOMETRIC: 8 mm)

Catt rightleg,(ISOMETRIC: 10 mm)

Catt rightleg,(ISOMETRIC: 12 mm)

Cat1 rightleg,(ISOMETRIC: 14 mm)

Cat1 rightleg,(ISOMETRIC: 16 mm)
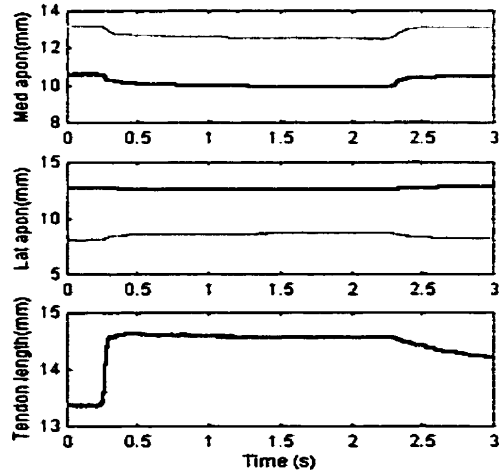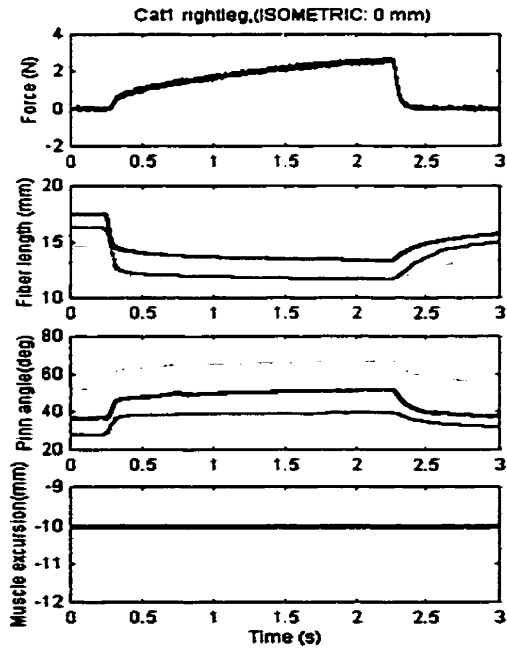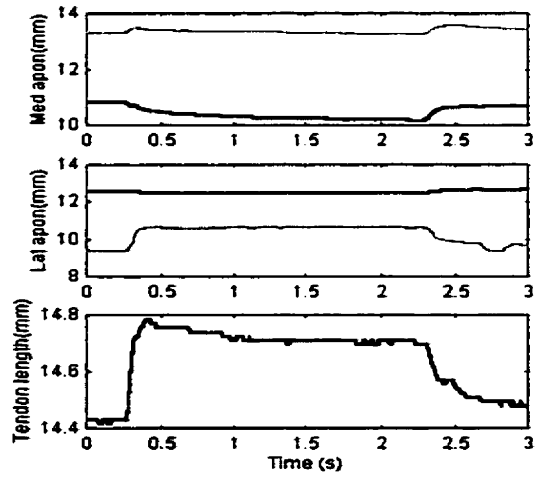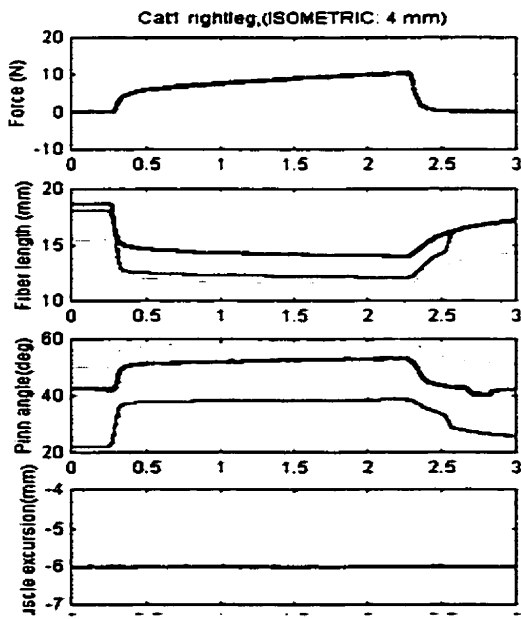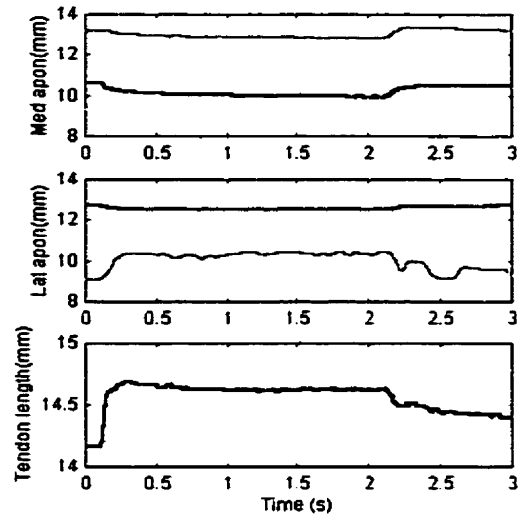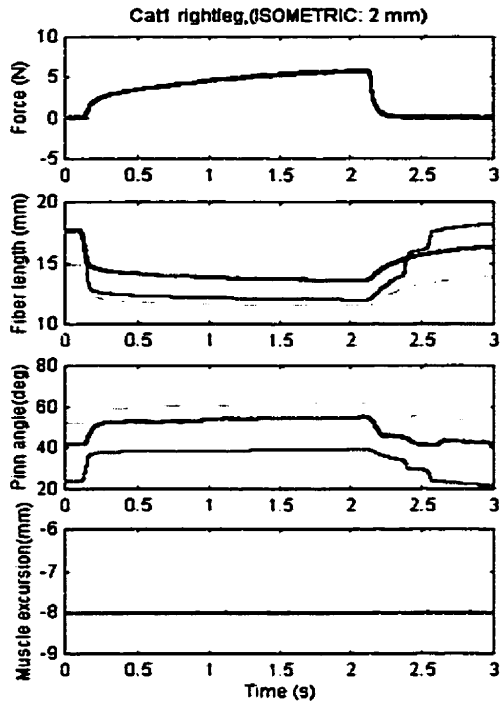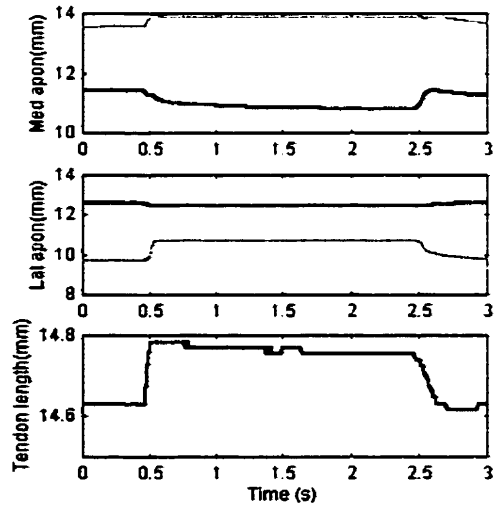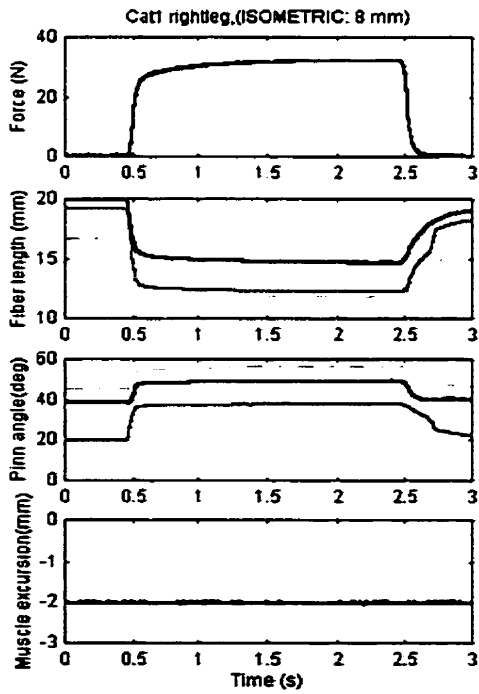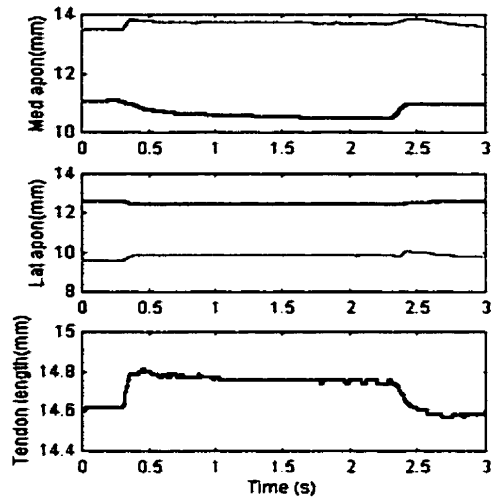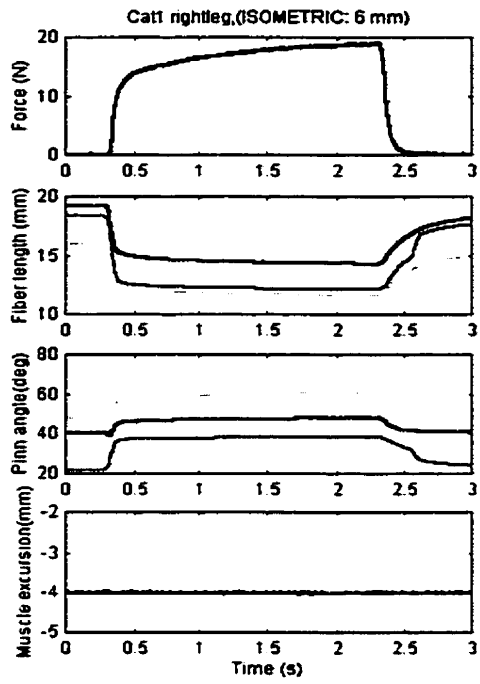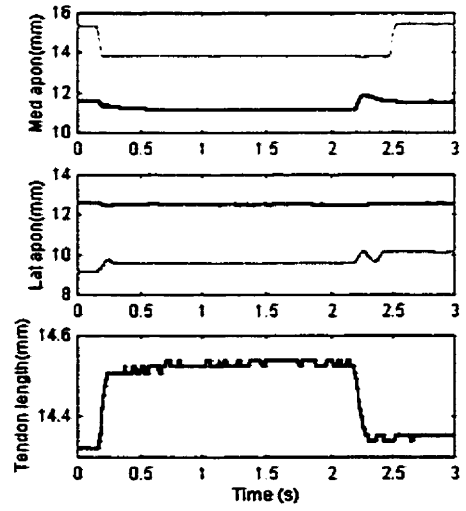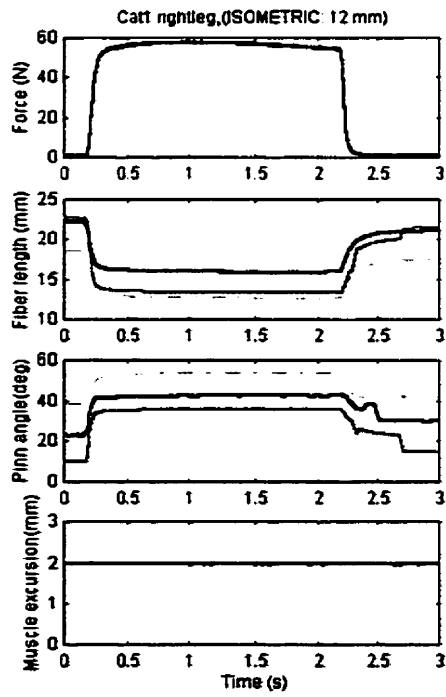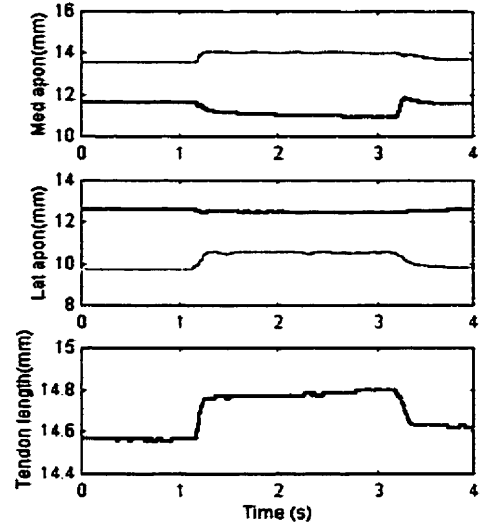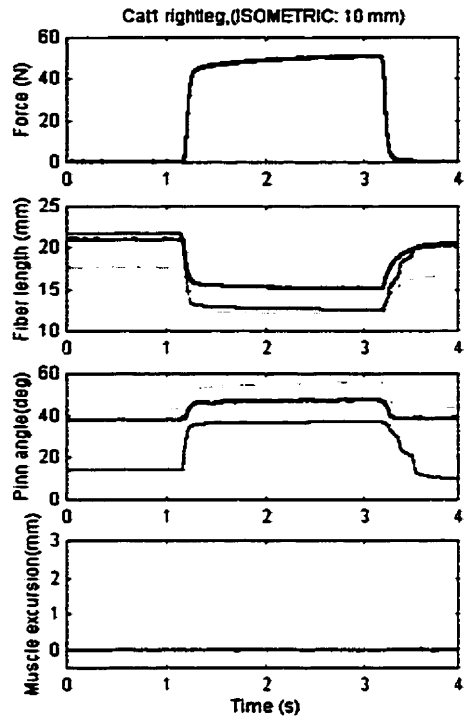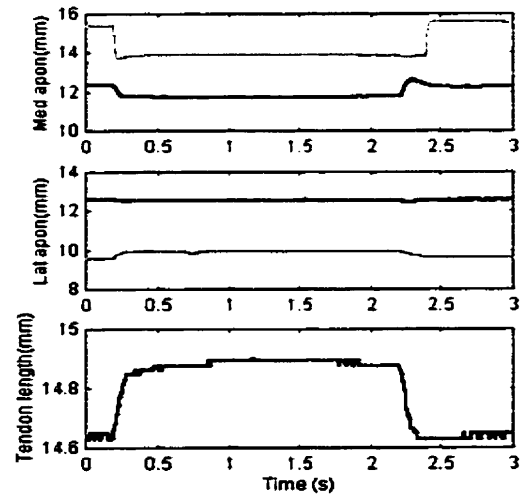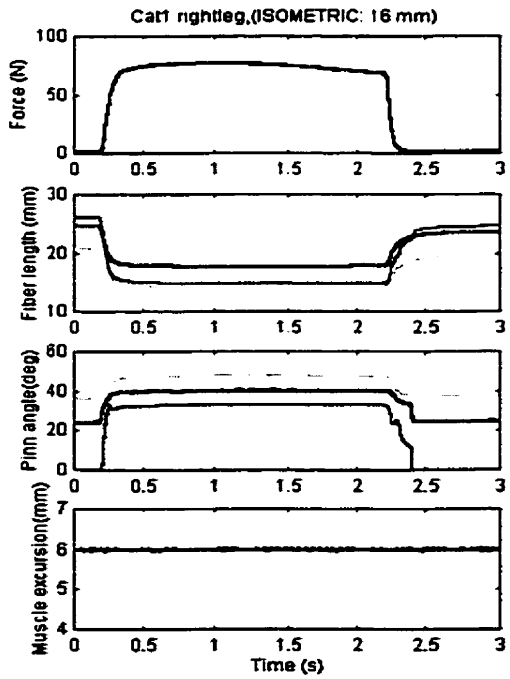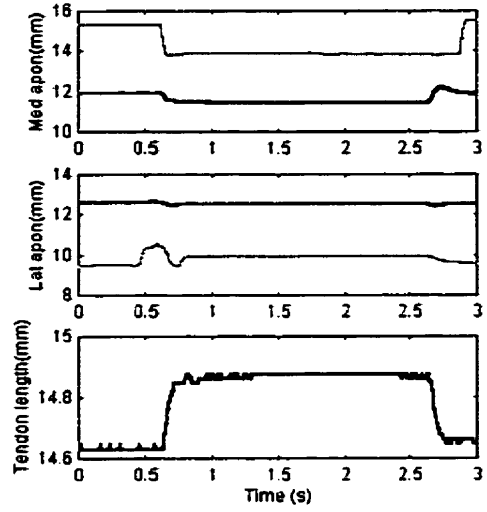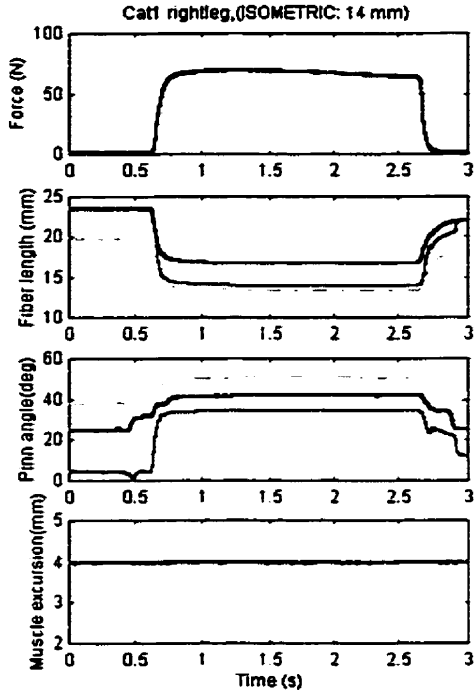
B3. Raw Data Traces For Cat 2 Left Leg

