THE UNIVERSITY OF CALGARY

Regulation of Sarcomere Number in the Growing Rabbit Tibialis Anterior

by

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A DISSERTATION

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF MEDICAL SCIENCE

CALGARY, ALBERTA

MARCH, 1997

ABSTRACT

Adaptations in the number of sarcomeres in series in muscle fibers or fascicles (sarcomere number) may be important in pathologic conditions, as well as normal muscle function. Despite the importance of sarcomere number adaptations, the signal(s) important in these adaptations remain to be elucidated.

The first purpose of this dissertation was to test the hypothesis that increasing muscle excursion results in increased serial sarcomere addition in growing animals. The tibialis anterior (TA) was released from its retinacular restraint at the ankle joint to increase muscle excursion in 4-week-old rabbits. Twelve weeks post-release, muscle excursion and sarcomere number were increased for released TAs compared with control TAs. However, *in vivo* TA force production was decreased in released TAs compared to control TAs and may have been responsible for the increase in serial sarcomere addition. Increasing force production of released TAs by partial ablation of the extensor digitorum longus did not inhibit the increase in serial sarcomere addition. Thus, the results of this study support the hypothesis that increasing muscle excursion results in increased serial sarcomere addition in growing animals.

The second purpose of this dissertation was to use the TA release model as an initial examination of the capability of a muscle to adapt following tendon transfer. The hypothesis tested was that increasing the moment arm and excursion of the TA muscle (via TA release) results in adaptation of muscle architecture that helps to achieve near normal TA torque at the ankle joint. Twelve weeks post-release, moment arm and excursion were increased in released compared with control TAs. Decreased *in vivo* TA force production compensated for the increased moment arm in released TAs such that *in vivo* TA ankle joint torque was similar between all groups. Physiological cross-sectional area was smaller, and sarcomere number was larger, in the released TA compared with the control TA. These adaptations were consistent with the smaller *in vivo* force for the released TA. In summary, muscle appears capable of adapting to increased moment arm and excursion in a manner that helps achieve near normal TA torque at the ankle joint.

PREFACE

Chapters 3 and 4 of this dissertation have been written as stand-alone manuscripts. Differences in style between manuscripts reflect different requirements of the journals to which the manuscripts were submitted. Since the methodology was similar for both chapters, there is repetition of information between chapters in the Methods sections. In addition, portions of Chapter 1 (Introduction) and Chapter 2 (Review of Literature) are repeated in the Introduction sections of Chapters 3 and 4.

Chapters 3 and 4 are based on the following manuscripts, respectively:

- Koh TJ, Herzog W. Excursion is important in regulating sarcomere number in the growing rabbit tibialis anterior. *J. Physiol. (Lond.)* Submitted.
- Koh TJ, Herzog W. Increasing the moment arm of the tibialis anterior induces structural and functional adaptation: Implications for tendon transfer. *J. Biomech.* Submitted.

ACKNOWLEDGMENTS

I would like to express my appreciation and thanks to:

Dr. Walter Herzog, my supervisor, for providing encouragement, support, and guidance throughout my Ph.D. studies. His unwavering enthusiasm for research and life will inspire me long beyond my dissertation days.

Drs. Ronald Zernicke and Brian MacIntosh for serving on my supervisory committee, and for providing guidance for my Ph.D. studies and my future career.

Dr. John Matyas for serving on my dissertation committee and for many discussions on science and my future.

Dr. Anthony Russell for serving on my dissertation committee.

Dr. James Tidball for serving as the external examiner on my dissertation committee and for venturing into the Great White North for the exam.

Timothy Leonard for teaching me everything I know about *in vivo* measurements, spending long days helping with experiments, helping me to develop the TIMI, and for discussion of life during lunch at Pho Hoa.

Hoa Nguyen for helping with experiments, answering many questions about computer applications (for a small fee) and introducing me to Pho Hoa (and other Vietnamese cuisine).

Andrzej Stano for helping me design and build equipment for experiments in and out of the dissertation.

Lorraine Poulin, Heather Finch, and Sue Henderson for their fantastic help in caring for the rabbits.

Julianna Gal for her help with digitizing the video records of endless hops on the treadmill.

The following institutions for financial support: Alberta Heritage Foundation for Medical Research, The Faculty of Graduate Studies and the Department of Medical Science at the University of Calgary, The Olympic Oval Endowment Fund, and The Killam Foundation.

Aaron Tubman and Marco Vaz for spending the majority of the past 4+ years in a small office with me, being good friends in good times and bad.

Dilson Rassier for discussions on science and philosophy while quaffing beer and inhaling burgers.

Stefan Judex for discussions on mechanotransduction during 2 hour runs and skis.

Gordon Hamilton for being Gord, and for his family ties.

The HPL volleyball team and Jasper-Banff relay teams, for providing fun outside the lab.

My brothers, Song and Jeff, and sisters, Robin and Amy, for their laid-back support.

My parents for supporting my career as an eternal student, and for teaching me that hard work is the key to everything.

Gillian Hamilton, for being Gord's sister and my best friend, and for providing growth experiences over the past 3 years.

DEDICATION

to my parents, Choong and Shirley

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1:

Introduction

Musculoskeletal tissues have a remarkable ability to adapt to changing mechanical demands. In general, the response to increased (non-injurious) loading is to increase the quantity and/or quality of tissue, and the response to decreased loading is to decrease the quantity and/or quality of tissue (e.g. Roy et al. 1991, Rubin and Lanyon 1987, Tipton et al. 1986). Functional properties of the tissues are enhanced by increased loading and degraded by decreased loading, and are thus matched to the imposed mechanical demand. The cell is thought to be responsible for transducing mechanical signals and responding to altered mechanical demands (Frank and Hart 1989). Cells increase protein synthesis and can increase or decrease protein degradation in response to increased loading, and decrease protein synthesis and increase protein degradation in response to decreased loading (Banes et al. 1996, Burger et al. 1992, Vandenburgh et al. 1989).

The abilities of bone and cartilage to respond to altered mechanical demands are appreciated as being clinically important in diseases such as osteoporosis and osteoarthritis (Forwood and Burr 1993, Moskowitz 1992). Preventing muscle atrophy associated with decreased loading following joint injury and immobilization and during spaceflight, and reversing atrophy once it has occurred, have been appreciated as being important in maintaining and/or restoring normal function (Ingemann-Hansen and Halkjaer-Kristensen 1985, Roy et al. 1991). Probably because of the clinical importance of the above adaptations, much research has been performed on them. Although mechanical factors appear to be important in adaptations of muscle fiber or fascicle length (Goldspink 1985, Herring et al. 1984), and the importance of such adaptations is beginning to be appreciated in pathological conditions (e.g. cerebral palsy) and clinical procedures (e.g. tendon transfer, bone lengthening), comparatively little research has been performed on this topic. Research into the signals involved in the adaptation of the number of sarcomeres in series in skeletal muscle fibers or fascicles (sarcomere number) will be important in understanding the role of

muscle adaptation in pathological conditions and clinical procedures, and in improving the outcomes of these conditions and procedures.