Cat2 leftleg,(ISOMETRIC: 0 mm)

Cat2 leftleg,(ISOMETRIC: 2 mm)

Cat2 leftleg,(ISOMETRIC: 4 mm)

Cat2 leftleg,(ISOMETRIC: 6 mm)

Cat2 leftleg,(ISOMETRIC: 8 mm)

Cat2 leftleg,(ISOMETRIC: 10 mm)

Cat2 leftleg,(ISOMETRIC: 12 mm)

Cat2 leftleg,(ISOMETRIC: 14 mm)

Cat2 leftleg,(ISOMETRIC: 16 mm)
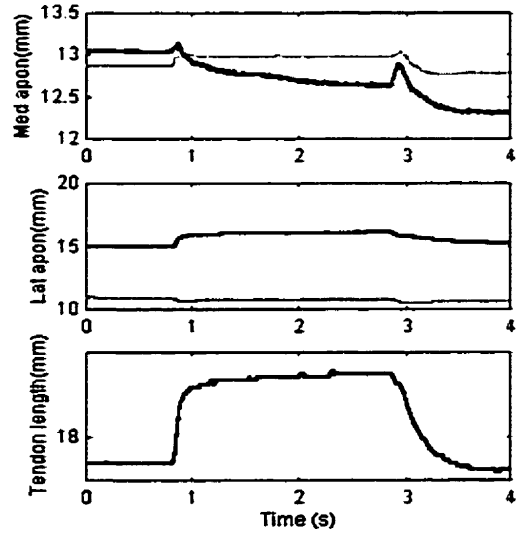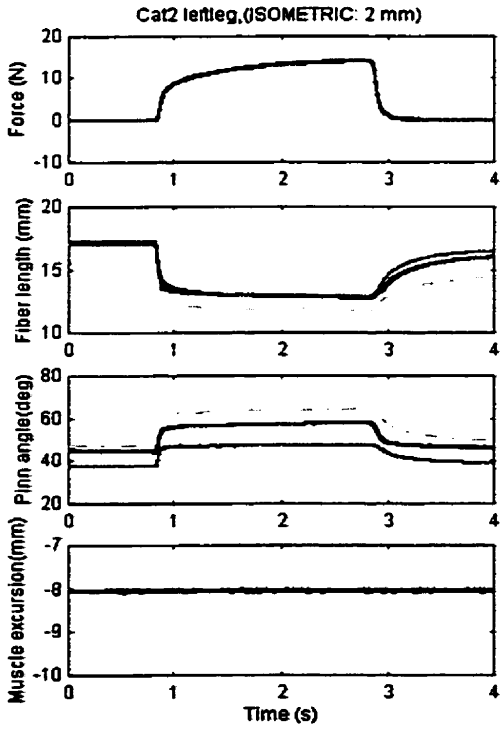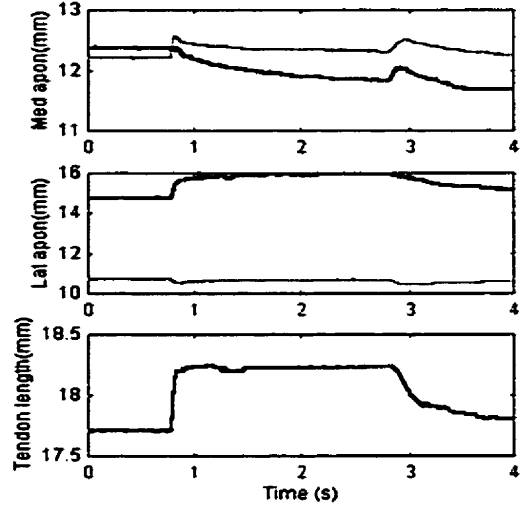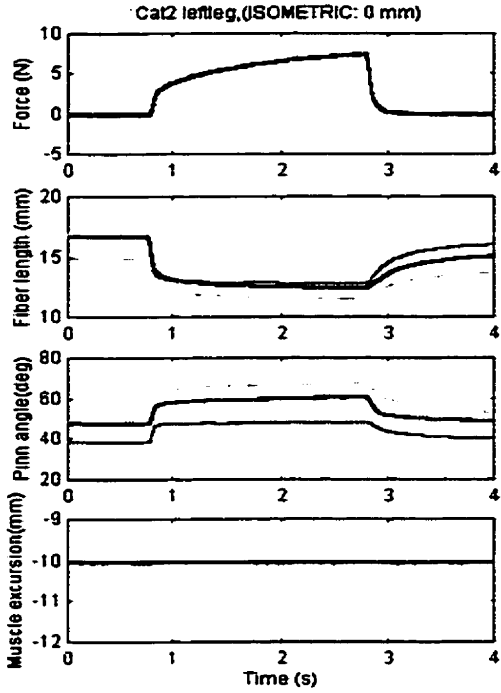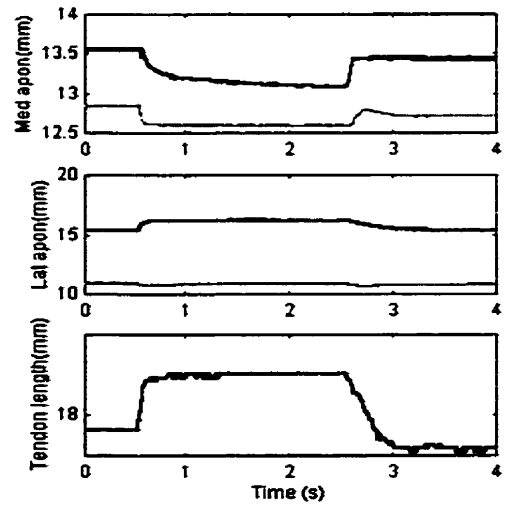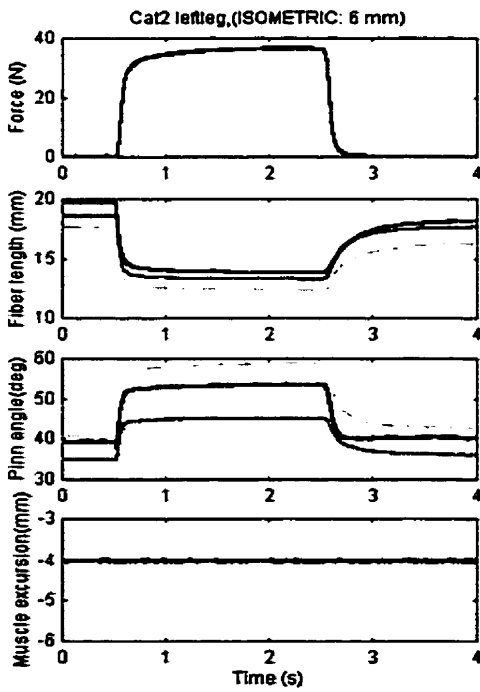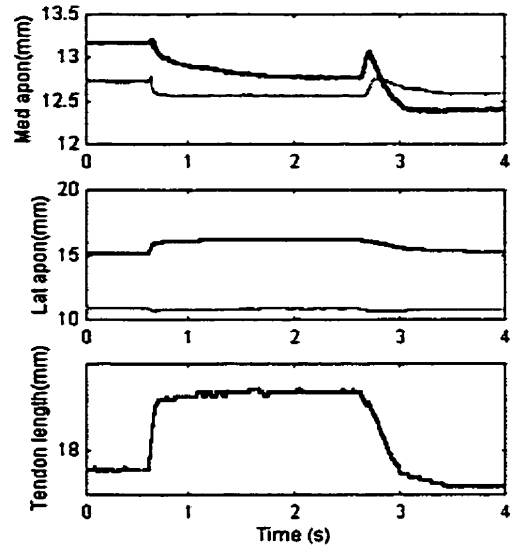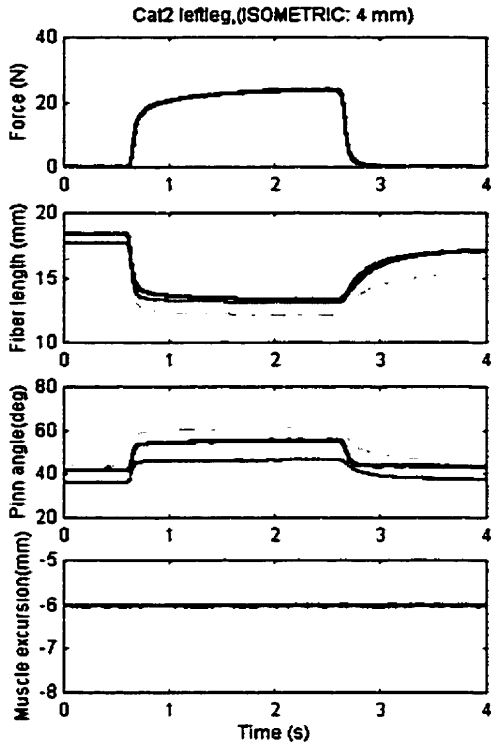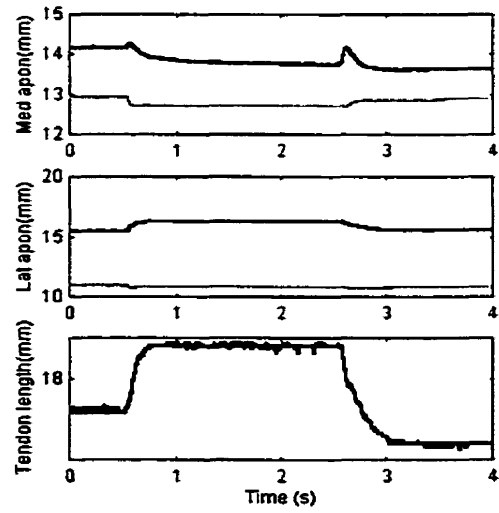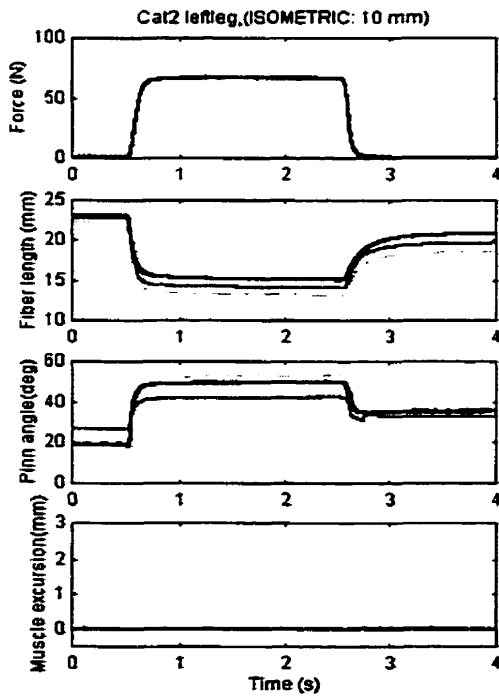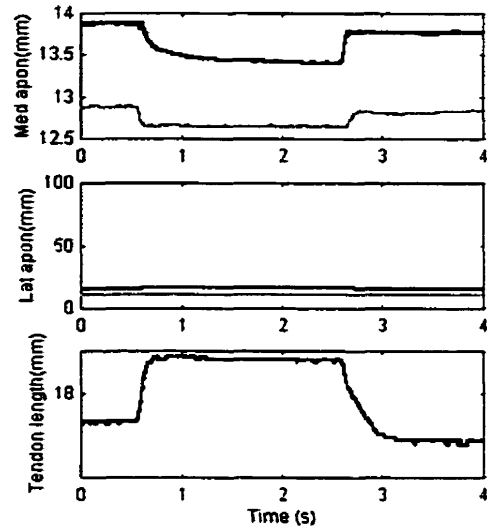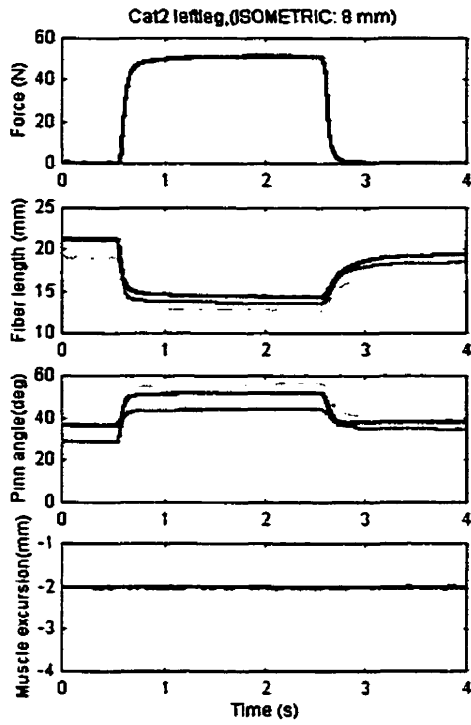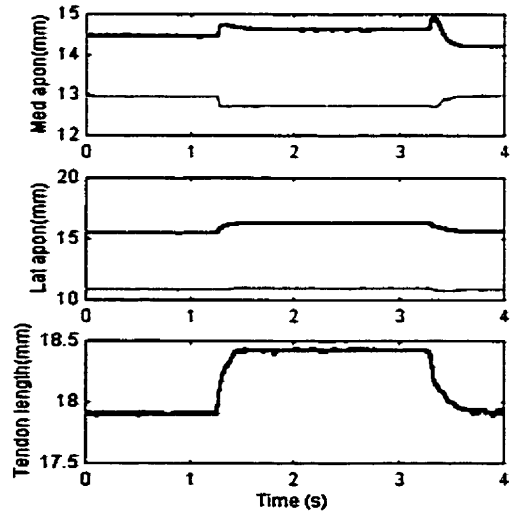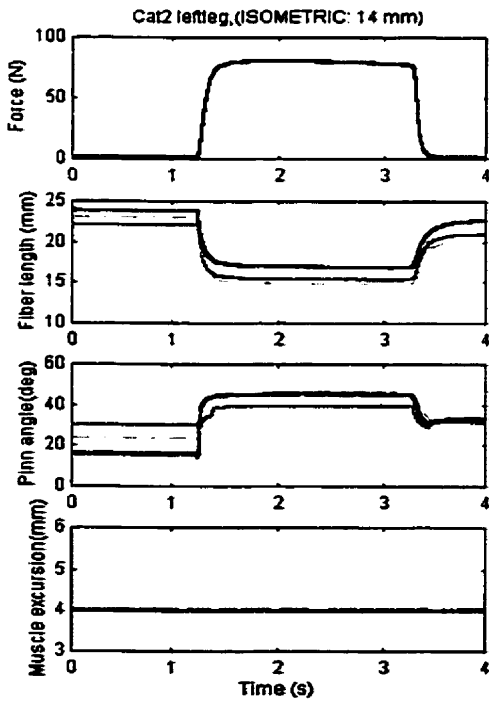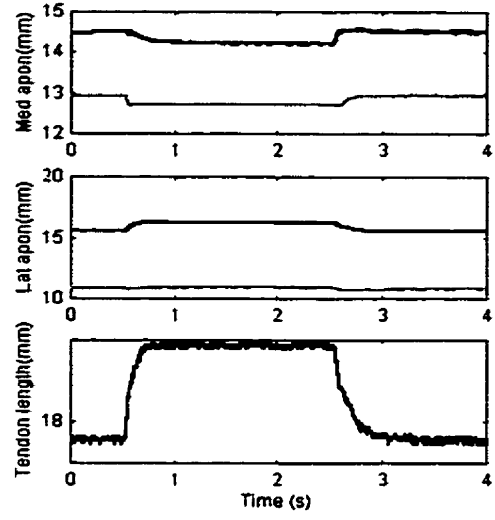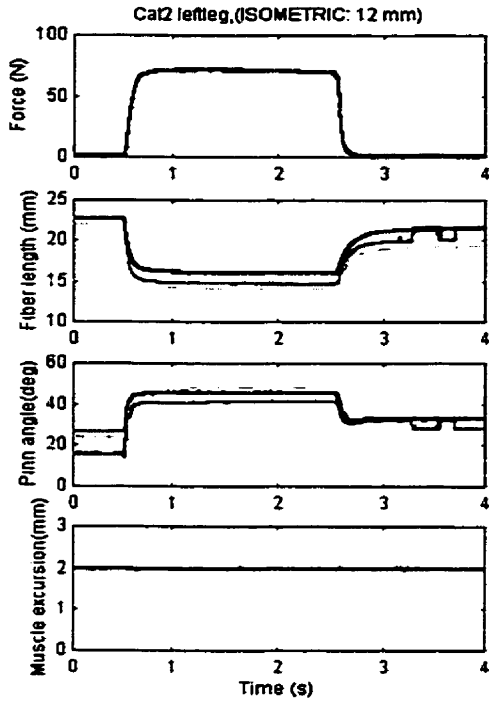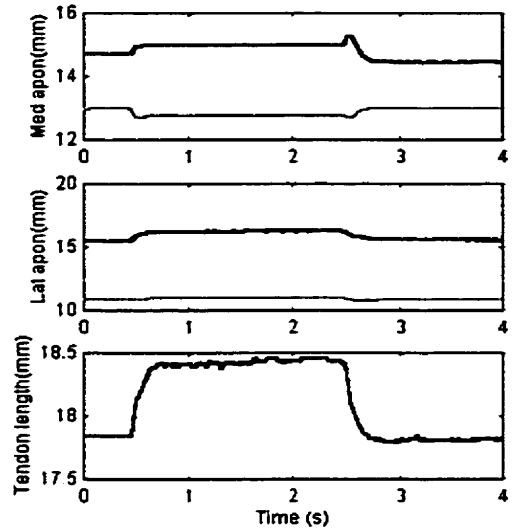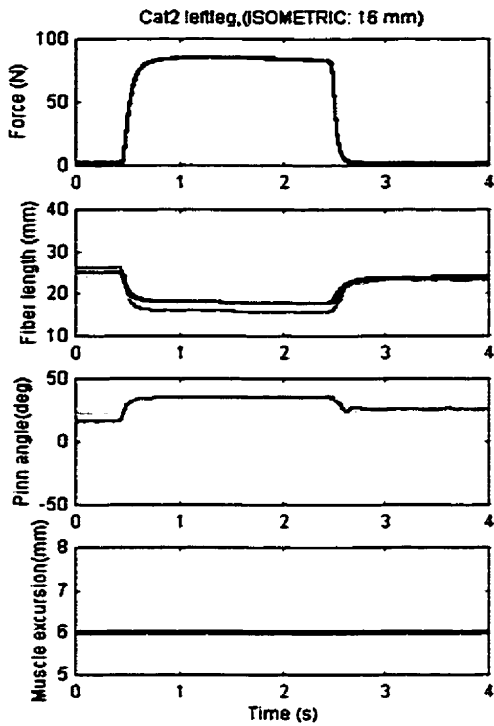
B4. Raw Data Traces For Cat 2 Right Leg

Cat2 rightleg,(ISOMETRIC: 0 mm)

Cat2 rightleg,(ISOMETRIC: 2 mm)

Cat2 rightleg,(ISOMETRIC: 4 mm)

Cat2 rightleg,(ISOMETRIC: 6 mm)

Cat2 rightleg,(ISOMETRIC: 8 mm)

Cat2 rightleg,(ISOMETRIC: 10 mm)

Cat2 rightleg,(ISOMETRIC: 12 mm)

Cat2 rightleg,(ISOMETRIC: 14 mm)

Cat2 rightleg,(ISOMETRIC: 16 mm)
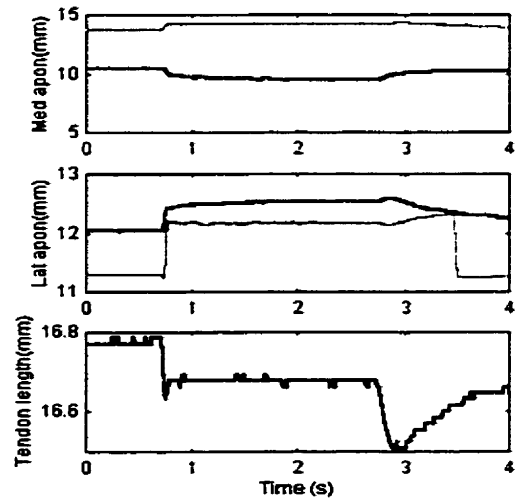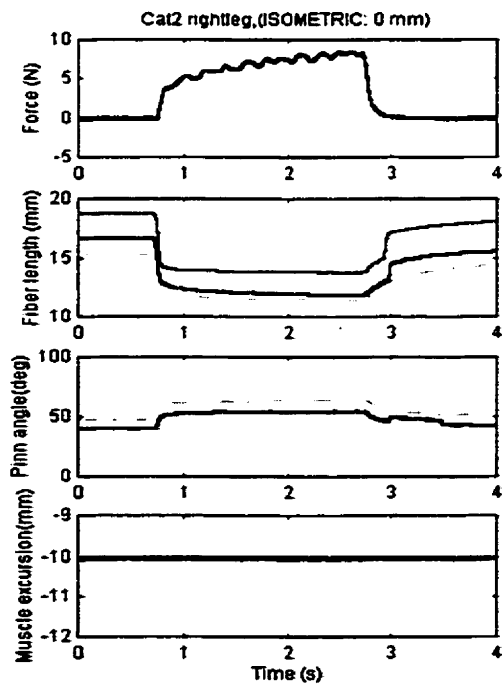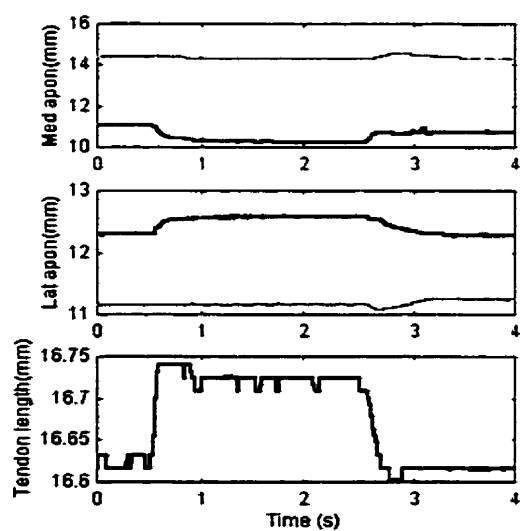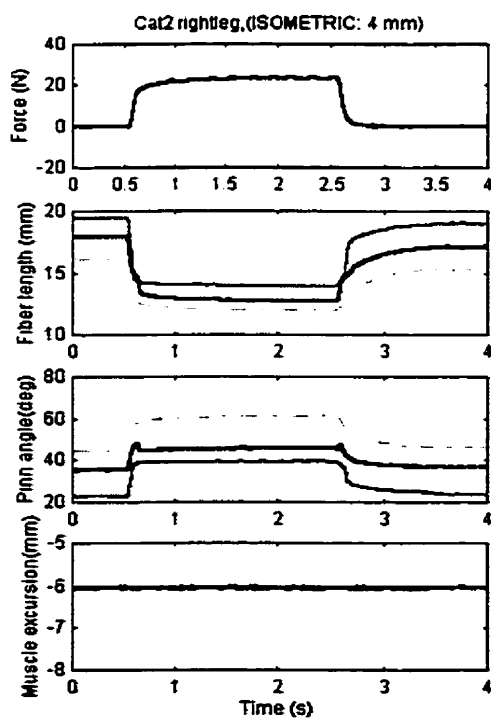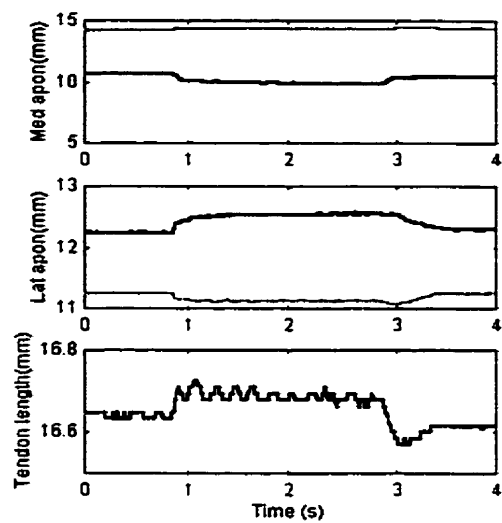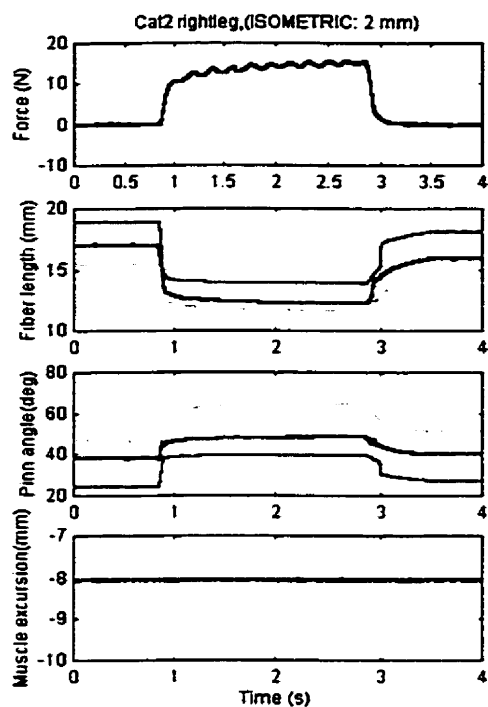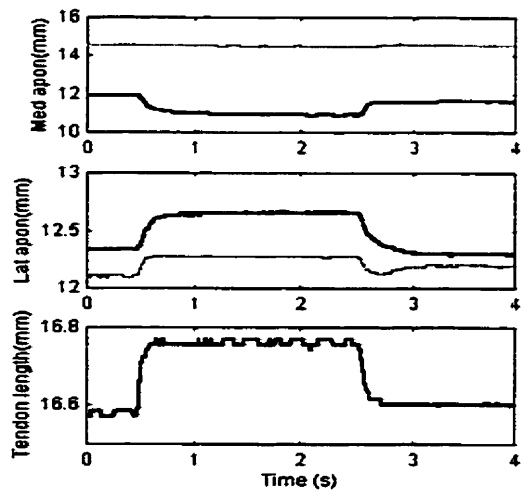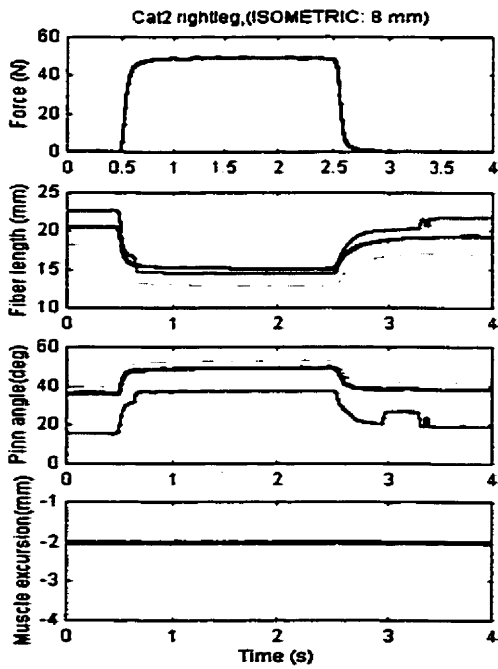