The increase in sarcomere number during post-natal growth appears to be influenced by mechanical factors. During post-natal growth, for many muscles, there is an increase in the distance between muscle attachments and a corresponding increase in muscle length (Carey 1921, Haines 1932, Stewart 1972). This association led to the suggestion that muscle grows in length in response to passive tension induced by longitudinal bone growth. Consistent with these early speculations, many experimental studies on sarcomere number adaptation using adult animals have supported the hypothesis that passive tension (or muscle length) is important in regulating sarcomere number. The models used in these experiments have included immobilization (Tabary et al. 1972, Tardieu et al. 1977, Williams and Goldspink 1971, 1978), bone lengthening (Simpson et al. 1995), and tendon lengthening (Tardieu et al. 1979). In these studies, sarcomere number was increased or decreased depending on whether the working length(s) of the muscle was chronically increased or decreased, respectively. Since these adaptations can take place in denervated muscle (Goldspink et al. 1974), the mechanism of adaptation does not necessarily involve neural factors or active muscle force.

In growing animals, muscle excursion (the change in muscle length required for producing full joint range of motion) may be important in regulating sarcomere number. For example, in growing mice and rabbits, immobilizing the ankle joint in either plantarflexion or dorsiflexion decreases the excursion of the soleus muscle and decreases serial sarcomere addition (Tardieu et al. 1977; Williams and Goldspink 1971, 1978). In addition, a procedure aimed at increasing muscle excursion has been shown to increase longitudinal muscle growth in growing rabbits (Crawford 1954, 1961). This procedure involved releasing the tibialis anterior (TA) tendon from its retinacular restraint at the ankle joint (Figure 1.1). After 4 months, muscle belly length was longer and tendon length was shorter compared with the contralateral control TA (Crawford 1954, 1961). If the increase in muscle length was accounted for by an increase in sarcomere number, these results may suggest a relationship between increased muscle excursion and increased sarcomere number. However,

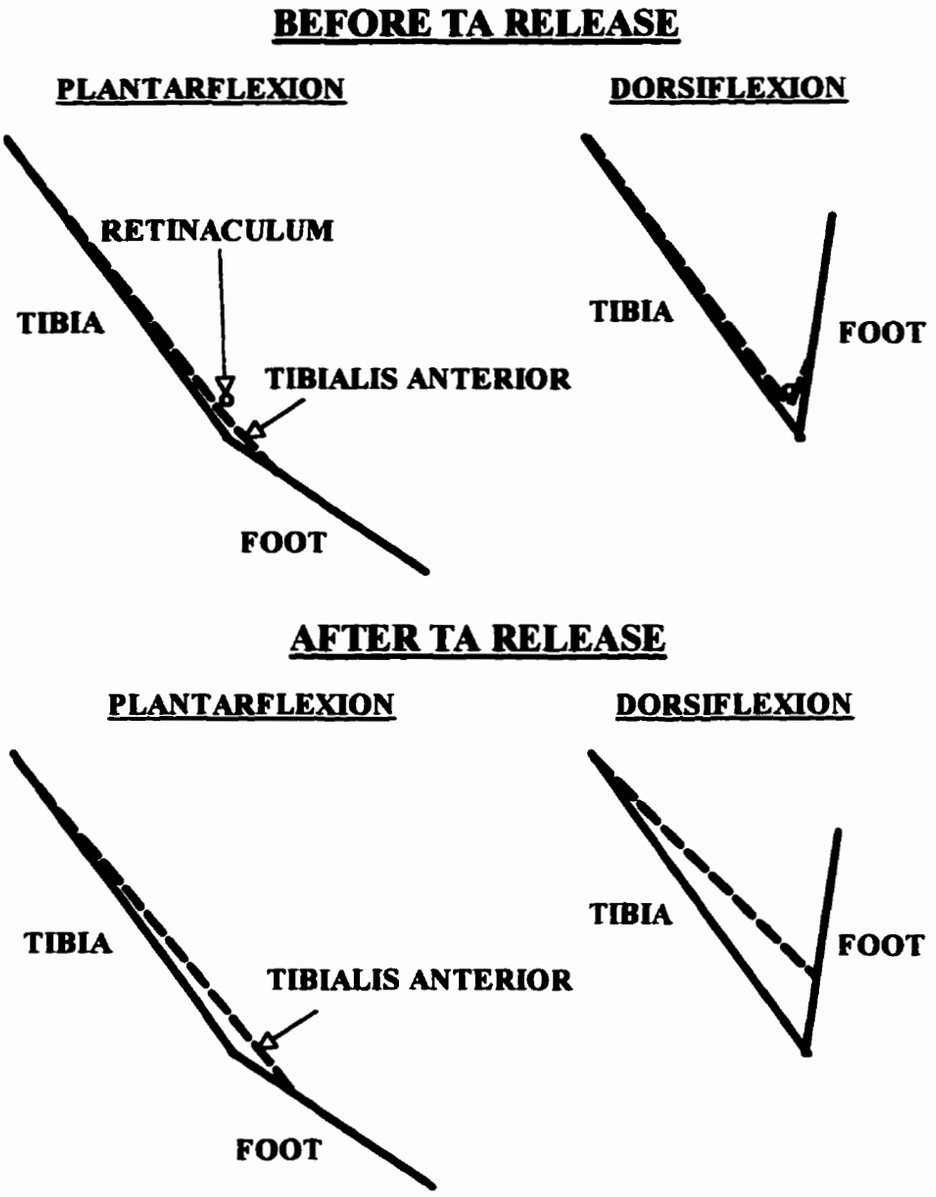

Figure 1.1. Schematic of releasing the tibialis anterior (TA) from its retinacular restraint at the ankle joint. Tibia and foot shown as solid lines, foot shown in plantarflexion and dorsiflexion. TA muscle-tendon unit shown as dashed line; length of line between origin and insertion represents TA muscle-tendon unit length in plantarflexion and dorsiflexion. Difference between TA muscle-tendon unit lengths in plantarflexion and dorsiflexion is TA muscle excursion. Release does not affect TA length in plantarflexion, but allows TA to follow shorter straight-line path from origin to insertion in dorsiflexion. Thus, TA muscle excursion is increased by TA release.

neither muscle excursion nor sarcomere number were measured; thus a relationship between the two remains speculative. In fact, in all studies of sarcomere number adaptation to date, the *in vivo* mechanical environment of muscle (e.g. muscle excursion, muscle force production) has not been defined. Thus, suggested links between the mechanical environment and sarcomere number adaptation in general have only been speculative.

TA release also provides a model for an initial examination of the capability of muscle to adapt following tendon transfer. TA release allows investigation of the architectural and functional adaptations associated with altering tendon path without the complications associated with transferring the tendon (e.g. tendon transection, healing, inflammation, scar formation). TA release increases the excursion and moment arm of the muscle, and both of these parameters may change after tendon transfer (Lieber et al. 1996). As muscle architecture is clearly capable of adapting to an altered mechanical environment (Booth and Thomason 1991, Goldspink 1985, Roy et al. 1991), muscle adaptation after tendon transfer seems likely. However, although the potential for post-tendon transfer muscle adaptation has been recognized (Lieber et al. 1996), a review of the literature revealed no experimental data, and models of tendon transfer have not included this possibility (Giat et al. 1994, Loren et al. 1995). Adaptations in TA muscle architecture following TA release could help to achieve near normal TA torque at the ankle joint. For example, sarcomere number may be increased to compensate for the increased muscle excursion, and physiological cross-sectional area (PCSA) may be decreased to compensate for the increased moment arm.

There were two main purposes of this dissertation. The first purpose was to test the hypothesis that increasing muscle excursion via TA release results in increased serial sarcomere addition in growing animals. The second purpose was to use the TA release model as an initial examination of the capability of muscle to adapt following tendon transfer. The hypothesis tested for the second purpose was that increasing the moment arm and excursion of the TA muscle (via TA release) results in adaptation of TA muscle architecture that helps to achieve near normal TA torque at the ankle joint. For both purposes, measurements of the *in vivo* mechanical environment of the TA were used to test

the stated hypotheses. *In vivo* TA muscle excursion and moment arm, TA force and torque production, ankle joint kinematics, and rabbit cage activity measurements provided the most complete characterization of the *in vivo* mechanical environment of muscle exposed to experimental perturbation to date. The results of the dissertation will improve understanding about the role of muscle excursion in sarcomere number adaptations, and will provide an indication of the capabilities of muscle to adapt following tendon transfer. These studies lay the groundwork for further investigations aimed at improving outcomes for tendon lengthening and transfer procedures, and for bone lengthening procedures.

Overview of dissertation

In the next section (Chapter 2), a review of relevant literature is presented to provide an historical framework for the hypotheses of the dissertation. The body of the dissertation (Chapters 3 and 4) addresses the main purposes in order, and were written as stand-alone manuscripts to be submitted for publication. The final section (Chapter 5) contains a summary of the significant findings, speculation on issues that was not appropriate for the manuscript chapters, and suggestions for future study.

The working hypothesis of this dissertation was that muscle excursion is a positive regulator of sarcomere number in growing animals. Tests of this working hypothesis were carried out at the organ and tissue level. Use of the terms "regulating" and "regulation" in the dissertation do not imply corroboration of the hypothesis at the cellular level. These terms are used to describe support for the working hypothesis based on statistical comparisons between treatment groups for parameters of the mechanical environment (e.g. muscle excursion) and sarcomere number.

CHAPTER 2:

Review of Literature

This review of literature is divided into two main parts. The first part contains a review of sarcomere number alterations during normal growth and during experimental perturbations and is focused on mechanical factors that may be involved in the regulation of sarcomere number. The second part contains a review of the effects of sarcomere number adaptations on muscle function in normal muscle, in pathologic conditions, and in clinical procedures.

Sarcomere number regulation during growth and adaptation

Longitudinal growth of muscle. Mammalian parallel-fibered skeletal muscle grows in length primarily by addition of sarcomeres in series (Williams and Goldspink 1971). It is widely accepted that sarcomeres are added to the ends of muscle fibers (Griffin et al. 1971, Williams and Goldspink 1971). This acceptance is based primarily on studies using radiolabeled adenosine injected into growing mice (Griffin et al. 1971, Williams and Goldspink 1971); these studies showed that most of the label found in muscle was incorporated into the ends of the muscle fibers, presumably into actin filaments. In addition, histological evidence has been presented for serial sarcomere assembly at the muscle-tendon junction in growing mice (increased concentration of ribosomes, free myofilaments and assemblies of filaments not incorporated into myofibrils; Williams and Goldspink 1971).

On the other hand, mammalian parallel-fibered skeletal muscles, with wire markers implanted to follow longitudinal growth, appear to grow uniformly along the entire length of the muscle belly (Alder et al. 1958, Crawford 1954, Mackay and Harrop 1969, Muhl and Grimm 1974), suggesting that serial sarcomere addition may occur throughout the length of muscle. A study using radiolabeled leucine and adenosine has shown uniform deposition of radiolabel throughout the length of developing insect flight muscle, consistent with serial sarcomere addition throughout the length of muscle (Houlihan and Newton 1979). In

addition, histological evidence (Z-band splitting, "extra" sarcomeres in certain myofibrils) has been presented for interstitial increases in sarcomere number in mammalian muscle (Friden 1984, Jakubiec-Puka 1985). In muscle subjected to stretch-immobilization, increased density of myosin heavy chain mRNA, ribosomes, and new myofibril assemblies were found at the muscle-tendon junction and under the sarcolemma in the midregion of stretched fibers compared to control fibers (Dix and Eisenberg 1990, 1991), suggesting sarcomere addition may occur in both regions. In summary, addition of sarcomeres in series appears to take place primarily at the ends of muscle fibers, although interstitial sarcomere addition may also be possible.

Longitudinal growth of tendon. Since tendon is arranged in series with muscle, longitudinal growth of tendon may affects that of muscle and vice versa (Figure 2.1). Recent studies have focused on revealing the mechanisms of tendon assembly during embryonic development (reviewed in Birk and Zycband 1994, Kadler et al. 1996). During tendon development, collagen fibrils are assembled in extracellular compartments formed by the fibroblast cell membrane (Birk and Zycband 1994). Fibril segments (10 μm in length) appear to be intermediates in tendon development that may fuse laterally and longitudinally to produce mature fibrils.

Little is known about the mechanisms involved in the post-natal longitudinal growth of tendon. Longitudinal tendon growth, measured using ink marks or implanted sutures, occurs along the entire tendon. In the rabbit tibialis anterior, the greatest increase in length occurs near the muscle-tendon junction (Alder et al. 1960, Crawford 1950, Fujio et al. 1994), whereas in the rabbit Achilles tendon and chicken flexor digitorum profundus tendon longitudinal growth was similar at all places marked along the tendon (Fujio et al. 1994, Nishijima et al. 1994).

Consistent with these observations on post-natal tendon growth, Davison (1992) has proposed a hypothesis for longitudinal tendon growth by strain-induced hydrolysis of immature (reducible) crosslinks leading to slipping of neighboring collagen molecules. Tension-induced sliding of collagen molecules is supported by X-ray diffraction studies (Mosler et al. 1985), and speculation of the involvement of such sliding in longitudinal

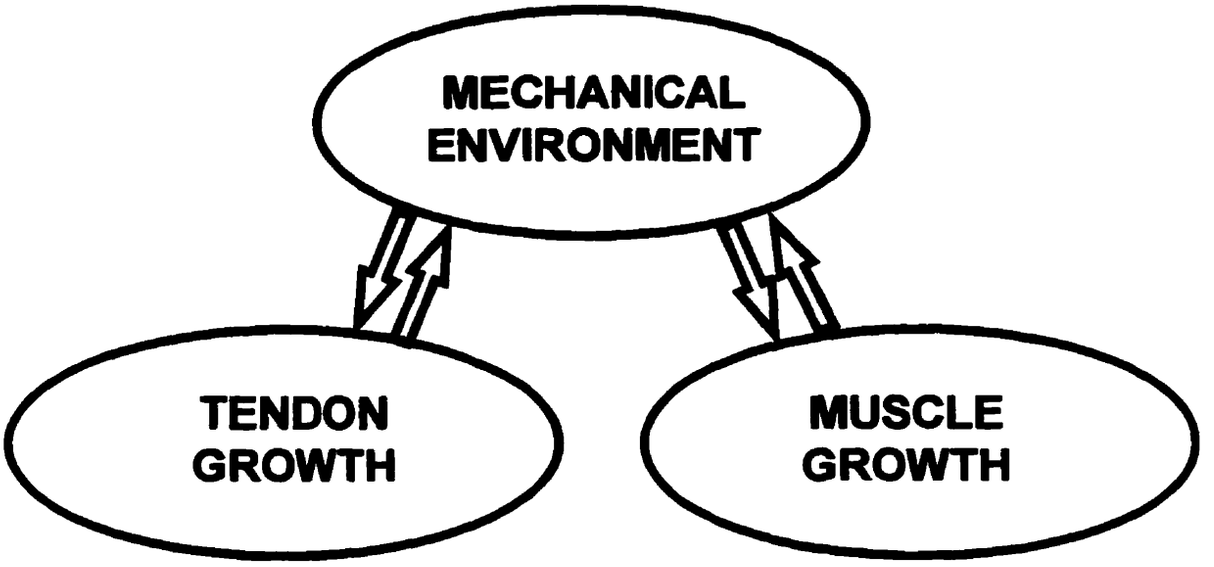

Figure 2.1. The mechanical environment may affect longitudinal growth of both muscle and tendon. In turn, the longitudinal growth of muscle and tendon will affect their mechanical environment.

growth has been made previously (Nemetschek et al. 1980). Under Davison's hypothesis, after sliding and thus tendon lengthening, new crosslinks are formed, and local synthesis fills in structural voids. Tendon with immature (reducible) crosslinks would be susceptible to strain-induced longitudinal growth. As mature (non-reducible) crosslinks replaced immature crosslinks in tendon (Bailey et al. 1974), the capacity for changes in length would diminish. This is consistent with the observations that tendon growth can be altered by experimental perturbation in young animals (Blanchard et al. 1985, Tardieu et al. 1983), but changes in tendon length do not generally occur in response to experimental perturbation in adult animals (Tardieu et al. 1977).