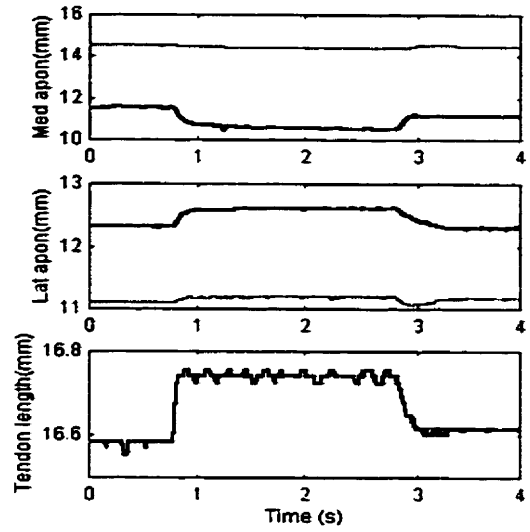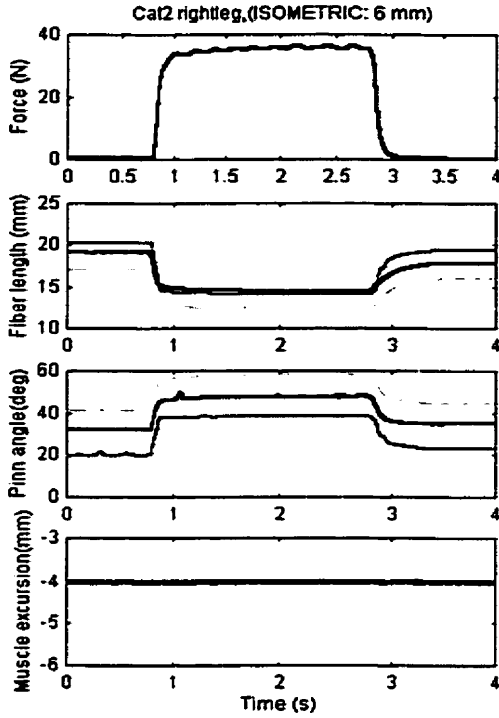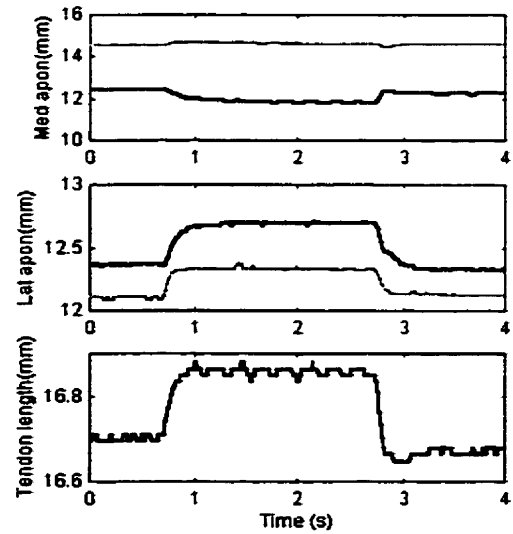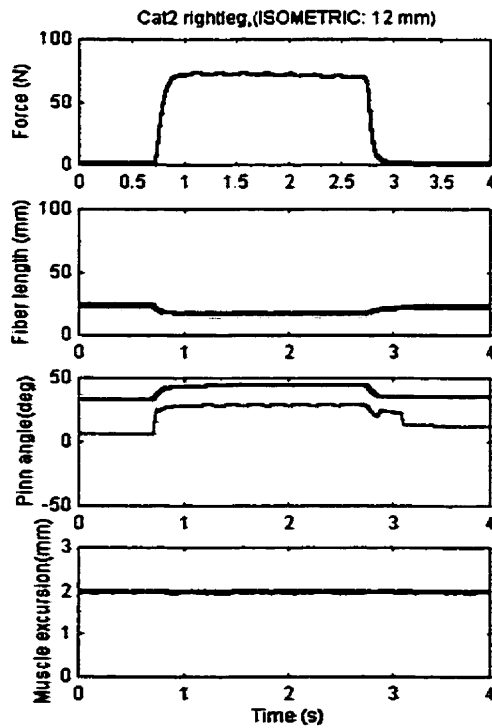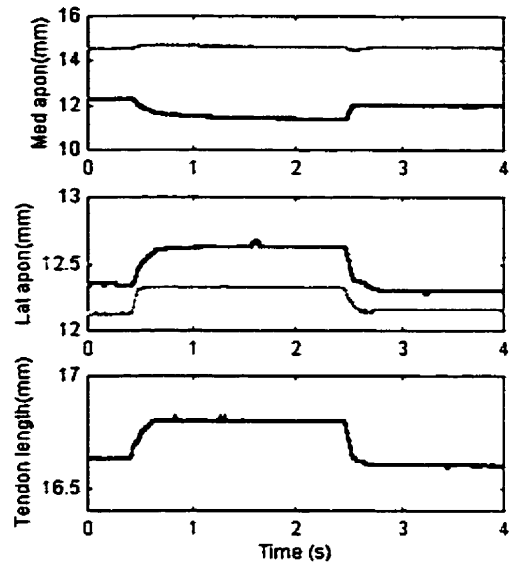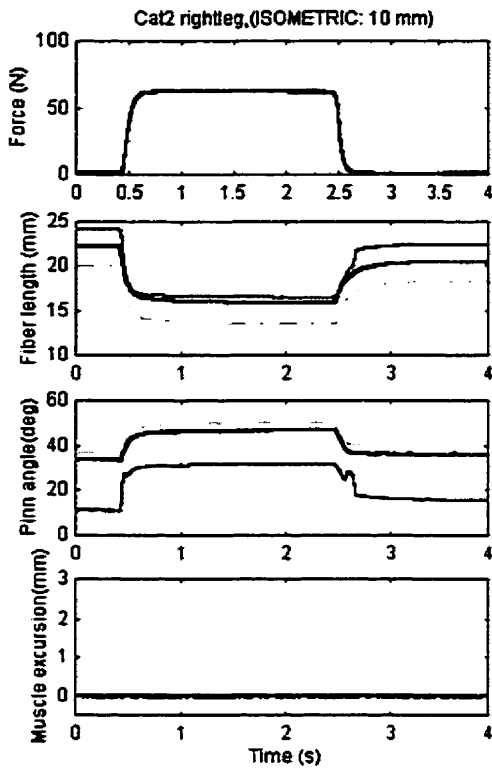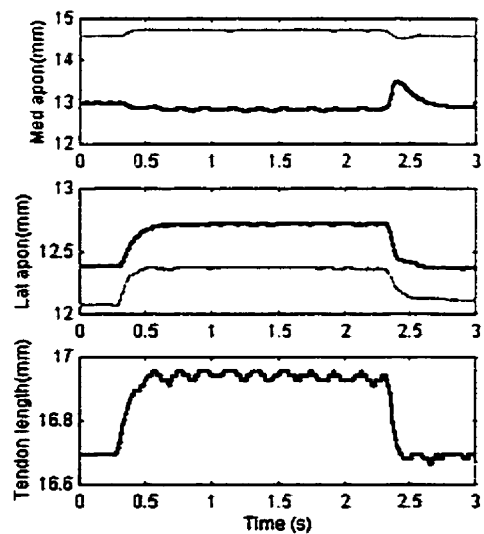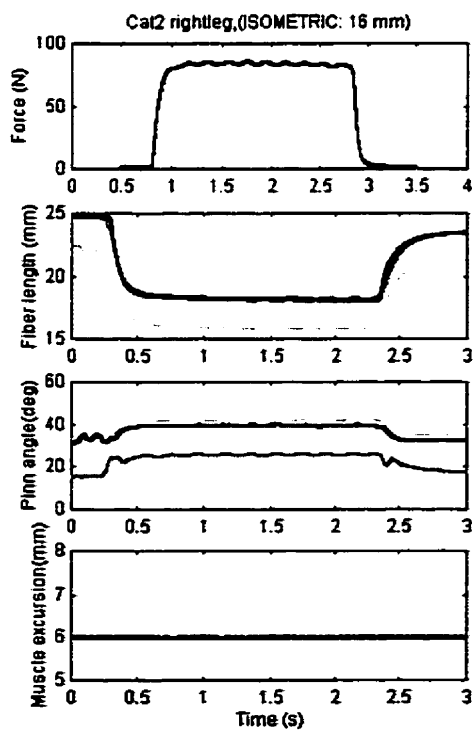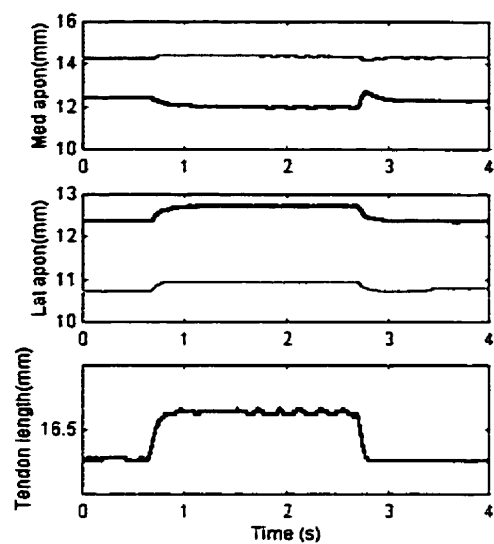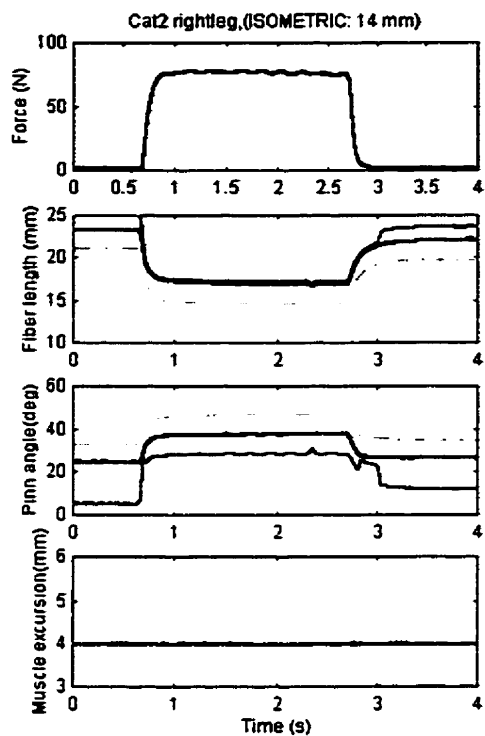
B5. Raw Data Traces For Cat 3 Left Leg

Cat3 leftleg,(ISOMETRIC: 0 mm)

Cat3 leftleg,(ISOMETRIC: 2 mm)

Cat3 leftleg,(ISOMETRIC: 4 mm)

Cat3 leftleg,(ISOMETRIC: 6 mm)

Cat3 lefleg,(ISOMETRIC: 8 mm)

Cat3 lefleg,(ISOMETRIC: 10 mm)