Regulation of sarcomere number by muscle length (passive tension). During growth, for many muscles, there is an increase in the distance between bony attachments of a muscle, and a corresponding increase in muscle length (Carey 1921; Haines 1932, Stewart 1972). This association led to the suggestion that muscle fibers grow in length in response to passive tension induced by longitudinal bone growth. In support of this notion, a strong linear correlation ($r = 0.99$) was reported between tibia length and gastrocnemius muscle length during growth (Comer 1956), suggesting that longitudinal bone growth may be important in regulating longitudinal muscle growth.

Immobilizing adult muscle in stretched or shortened positions has provided evidence supporting the hypothesis that muscle length or passive tension is a positive regulator of sarcomere number (passive tension hypothesis). Immobilizing the adult cat ankle joint in full dorsiflexion or in full plantarflexion resulted in a 20% increase or a 40% decrease, respectively, in the sarcomere number of the soleus after 4 weeks (Tabary et al. 1972). Similar results have been reported for stretch- or shorten-immobilization of parallel-fibered muscles of the mouse, rat, guinea pig, and rabbit (Heslinga et al. 1995, Huet de la Tour et al. 1979, Spector et al. 1982, Tardieu et al. 1977, Williams and Goldspink 1976, 1978, Williams et al. 1986). In these studies, increased or decreased passive tension induced by immobilization may signal that sarcomeres in series should be added or removed, respectively. The adaptation in sarcomere number appeared to restore sarcomere length to near optimal (i.e. maximal thick and thin filament overlap) at the immobilized muscle length.

Non-immobilization models that increase or decrease the working lengths of muscle have also provided results consistent with the passive tension hypothesis. Experimental lengthening of the rabbit tibia using an external fixator, which would increase the working lengths of the tibialis anterior muscle, produced increases in sarcomere number in this muscle (Simpson et al. 1995). Tibia lengthening of 20% produced an increase in sarcomere number of approximately 20% if lengthening was performed at 1 mm per day or less. Surgical lengthening of the Achilles tendon by Z-plasty in cats, which would decrease the working lengths of the soleus muscle, produced a decrease in sarcomere number in this muscle (Tardieu et al. 1979). Lengthening of the Achilles tendon by 10 to 25 mm resulted in reductions of sarcomere number of 30 to 60% after 2 to 7 months. Thus, chronically increasing or decreasing passive tension of the muscle fibers using methods that allow mobility also may signal the fiber that sarcomeres in series should be added or removed, respectively.

Regulation of sarcomere number by muscle excursion. Muscle excursion has also been proposed as a factor regulating sarcomere number, especially in growing animals. Based on observations of muscle fascicle lengths in human cadavers, Haines (1932) suggested that the length to which muscle fibers grow is determined, in part, by the change in fiber length required for the full joint range of motion (hereafter termed muscle excursion). Haines postulated that, for most muscles in the human body, the ratio of this change in fiber length to the maximum *in vivo* fiber length was 57%. Comer (1956) criticized Haines for not giving due credit for earlier observations on the relation between fiber length and muscle excursion (Fick 1860, Weber 1851). These earlier authors had proposed that the ratio of muscle excursion to fiber length was 67%, which was similar to the value proposed by Haines. Comer (1956) presented data supporting a relation between muscle excursion and muscle length in growing rats (20 to 60 days old). Muscle excursion was strongly correlated ($r = 0.84$) with muscle length, suggesting that muscle excursion may be important in regulating muscle length.

Immobilizing growing muscle in stretched or shortened positions has provided evidence that muscle excursion is important in sarcomere number regulation in growing

muscle. Long-term immobilization (for up to 11 weeks) of the ankle joint in young (1-week-old) mice in either plantarflexion or dorsiflexion resulted in decreased soleus serial sarcomere addition such that sarcomere number was approximately 50% smaller compared with contralateral control muscles (Williams and Goldspink 1971, 1978). Long-term immobilization (for up to 42 weeks) of the ankle joint in plantarflexion or dorsiflexion in young (4-week-old) rabbits reduced longitudinal growth of the tibialis anterior muscle belly (Alder et al. 1959) and muscle fascicles (Crawford 1973), relative to the contralateral control muscle. Immobilizing the ankle joint of 4-week-old rabbits in dorsiflexion initially increased serial sarcomere addition in the soleus muscle (after 7 days) and then decreased serial sarcomere addition (after 10-18 days; Tardieu et al. 1977). The initial response of sarcomere number to stretch immobilization in growing muscle may thus be similar to that of adult muscle (regulation by passive tension), but in the long-term, muscle excursion appears to be important in regulating serial sarcomere addition.

Studies attempting to increase muscle excursion have also provided evidence that muscle excursion is important in regulating longitudinal muscle growth in young animals. Releasing the tibialis anterior (TA) from its retinacular restraint at the ankle joint likely increases the excursion of the muscle (Crawford 1954, 1961). In growing rabbits, release of the TA resulted in increased longitudinal growth of the muscle belly and decreased longitudinal growth of the tendon compared with the contralateral control TA. Since passive tension is probably not altered or decreased by TA release, passive tension likely does not regulate longitudinal muscle growth after TA release. If the increase in muscle length was accounted for by an increase in sarcomere number, these results may suggest a relationship between increased muscle excursion and increased sarcomere number. However, neither muscle excursion nor sarcomere number were measured; thus a relationship between the two remains speculative. In fact, in all studies of sarcomere number adaptation to date, the *in vivo* mechanical environment of muscle has not been defined. Thus, suggested links between the mechanical environment and sarcomere number adaptation in general have only been speculative.

Regulation of sarcomere number by active force/neural factors. Herring et al. (1984) have suggested that sarcomere number is regulated such that sarcomere length is optimal at the joint position for which maximal force is produced during normal daily activity. In support of this hypothesis, Herring et al. (1984) reported that sarcomere length of a number of jaw muscles in the pig and rabbit were near optimal at the jaw angle for which maximal electromyographic activity was observed during feeding. However, since (1) muscle force was not measured and (2) a clear relationship between dynamic electromyographic measurements and muscle force has not been defined, this support is tenuous.