Cat3 leftleg,(ISOMETRIC: 12 mm)

Cat3 leftleg,(ISOMETRIC: 14 mm)

Cat3 leftleg,(ISOMETRIC: 15 mm)
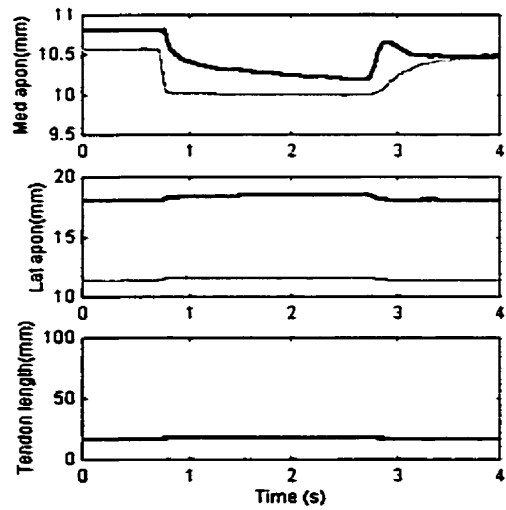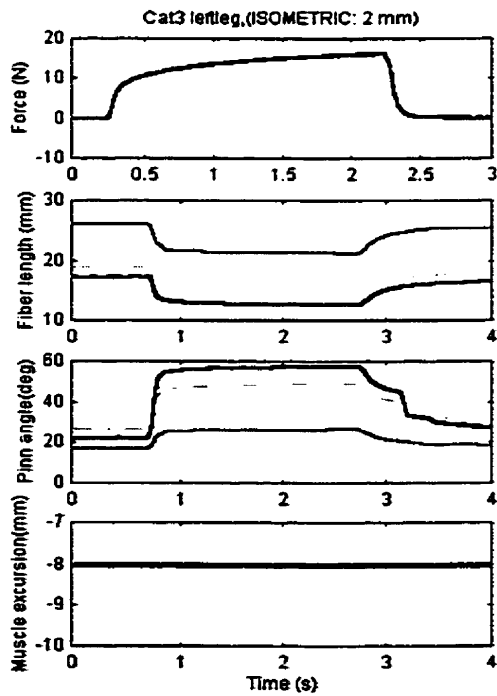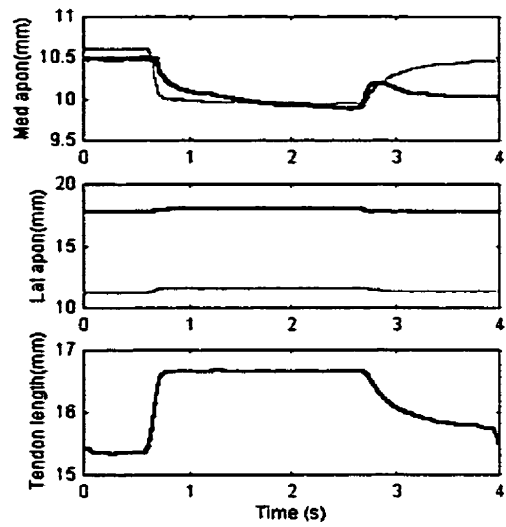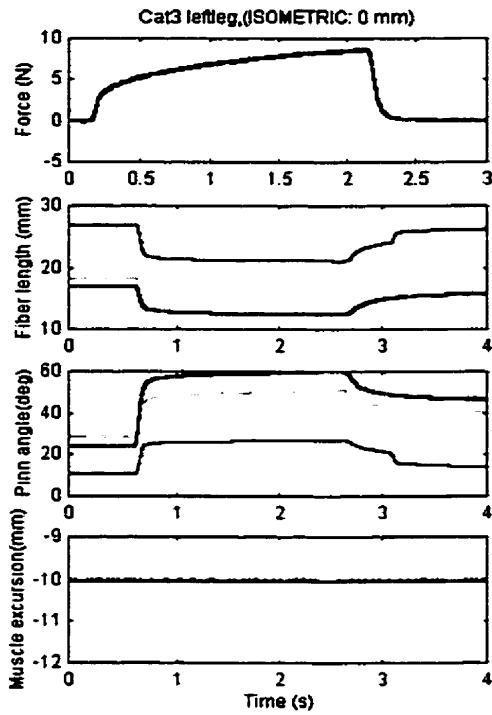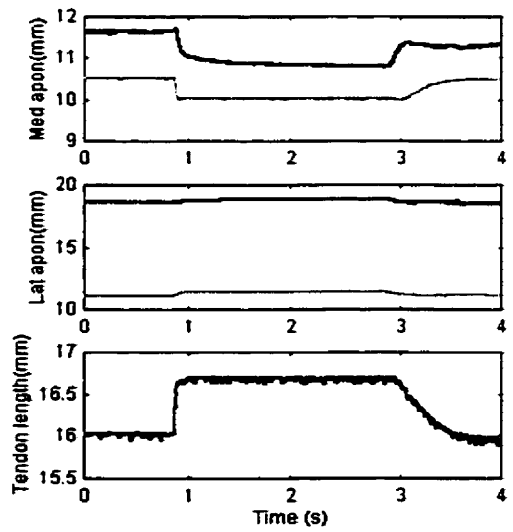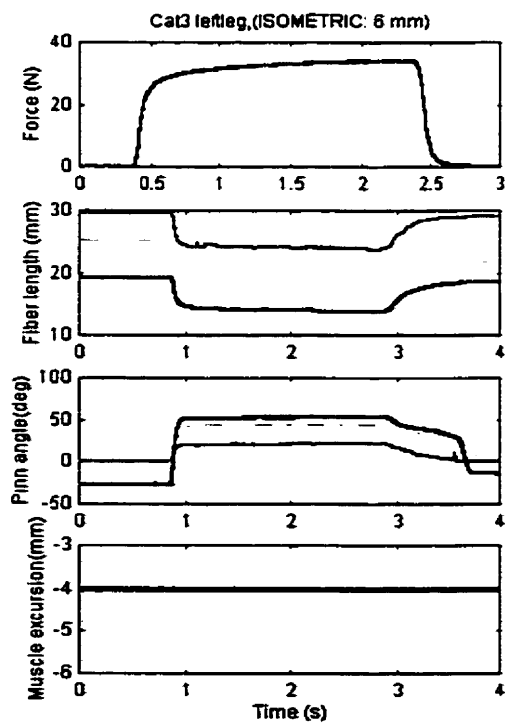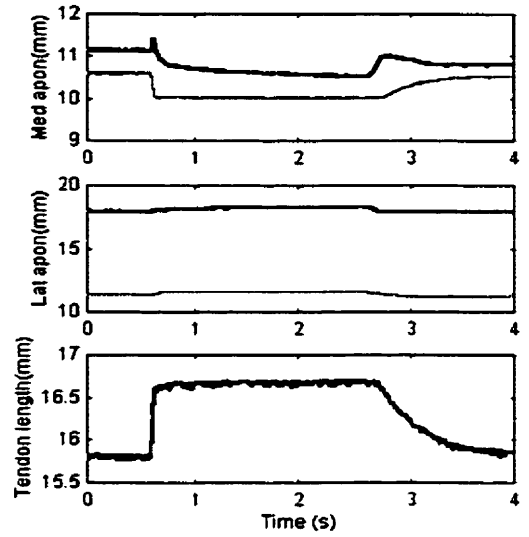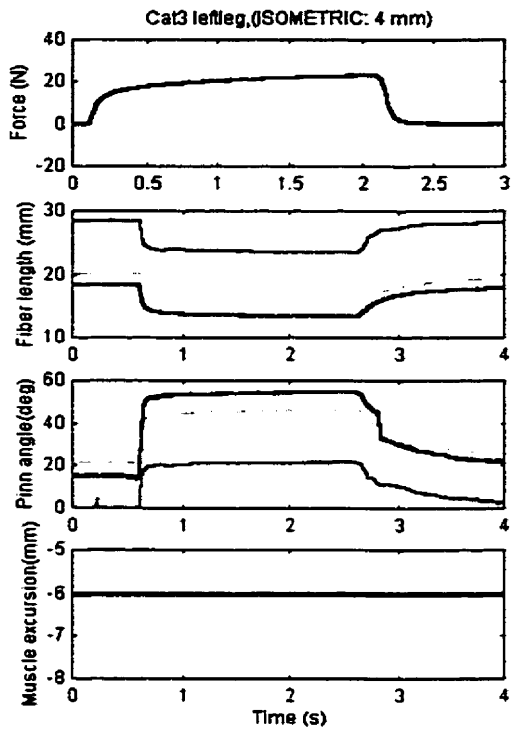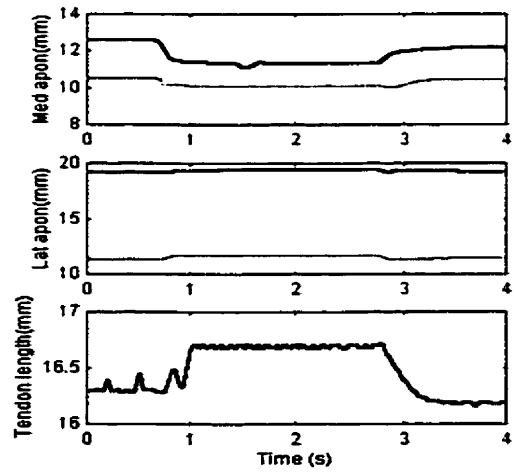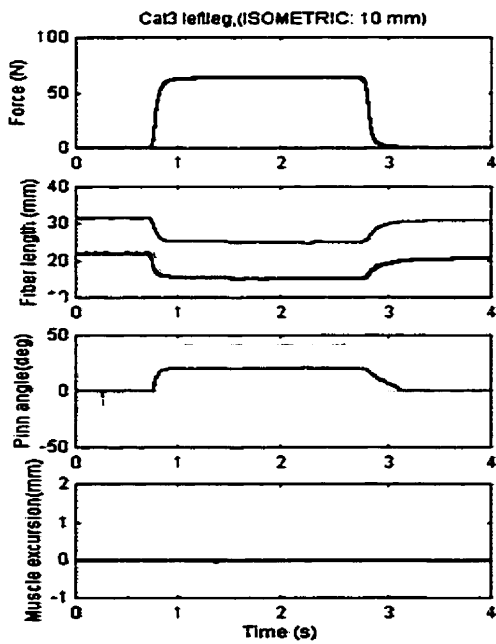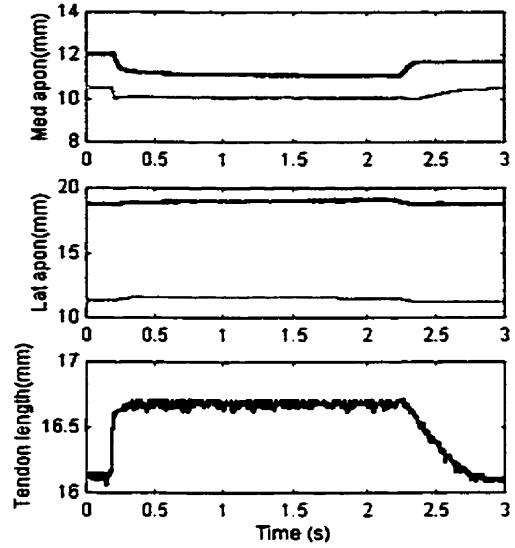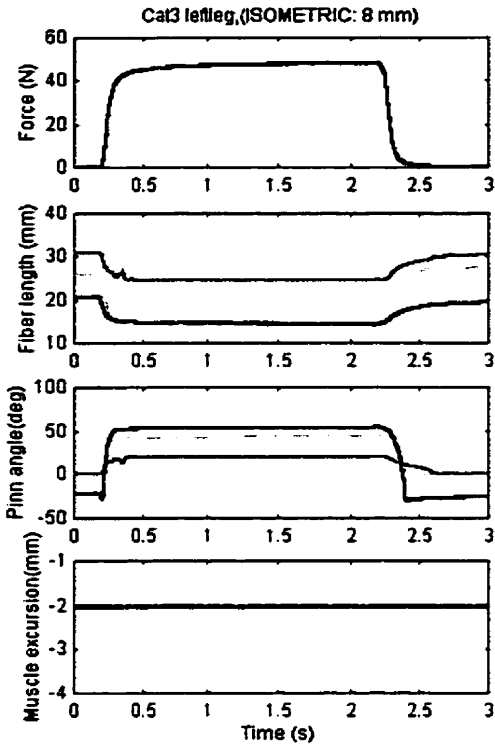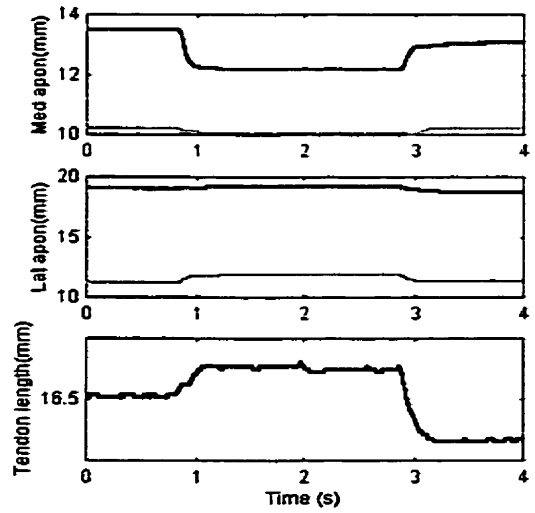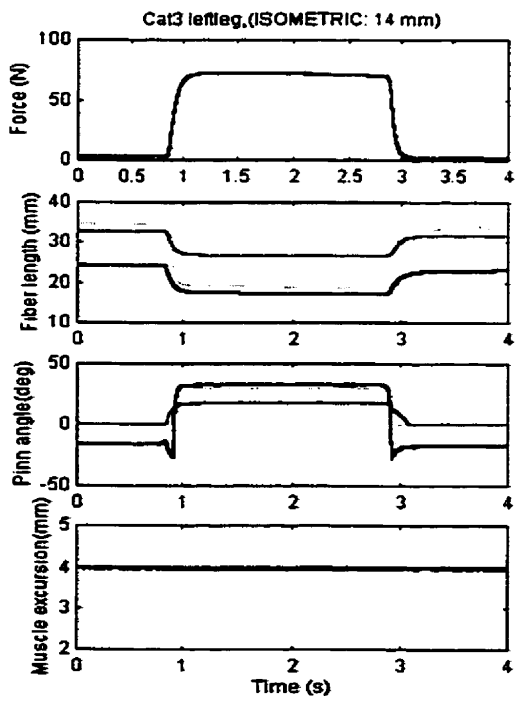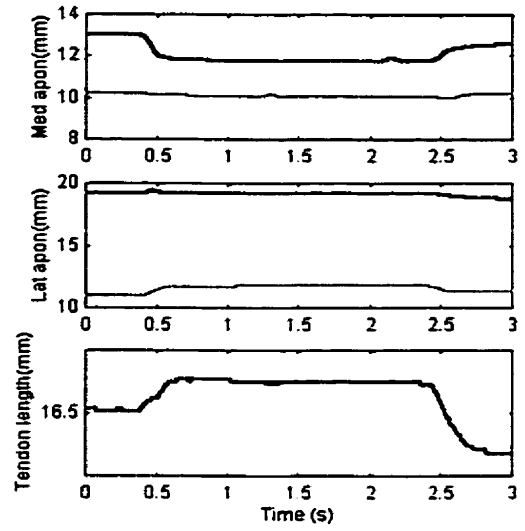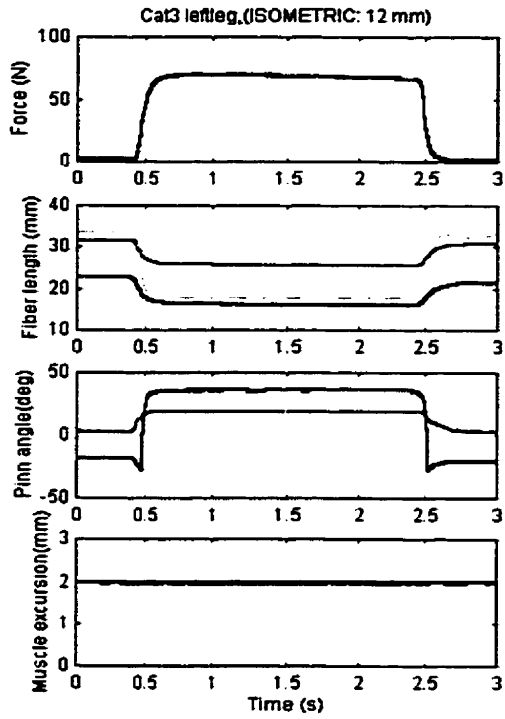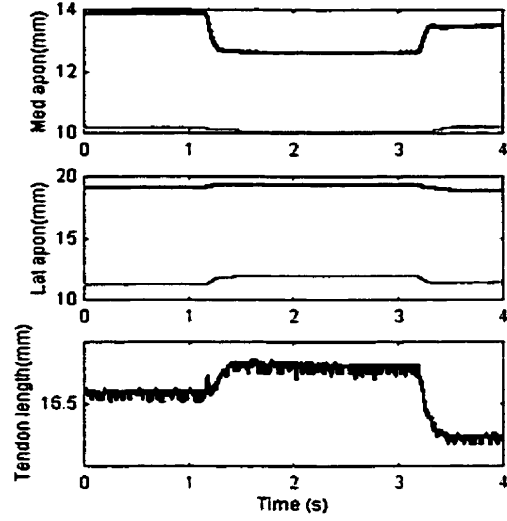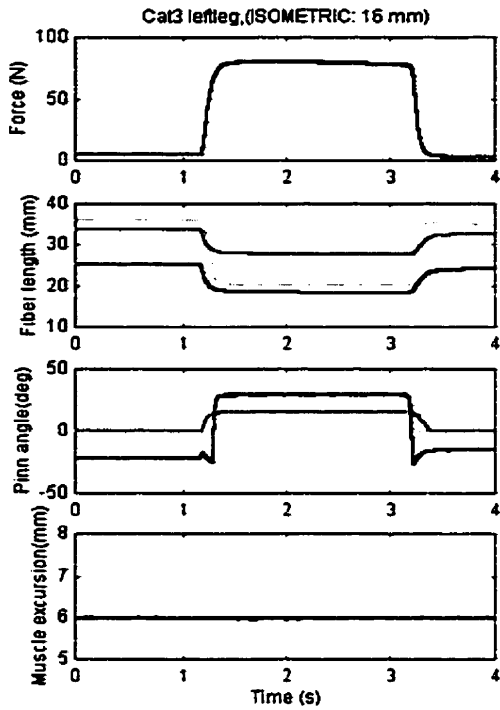
B6. Raw Data Traces For Cat 3 Right Leg