Another study suggested that eccentric muscle training may increase sarcomere number (Lynn and Morgan 1994). Rats trained by downhill treadmill running showed greater sarcomere numbers in the vastus intermedius than rats trained by uphill running. The authors attributed the greater sarcomere number in the downhill-trained rats to the greater amount of eccentric muscle activity involved in downhill running. It is difficult to attribute the greater sarcomere number specifically to eccentric activity in this study because the joint kinematics and muscle forces likely also differed between uphill and downhill running. In addition, controlled eccentric training of rabbit dorsiflexor muscles produced little or no change in sarcomere number (Koh and Herzog, unpublished data). However, the study of Lynn and Morgan does suggest that exercise training can produce adaptations in sarcomere number, and it seems likely that active force production may have been involved in this process.

Intact innervation does not appear to be required for adaptations in sarcomere number, but may affect the rate of adaptation. Denervating the cat soleus did not affect the adaptation in sarcomere number to immobilization in either plantarflexed or dorsiflexed positions (Goldspink et al. 1974). Likewise, denervating the mouse soleus and biceps brachii had no effect on the extent of sarcomere number adaptation to stretch immobilization after two weeks (Williams and Goldspink 1976). Denervation of the mouse soleus appeared to decrease the rate of sarcomere removal from muscle subjected to tenotomy, suggesting that neural factors or active muscle force may influence the rate of sarcomere removal (McLachlan and Chua 1983). Electrical stimulation of the rabbit tibialis anterior

immobilized in a stretched position increased the number of sarcomeres added after 4 days (Williams et al. 1986). This result supports the idea that stimulation of a muscle may increase the rate of sarcomere adaptation.

Effects of sarcomere number adaptation on muscle function

Sarcomere number and normal muscle function. Studies addressing the functional implications of muscle architecture typically assume that maximal isometric tension is directly proportional to the number of sarcomeres in parallel in muscle (physiological cross-sectional area is an index of the number of sarcomeres in parallel), and that maximal muscle excursion and maximal velocity of shortening are directly proportional to serial sarcomere number (Gans 1982, Lieber and Blevins 1989, Sacks and Roy 1982, Wickiewicz et al. 1983). All other things being equal, increasing sarcomere number should decrease excursion of each sarcomere for a given muscle excursion. Thus increasing serial sarcomere number should (1) decrease the change in isometric force for a given change in muscle length, and (2) decrease the change in isotonic force for a given change in muscle shortening velocity.

Experimental support for the influence of sarcomere number on functional muscle properties has been reported. Changes in muscle length have been correlated with changes in muscle force-length properties. Decreased longitudinal growth of the TA in response to immobilization of the ankle joint of young rabbits has been correlated with force-length relations that show peak forces at shorter muscle lengths, and greater changes in force with changes in muscle length, compared with contralateral control muscles (Alder et al. 1959, Crawford 1973). Increased longitudinal growth of the TA in response to TA release in young rabbits has been correlated with force-length relations that show peak forces at longer muscle lengths, and smaller changes in force with changes in muscle length, compared with control muscles (Crawford 1961). In these studies, sarcomere number was likely the major contributor to altered muscle length and thus sarcomere number likely influenced muscle force-length properties. Sarcomere number has also been directly correlated with muscle force-length properties (Williams and Goldspink 1978); immobilization of the mouse soleus in lengthened or shortened positions was correlated with peak forces at long or short muscle

lengths, respectively, and with wide or narrow force-length relations, respectively. In addition, the difference between the force-velocity properties of the cat soleus and medial gastrocnemius has been partly attributed to differences in sarcomere number (Spector et al. 1980). In short, muscle force-length and force-velocity properties appear to be influenced by sarcomere number.

The torque produced by a muscle at a joint is the product of muscle force and its moment arm at the joint. Since muscle force and moment arm both tend to vary with joint angle (muscle length), the shape of the joint torque-angle relation depends on the shapes of both the muscle force and moment arm-joint angle relations (Hoy et al. 1990, Koh 1995, Lieber 1992). Since sarcomere number can influence the shape of the force-length relation, sarcomere number can influence the joint torque-angle relation. Evidence that adaptations in sarcomere number could explain changes in joint torque-angle relations with muscle training was presented in a previous publication (Koh 1995).

Sarcomere number and muscle function in disease and clinical procedures. Adaptations in sarcomere number may be important in pathological conditions and may influence the outcome of clinical procedures. Cerebral palsy is a prevalent neuromuscular disorder originating in childhood and is associated with muscle spasticity and muscle contractures. When the triceps surae muscles are involved, "toe-walking" is a result (Tardieu et al. 1989). When the hamstrings muscles are involved, "crouch-gait" is a result (Thometz et al. 1989). Triceps surae muscle contractures have been attributed partly to decreased growth in sarcomere number in spastic muscle (O'Dwyer et al. 1989, Tardieu et al. 1982). Although direct measurements have not been made (and likely will not be made) in humans, passive joint torque-angle relations have supported the idea that muscle length is decreased in muscle contractures (Tardieu et al. 1982). In addition, in spontaneously spastic mice, the longitudinal growth of the gastrocnemius muscle was reduced, resulting in muscle contracture (Ziv et al. 1984). Tendon lengthening is commonly performed in an attempt to relieve muscle contracture and allow normal joint motion in patients with cerebral palsy (Thometz et al. 1989, Truscelli et al. 1979). However, the likelihood of further decreased muscle growth after tendon lengthening has largely been ignored, especially in models of the

functional effects of tendon lengthening (Delp and Zajac 1992), and is perhaps a factor in the redevelopment of contractures post-surgery (Truscelli et al. 1979). Understanding the factors responsible for sarcomere number adaptation could aid in designing approaches to prevent redevelopment of contractures post-surgery, and better yet, may help in developing non-surgical methods to effectively treat contractures

Bone lengthening is often performed in patients with short limbs of congenital or developmental etiology (Paley 1990). Although bone responds well to lengthening, the soft tissues surrounding the bone appear to limit the rate and extent of lengthening possible (Lehman et al. 1991, Paley 1990). For example, muscle injury and abnormal proliferation of fibrous tissue occurred with lengthening rates greater than 1 mm per day (Simpson et al. 1995). In addition, passive muscle properties were maintained only with the slowest lengthening rate (0.4 mm per day). Thus the rate of sarcomere number addition along with concomitant muscle connective tissue adaptation may partly limit the success of bone lengthening procedures. Understanding the factors that stimulate sarcomere number addition could help in designing strategies to improve the rate and extent of longitudinal muscle growth possible in bone lengthening procedures.

Tendon transfer is often performed to restore function to a joint in patients with peripheral nerve damage, spinal cord injury, brain damage, and neuromuscular disease (e.g. cerebral palsy). Many factors are considered when selecting a donor muscle for tendon transfer. These include donor muscle availability, expendability, integrity, transfer route and surgeon preference (Lieber et al. 1992). Recent studies have emphasized the importance of muscle architecture when selecting donor muscles (Lieber et al. 1992, Zajac 1992). Lieber et al. (1996) have proposed using sarcomere length, measured intra-operatively, as a guide for the length at which muscle should be transferred. Predictions of muscle function post-transfer were made based on these measurements. Although the potential for post-transfer muscle adaptation was recognized, this possibility was not included in the discussion of the post-surgical functional outcome. Other mathematical models of tendon transfer also have not included this possibility (Giat et al. 1994, Loren et al. 1995). As muscle architecture is clearly capable of adapting to an altered mechanical environment (Booth and Thomason

1991, Goldspink 1985, Roy et al. 1991), post-transfer adaptation seems likely. Post-transfer adaptation would influence the functional outcome of tendon transfer procedures. Understanding the factors responsible for sarcomere number adaptation could help to optimize selection of donor muscles and placement of transferred muscles for tendon transfer procedures.