Cat3 rightleg,(ISOMETRIC: 0 mm)

Cat3 rightleg,(ISOMETRIC: 2 mm)

Cat3 rightleg,(ISOMETRIC: 4 mm)

Cat3 rightleg,(ISOMETRIC: 6 mm)

Cat3 rightleg,(ISOMETRIC: 8 mm)

Cat3 rightleg.(ISOMETRIC: 10 mm)

Cat3 rightleg.(ISOMETRIC: 12 mm)

Cat3 rightleg,(ISOMETRIC: 14 mm)

Cat3 rightleg,(ISOMETRIC: 16 mm)
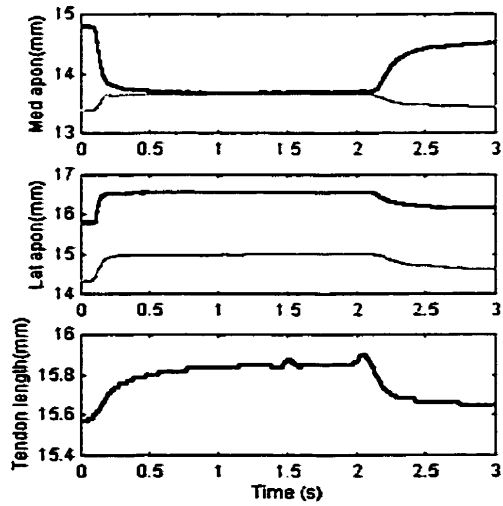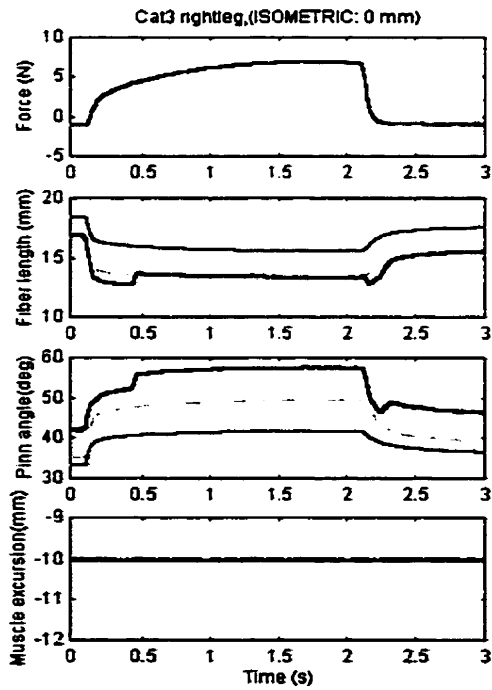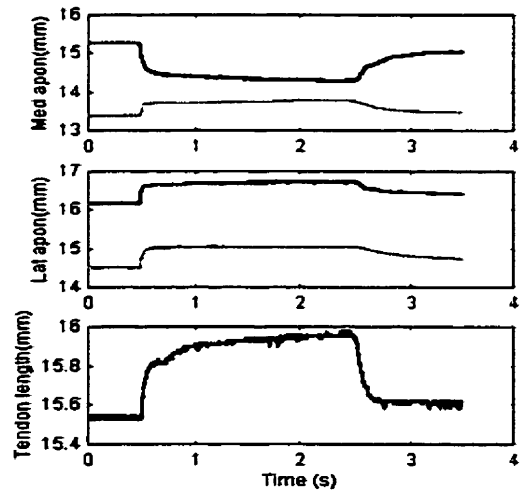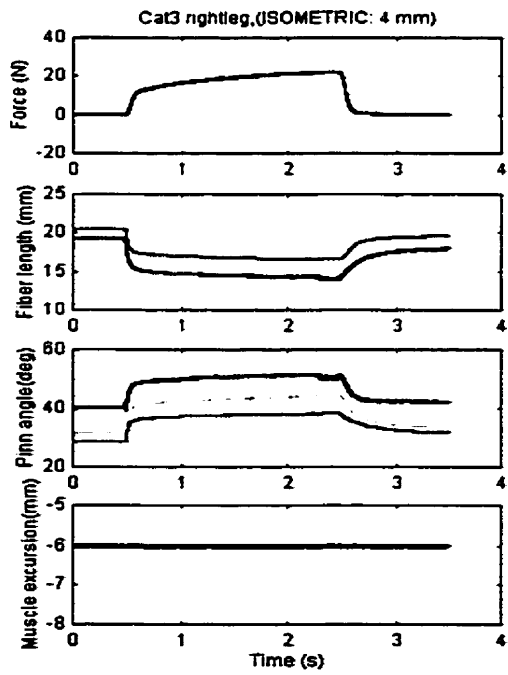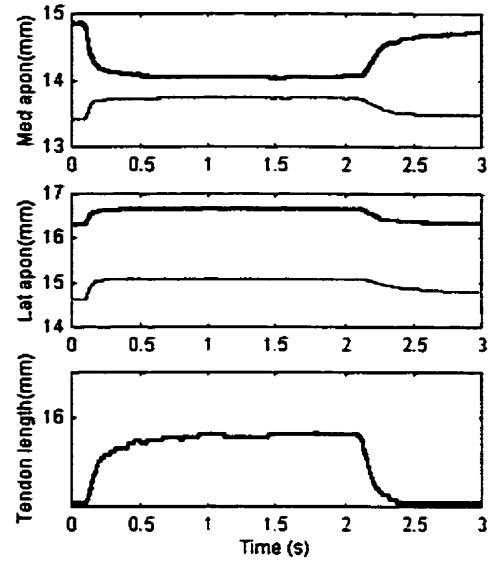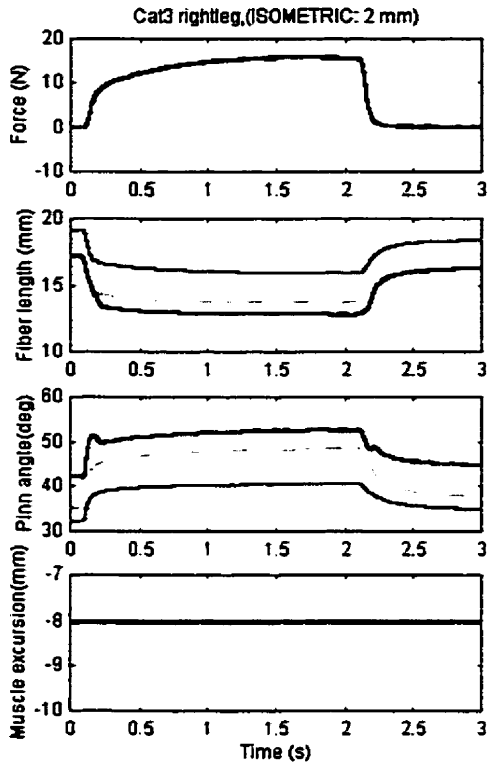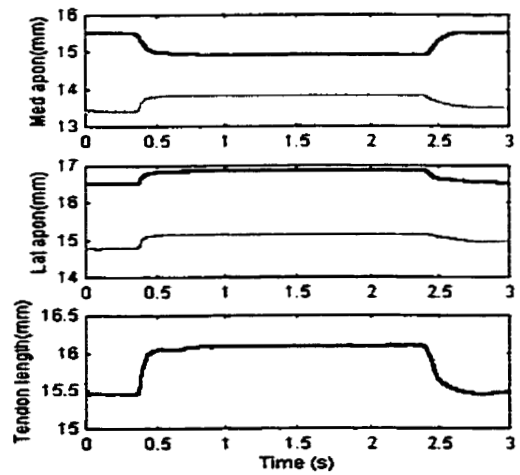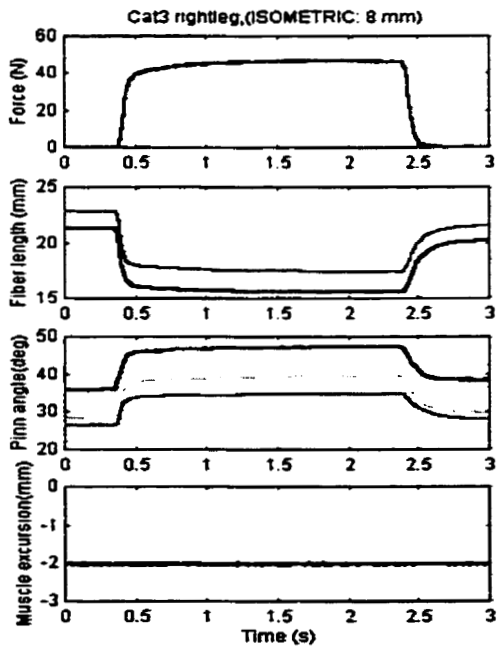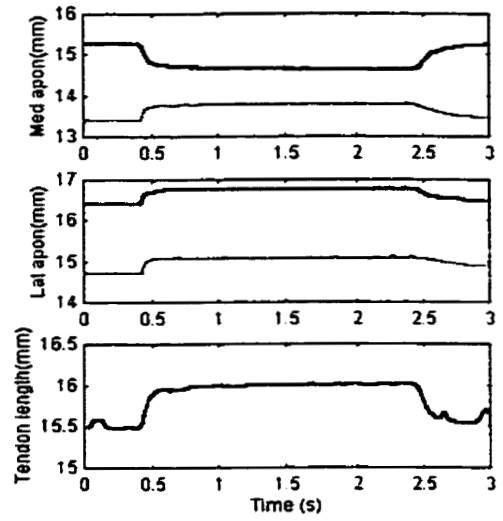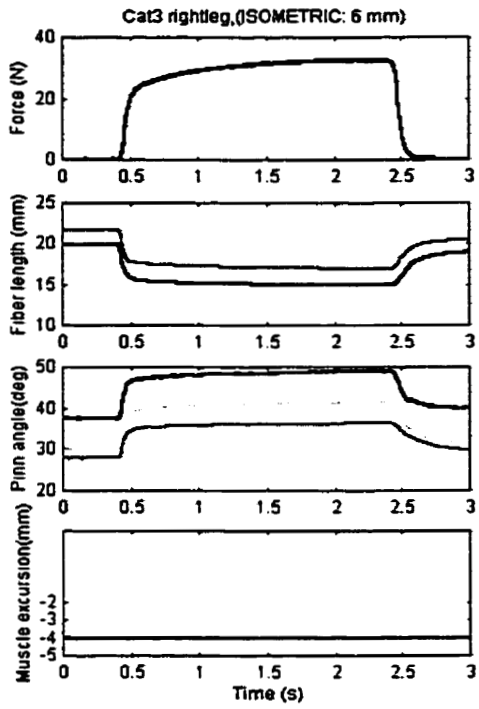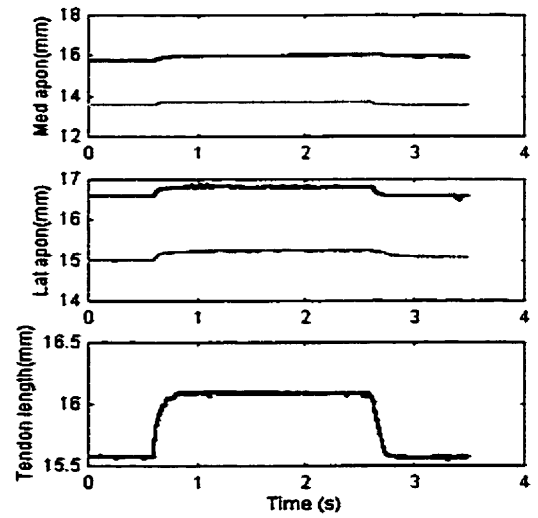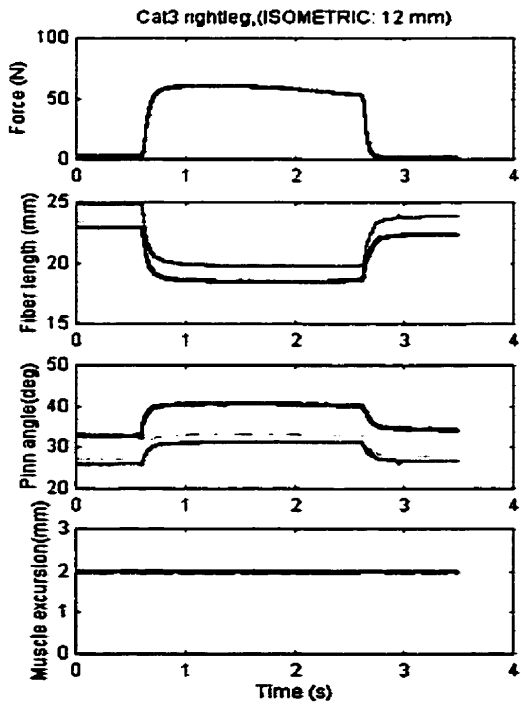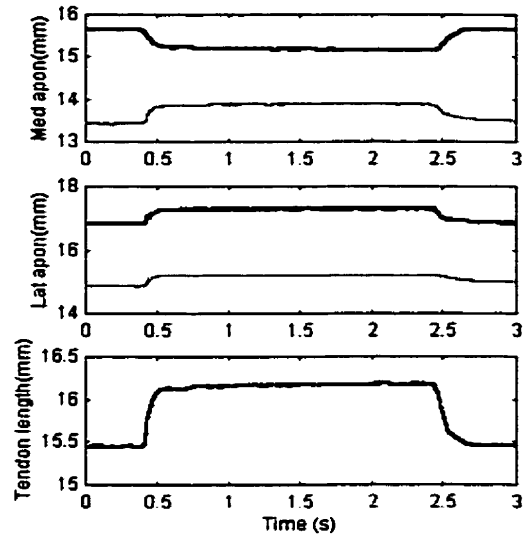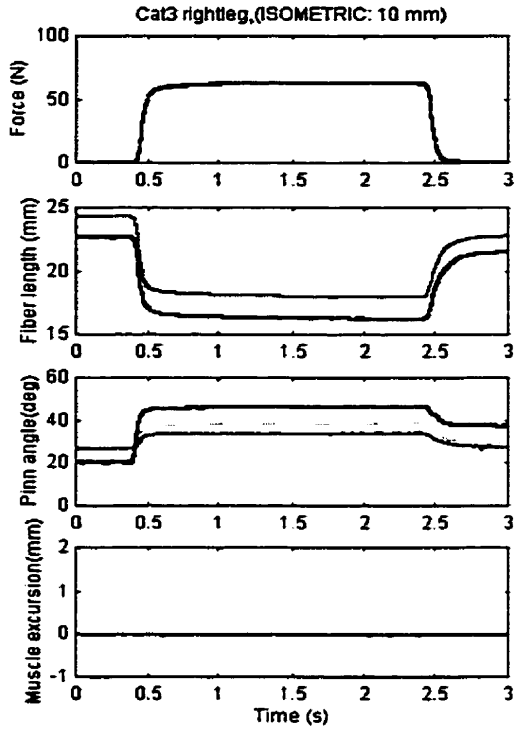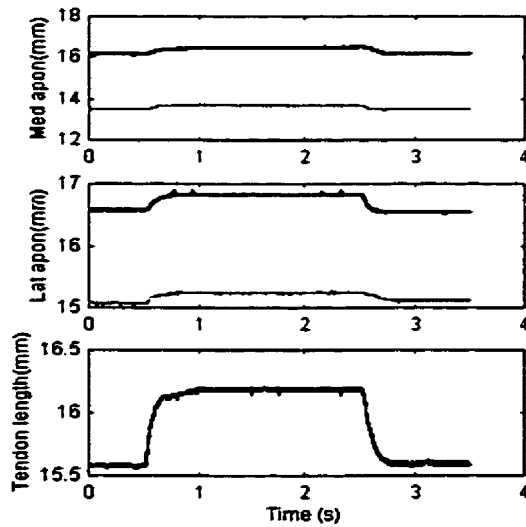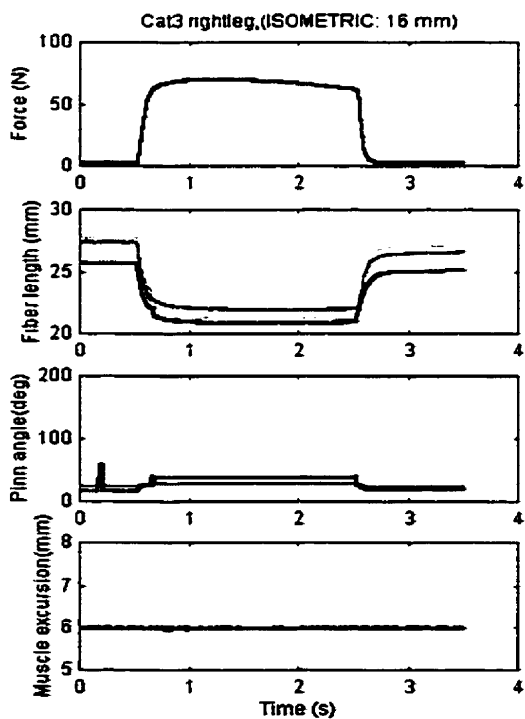
B7. Raw Data Traces For Cat 4 Left Leg

Cat4 leftleg,(ISOMETRIC: 0 mm)

Cat4 leftleg,(ISOMETRIC: 2 mm)

Cat4 leftleg,(ISOMETRIC: 4 mm)

Cat4 leftleg,(ISOMETRIC: 6 mm)

Cat4 leftleg,(ISOMETRIC: 8 mm)

Cat4 leftleg,(ISOMETRIC: 10 mm)

Cat4 lefleg.(ISOMETRIC: 12 mm)

Cat4 leftleg.(ISOMETRIC: 14 mm)

Cat4 leftleg,(ISOMETRIC: 16 mm)
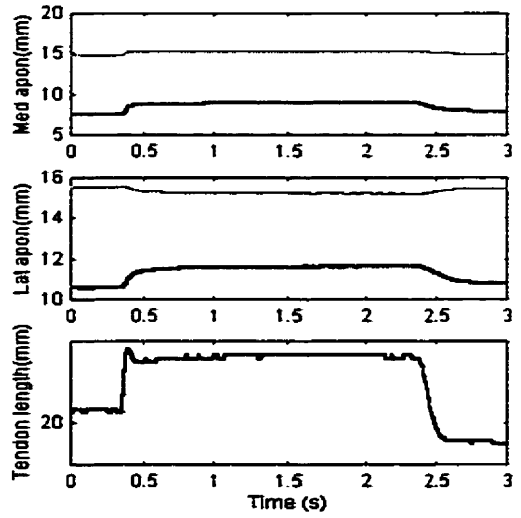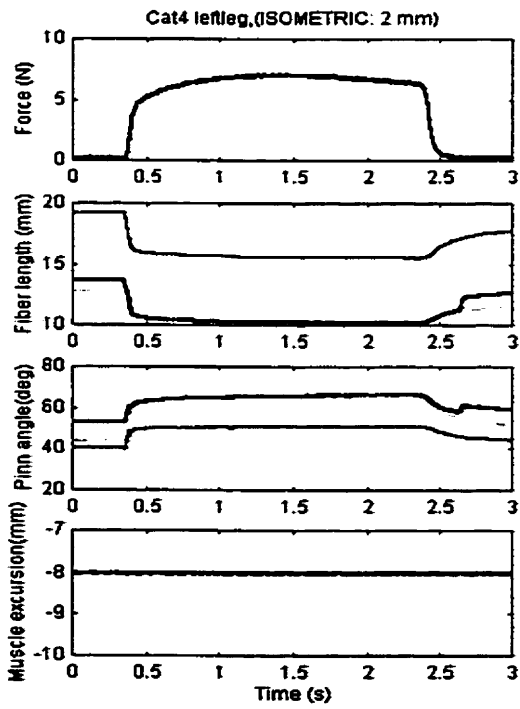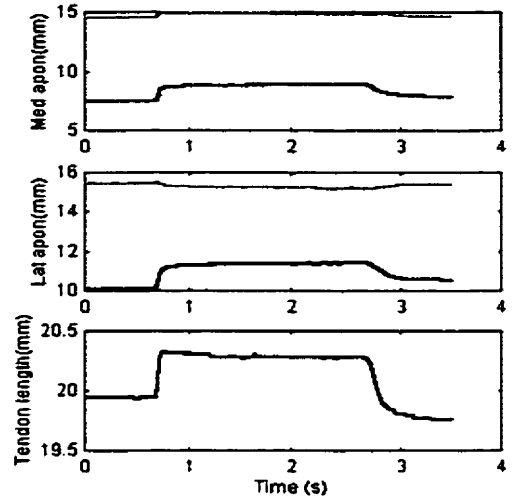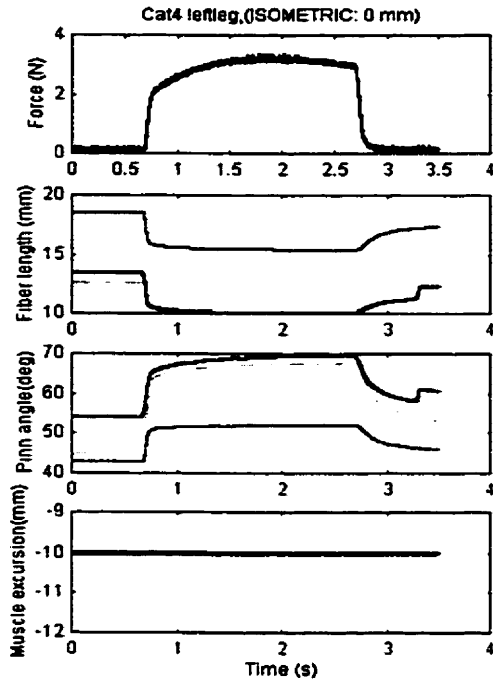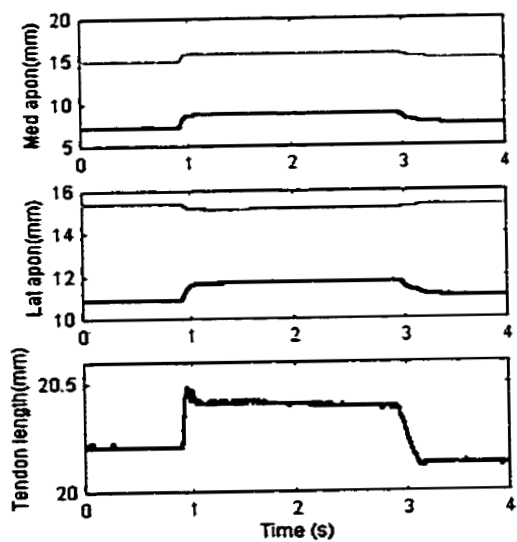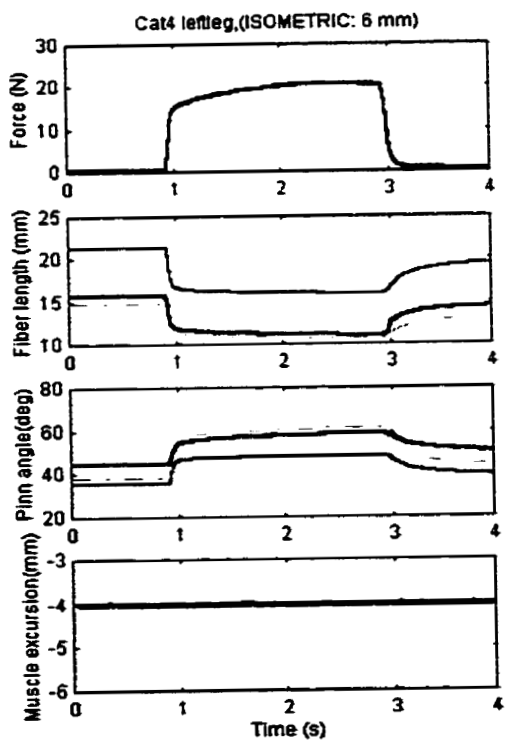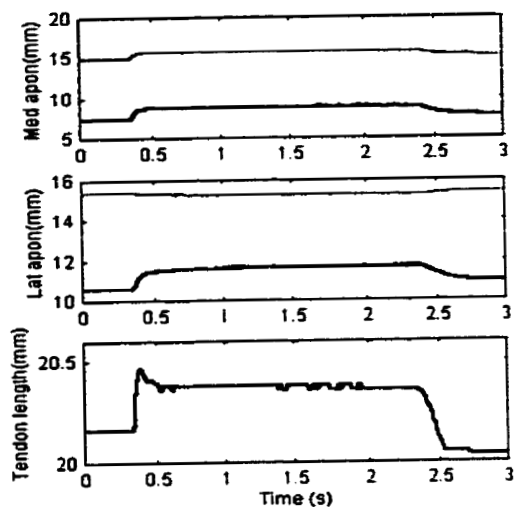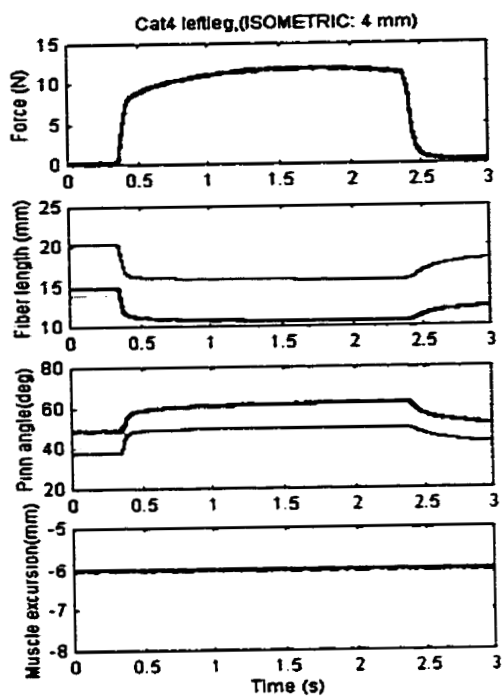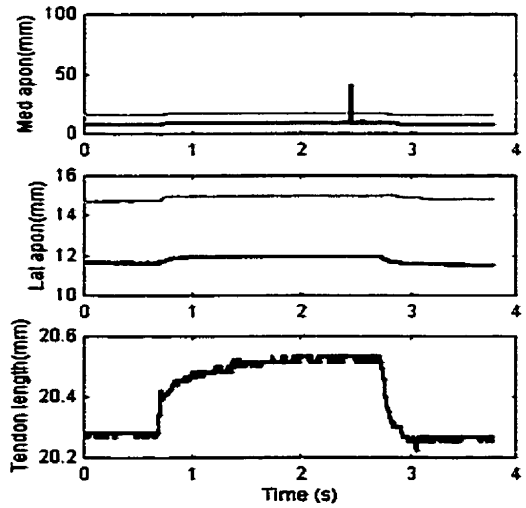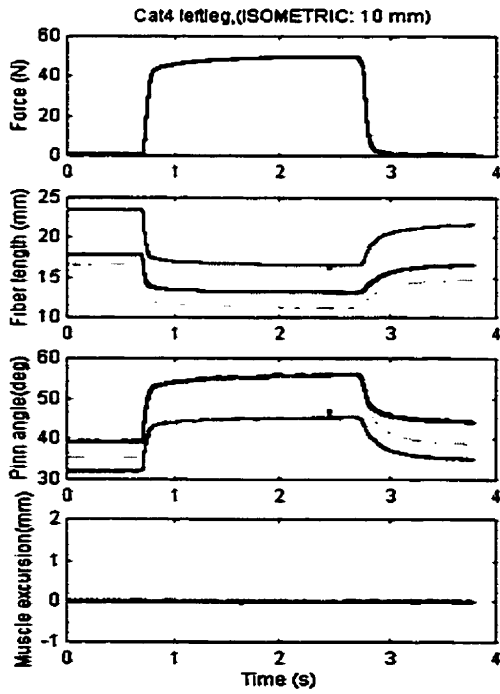