Summary

Sarcomere number is responsible for most of the increase in muscle length during post-natal growth of mammalian parallel-fibered skeletal muscle. Muscle length or passive tension appears to be important in regulating sarcomere number in adult muscle. Muscle excursion appears to be important in regulating sarcomere number in growing muscle. Sarcomere number is an important determinant of muscle force-length and force-velocity properties. Adaptations in sarcomere number may be important in pathology associated with cerebral palsy and may play a role in the outcome of bone lengthening, tendon lengthening, and tendon transfer. Understanding the factors responsible for sarcomere number adaptation could help in improving treatment of muscle contractures in cerebral palsy, in preventing contractures in bone lengthening, and in improving the outcome of tendon lengthening and tendon transfer procedures.

CHAPTER 3:

Muscle Excursion is Important in Regulating Sarcomere Number in the Growing Rabbit Tibialis Anterior

Introduction

Adaptation in the number of sarcomeres in series in skeletal muscle fibers or fascicles (sarcomere number) may be important in pathologies associated with cerebral palsy (O'Dwyer et al. 1989), tendon transfer (Chapter 4), and with bone lengthening (Lehman et al. 1991, Simpson et al. 1995). Adaptations in sarcomere number also have implications for the function of normal muscle: muscle force-length properties (passive and active; Williams and Goldspink 1978) and force-velocity properties (Spector et al. 1980) can be influenced by sarcomere number. Despite the likely importance of sarcomere number adaptation, the factors responsible for such adaptation remain unknown. Specifically, although mechanical signals appear to be important for inducing serial sarcomere addition or deletion (Goldspink 1985, Herring et al. 1984), the nature of these signals remain to be elucidated.

In growing animals, muscle excursion (the change in muscle length required to produce the full joint range of motion) may be important in regulating sarcomere number. For example, in growing mice and rabbits, immobilizing the ankle joint in either plantarflexion or dorsiflexion decreases the excursion of the soleus muscle, and decreases soleus sarcomere number relative to the contralateral control muscle (Tardieu et al. 1977, Williams and Goldspink 1971, 1978).

In addition, a procedure assumed by Crawford (1954, 1961) to increase muscle excursion increased longitudinal muscle growth in young animals. This procedure involved releasing the tibialis anterior (TA) tendon from its retinacular restraint at the ankle joint in 3-4-week-old rabbits (Figure 3.1). After 4 months, muscle belly length was 20% longer for the released TA compared with the contralateral TA. If the increase in muscle length was accounted for by an increase in sarcomere number, these results may suggest a relationship between increased muscle excursion and increased sarcomere number (Figure 3.2).

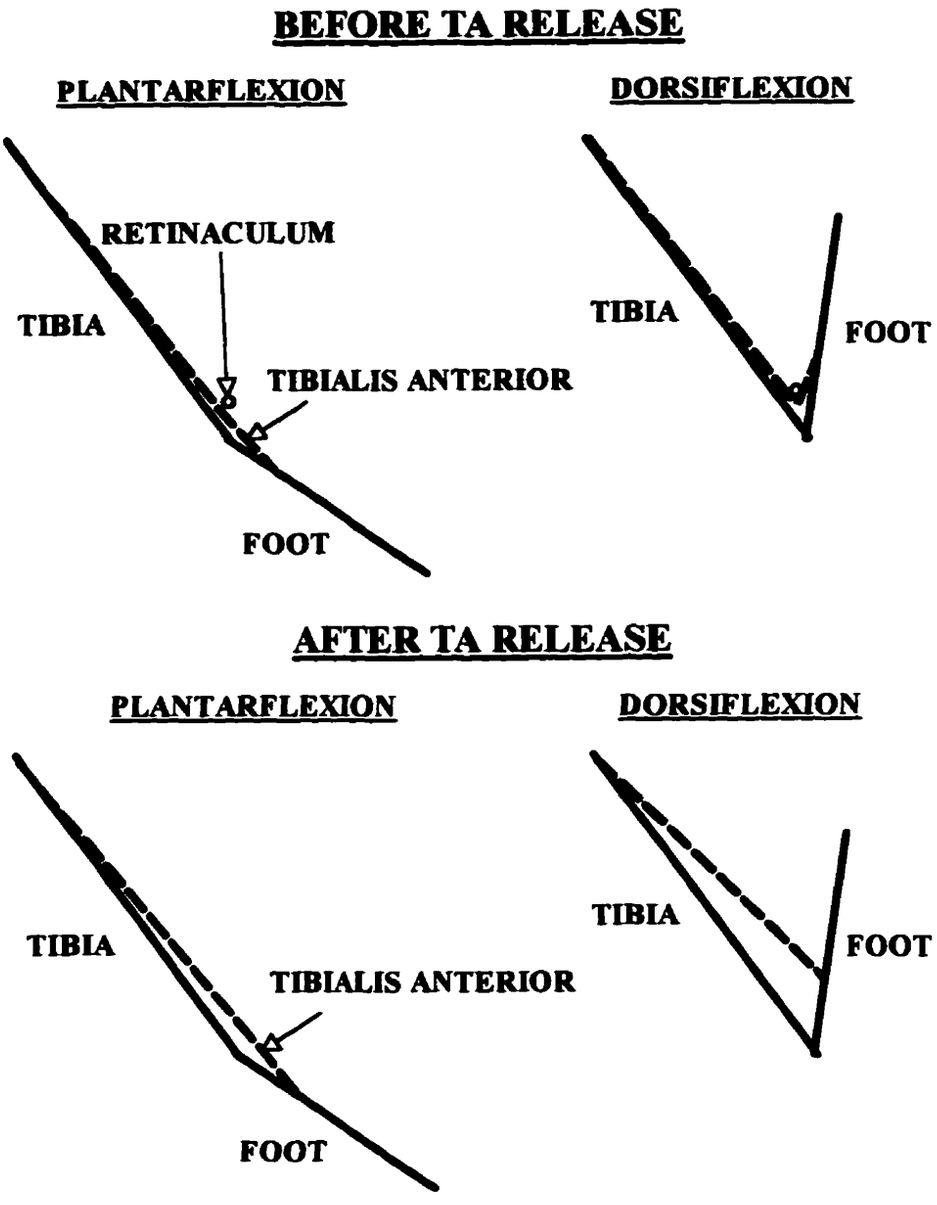

Figure 3.1. Schematic of releasing the tibialis anterior (TA) from its retinacular restraint at the ankle joint. Tibia and foot shown as solid lines, foot shown in plantarflexion and dorsiflexion. TA muscle-tendon unit shown as dashed line; length of line between origin and insertion represents TA muscle-tendon unit length in plantarflexion and dorsiflexion. Difference between TA muscle-tendon unit lengths in plantarflexion and dorsiflexion is TA muscle excursion. Release does not affect TA length in plantarflexion, but allows TA to follow shorter straight-line path from origin to insertion in dorsiflexion. Thus TA muscle excursion is increased by TA release.

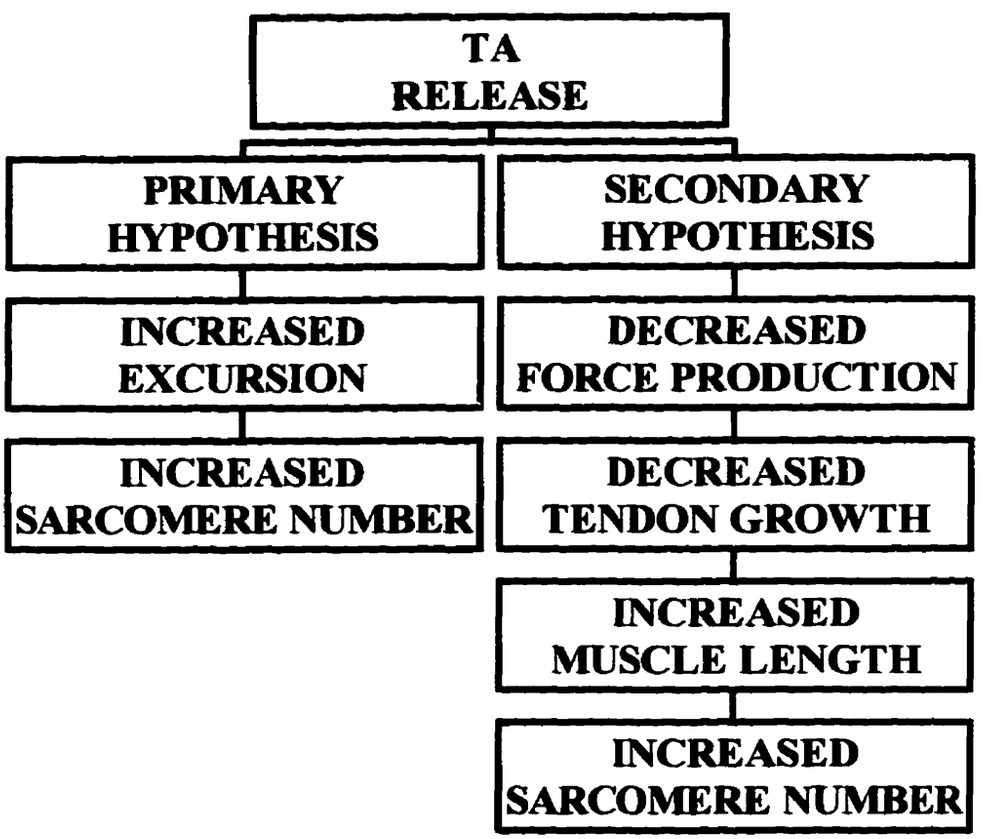

Figure 3.2. Two hypotheses for adaptation in sarcomere number following TA release. The primary hypothesis is that increased muscle excursion results in increased sarcomere number following TA release. The secondary hypothesis is that decreased force production results in increased sarcomere number following TA release.

However, neither muscle excursion nor sarcomere number were measured; thus a relationship between the two remains speculative. In fact, in all studies of sarcomere number adaptation in the literature, the *in vivo* mechanical environment of muscle has not been defined. Thus, suggested links between the mechanical environment and sarcomere number adaptation in general have only been speculative.

Another explanation for the increase in muscle length associated with TA release involves possible decreased force production of the TA (Figure 3.2). TA release increases the moment arm of the TA at the ankle joint, and thus decreased TA force production could help to achieve near normal TA torque at the ankle joint (cf. Chapter 4). This possibility is supported by the observation that, 4 months after release, the maximum isometric force produced by the released TA is less than that produced by its contralateral counterpart (Crawford 1961). Chronically decreased force production could be responsible for reduced longitudinal tendon growth; the released tendon was shorter than the contralateral tendon (Crawford 1954, 1961). This possibility is supported by data suggesting that tension is an important stimulus for longitudinal tendon growth (Blanchard et al. 1985, Davison 1992, Tardieu et al. 1983). Decreased tendon growth would result in a chronically increased working length of the released muscle, since muscle and tendon are arranged in series. A chronically increased working muscle length has been shown to be a stimulus for sarcomere number addition (Tabary et al. 1972, Tardieu et al. 1977). Thus, this sequence of events could be responsible for the increase in longitudinal muscle growth associated with release.

The working hypothesis of the present study was that muscle excursion is important in regulating sarcomere number in growing animals. The specific hypotheses of the study were (1) that increased muscle excursion results in increased serial sarcomere addition following TA release in growing animals (primary hypothesis), and (2) that decreased TA force production results in increased serial sarcomere addition following TA release (secondary hypothesis). The primary hypothesis was tested by determining whether sarcomere number and *in vivo* muscle excursion were increased following TA release. Cage activity and *in vivo* ankle joint kinematics were also recorded to determine whether altered animal or joint activity patterns could be related to sarcomere number adaptation. The

secondary hypothesis was tested by determining whether *in vivo* TA force production was decreased following TA release, and whether increasing the *in vivo* force production of the released TA (via ablation of the synergistic extensor digitorum longus -- EDL) inhibited the expected increase in sarcomere number associated with release. The present investigation appears to be the first study of sarcomere number adaptation in which the *in vivo* mechanical environment of muscle has been defined.

Methods

Animals. Female New Zealand White rabbits (4-week-old) were obtained from a single supplier (Vandermeer, Edmonton, AB, Canada). Twelve rabbits each were allotted into control, sham-operated, TA release, and TA release plus EDL ablation groups. Animals in the control group received no surgical treatment at 4 weeks of age. Animals in the sham-operated, TA release, and TA release plus EDL ablation groups received the surgical treatments described below at 4 weeks of age. Six animals in each group were used for measurements of *in vivo* TA muscle force, *in vivo* ankle joint kinematics and *in situ* maximum TA muscle force at 15-16 weeks of age, which required implantation of force transducers. The remaining 6 animals in each group were used for measurements of TA muscle excursion and muscle architecture at 16 weeks of age. All procedures were performed according to the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of Calgary.

Sham, TA release, and EDL ablation procedures. Animals were anesthetized with halothane for surgery. TA release was performed essentially following Crawford (1954). Under sterile conditions, a small (2 cm long) incision was made over the anterior part of the left ankle joint to expose the proximal extensor retinaculum (also called the crural ligament). For animals in the TA release only and TA release plus EDL ablation groups, the retinaculum was removed, and the fascia overlying the TA muscle belly and tendon was divided 1 cm proximal and distal to the ankle. For sham-operated animals, identical procedures were used, except the retinaculum was not removed. For rabbits receiving partial EDL ablation in addition to TA release, one-third of the distal muscle belly of the EDL was removed along with its tendon reaching 1 cm distal to the ankle. Care was taken not to damage nerve or blood supply to the TA. After closing the incision, the rabbit was given an injection of butorphanol (0.1 mg/kg) for analgesia. The rabbits recovered quickly from surgery; noticeable limping was absent after two days. Rabbits were housed singly (cage dimensions: 44 cm x 60 cm x 38 cm) and allowed normal cage activity.

Cage activity. Cage activity over a 24 hour period was assessed 11-12 weeks post-surgery to determine whether a possible lack of adaptation in sarcomere number was due to

a lack of animal activity. Cage activity was measured as the total time that the rabbit spent moving in the cage. Activity was monitored with a motion detector (Noma, Toronto, ON, Canada) placed above the cage. The sensitivity of the motion detector was set to monitor only entire body movements. The sampling rate of the detector was adjusted to 2 Hz. The total time that the rabbit spent moving in the cage was calculated by multiplying the total number of motion detector counts by 0.5 seconds.

In vivo TA muscle force. *In vivo* TA muscle force production was measured for 6 rabbits in each experimental group 11-12 weeks post-surgery to determine the influence of TA release and EDL ablation on *in vivo* TA force production. Muscle force was measured using surgically-implanted custom-made E-shaped tendon force transducers (Herzog et al. 1993). Briefly, under anesthesia, transducers were implanted on the left and right TA tendon just distal to the muscle belly through a small incision (2 cm long) over the anterior part of each leg. Another incision was made over the midline of the thoracic spine (5 cm long) to allow subcutaneous passage of the transducer leads from the leg to the back where they were sutured subcutaneously to fascia for strain relief. The leads terminated in a custom-made electrical connector. After closing the incisions, the rabbit was given an injection of butorphanol (0.1 mg/kg) for analgesia.