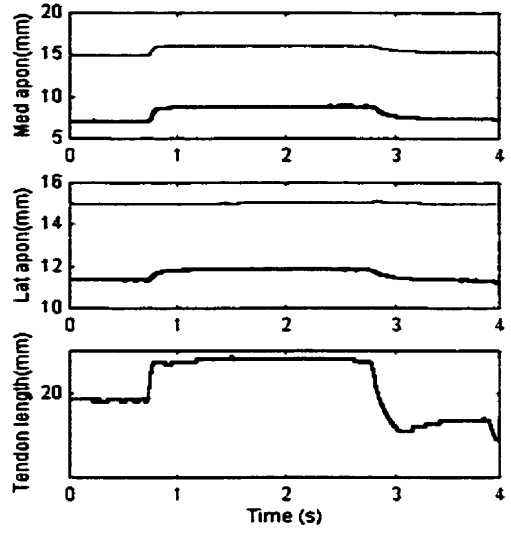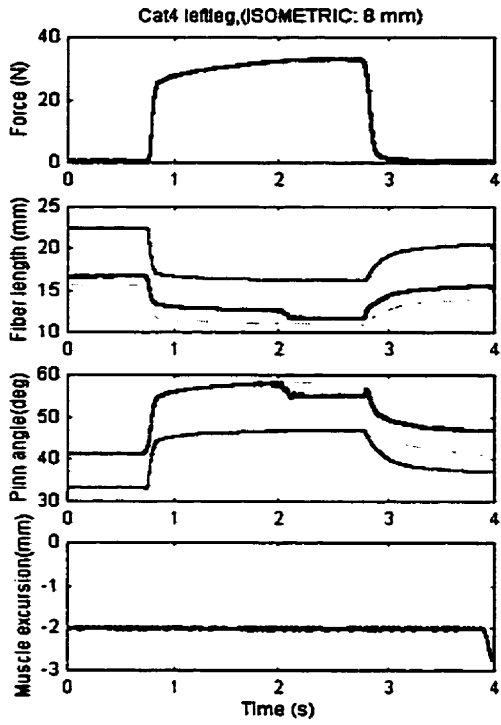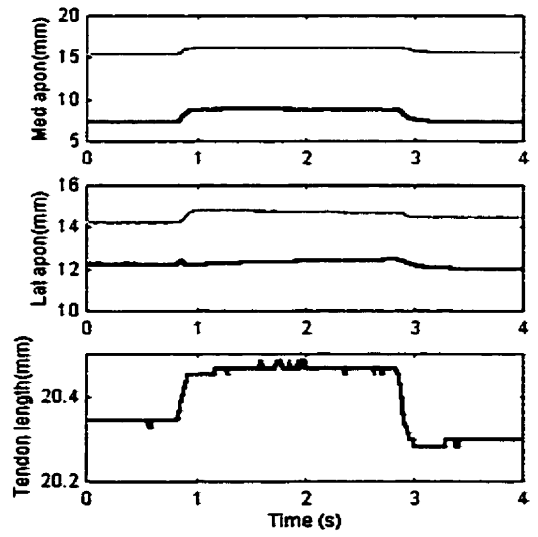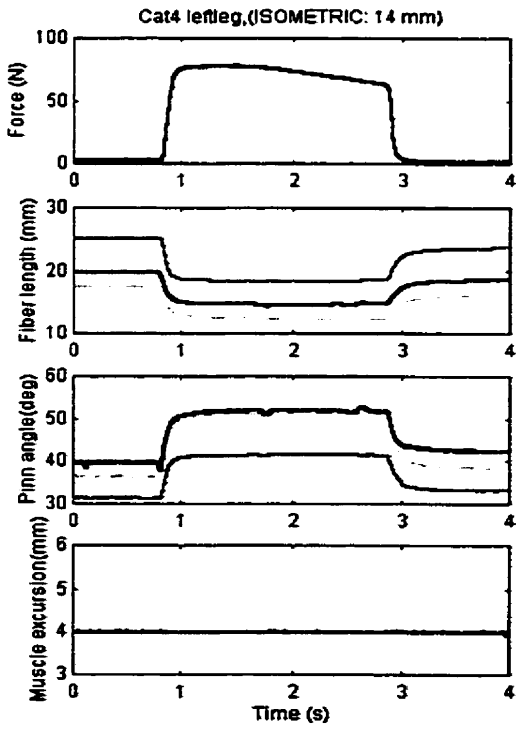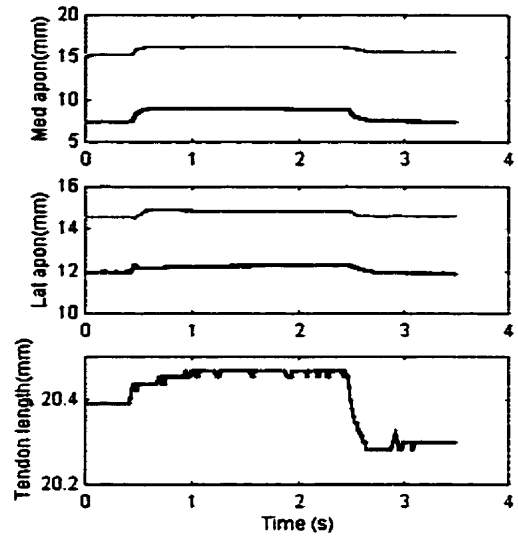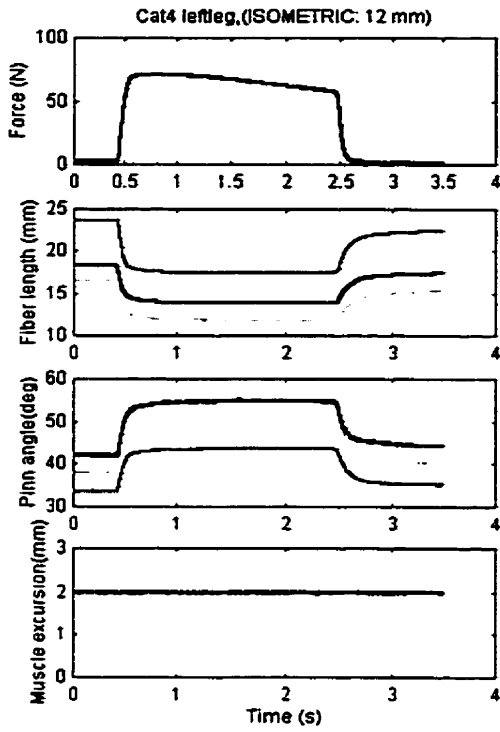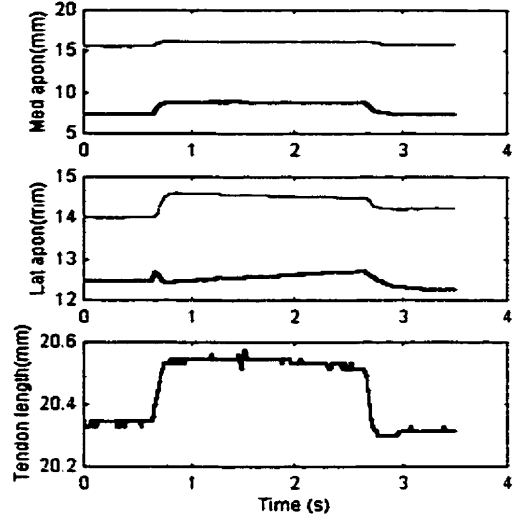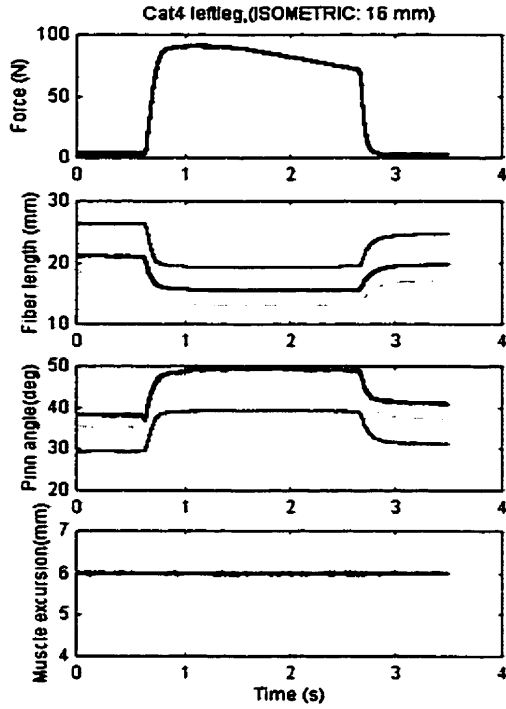
B8. Raw Data Traces For Cat 4 Right Leg

Cat4 rightleg,(ISOMETRIC: 0 mm)

Cat4 rightleg,(ISOMETRIC: 2 mm)

Cat4 rightleg,(ISOMETRIC: 4 mm)

Cat4 rightleg,(ISOMETRIC: 6 mm)

Cat4 rightleg,(ISOMETRIC: 8 mm)

Cat4 rightleg,(ISOMETRIC: 10 mm)

Cat4 rightleg,(ISOMETRIC: 12 mm)

Cat4 rightleg,(ISOMETRIC: 14 mm)

Cat4 rightleg,(ISOMETRIC: 16 mm)