Muscle forces were recorded 5-7 days after the transducers were implanted. Each animal was briefly anesthetized with isoflurane (< 1 minute), two sutures on the back were removed and the connector was exteriorized and linked by a cable to a strain-gage amplifier (Intertechnology, 2100 System, Toronto, ON, Canada). After the animal recovered from anesthesia, muscle force was recorded at 500 Hz via microcomputer while the rabbit was (a) hopping on a motor-driven treadmill at 0.3 m/s, (b) hopping on a walkway at a self-selected speed, and (c) performing a large force-producing flicking motion while attempting to remove a piece of foil taped to its foot. Twenty hops or flicks were chosen for analysis from each condition for each rabbit. Force data were low-pass filtered at 50 Hz (2nd-order recursive Butterworth filter), and peak force and impulse (the integral of force over time) for each hop or flick were measured with a computer program written for the purpose. For hopping, peak force and impulse were averaged over 20 hops to provide representative

values for each rabbit. For the flicking motion, values were averaged over flicks that produced the 5 largest peak force and 5 largest impulse values.

In vivo ankle joint kinematics. Bilateral saggital plane ankle joint kinematics were measured for the rabbits during treadmill hopping to determine if altered joint kinematics could be related to sarcomere number adaptations. Two high-speed (60 Hz) video cameras (Motion Analysis, Santa Rosa, CA, USA) were used to record rabbits hopping on the treadmill; one camera each was focused on the lateral surface of the left and right hindlimb. The 20 hops selected for TA force analysis (cf. previous paragraph) were also used for kinematic analysis for each rabbit. TA force data were synchronized with kinematic data using a time code generator (Datum, Model 9550, San Jose, CA, USA).

The swing phase was identified for each hop (separately for each leg) as the set of frames from the video record between takeoff and touchdown of each foot. For each hop, every frame showing the swing phase, plus 10 frames prior to, and 10 frames after, the swing phase, were analyzed. In each frame, two reflective markers placed on the lateral side of each foot (lateral malleolus, metatarsal-phalangeal joint), and two points on the anterior surface of the leg (one near the knee, one near the ankle) were digitized manually (Motion Analysis, Manual Input System). These points were selected to minimize artifacts from soft tissue movement, and they defined the long axes of the foot and leg. Ankle joint angles were defined as the included angle between the long axes of the foot and leg, and these angles were low-pass filtered (2nd-order recursive Butterworth) at 10 Hz. For each hop, the duration of the swing phase (swing time), the maximum and minimum angles achieved in each hop (typically at takeoff and mid-swing), the difference between these values (joint excursion) and the maximum velocity of dorsiflexion were calculated using a computer program written for the purpose. Each parameter was averaged over the 20 hops to provide representative values for each rabbit.

In situ TA muscle force. After the *in vivo* force and kinematic measurements, maximum *in situ* TA muscle force was assessed. Each animal was anesthetized with isoflurane, and nerve cuff stimulating electrodes were placed on the left and right common peroneal nerves. The animal was then placed in a device designed to secure the lower limb

with the knee joint fixed at 90 degrees. The foot was strapped to a footplate, which could be fixed at any desired ankle joint angle. The nerve cuff was connected to an isolated stimulator (Grass, Model S88, West Warwick, RI, USA) and supramaximal stimulation intensity (pulse duration = 0.1 ms, frequency = 150 Hz, train duration = 400 ms, intensity = 1-2 Volts) was determined as that which produced maximum force (measured using the tendon force transducer). Maximum developed isometric TA force was measured for ankle joint angles from 60 to 160 degrees in 20 degree increments (full plantarflexion = 160 degrees) to determine the maximum force-producing capability of the muscle. Following this protocol, the animal was euthanized with an overdose of pentobarbital, the TA tendon was freed from its distal attachment, and the implanted tendon force transducers were calibrated immediately by hanging a series of known weights on the tendon.

TA muscle excursion. TA muscle excursion was determined for the left leg of the remaining 6 rabbits in each group at 12 weeks post-surgery to quantify the increase in muscle excursion associated with TA release. Each rabbit was anesthetized with isoflurane, and a nerve stimulating cuff was implanted on the left common peroneal nerve. Radiopaque wire markers were implanted (1) at the tibial tubercle and (2) on the TA tendon near the muscle-tendon junction; these landmarks allowed for consistent placement of markers between animals. The rabbit was placed on its side on an x-ray table. A fluoroscope was focused on the left hindlimb, and the displacements of the markers were recorded on fluoroscopic video during three trials of dorsiflexion. The foot was first held in a fully plantarflexed position, the TA was then submaximally stimulated (pulse duration = 0.1 ms, frequency = 40 Hz, train duration = 2 seconds, intensity = 1-2 Volts), the foot was allowed to dorsiflex slowly while the muscle was contracting, and the movements of the bones and wire markers were recorded. A scale was placed in the field of view of the fluoroscope, and in the plane of the leg, to calibrate the distances measured on the fluoroscopy images.

For each trial, every frame of the fluoroscopy video record from the onset of stimulation with the foot plantarflexed until the foot reached full dorsiflexion was analyzed. Eight points were manually digitized (Motion Analysis) in each frame: the two ends of the scale, the two wire markers, two arbitrary points defining the long axis of the tibia, and two

arbitrary points defining the long axis of the foot. The ankle joint angle and the distance between the wire markers were calculated for each frame. Data were then grouped over the three trials. Muscle excursion was determined as the difference between the maximum (at full plantarflexion) and minimum (at full dorsiflexion) distances between the wire markers.

TA muscle and tendon architecture. After measurement of TA muscle excursion, rabbits were euthanized with an overdose of pentobarbital. Both lower limbs were removed, skinned, and cleaned of excess muscle and connective tissue. The limbs were then immersed and stored (> 1 month) in 10% neutral buffered formalin with the TA left attached to the bones and the ankle joint positioned at 90°.

After fixation, each TA was dissected carefully from its bony attachments. The length of the tibia was measured with vernier calipers. The length of the external tendon and proximal and distal aponeuroses were measured with a steel ruler (Figure 3.3). The tendon was excised at the muscle-tendon junction (at the distal end of the most distal muscle fibers). The muscle was blotted dry and fixed muscle mass was measured.

The muscle belly was divided into thirds with longitudinal cuts. The central third of each muscle was then placed in 30% nitric acid (Loeb and Gans 1986) for approximately 10 hours until the connective tissue had been softened sufficiently to allow teasing of small fascicles of fibers (approximately 20 fibers in diameter) that spanned the distance between proximal and distal aponeuroses (Figure 3.3). Twelve fascicles were teased from each muscle (6 each from the superficial and deep surfaces of the muscle). Fascicles were mounted in glycerol jelly on microscope slides, and fascicle length was measured using a video analysis system (Media Cybernetics, ImagePro Plus, Silver Spring, MD, USA). Sarcomere length was measured at five points along the length of each fascicle using a laser diffraction system (beam diameter -- 0.8 mm; Allinger 1995); (1) center of the fascicle, (2 and 3) midway between the center and each end of the fascicle, and (4 and 5) 2 mm from each end of the fascicle. Sarcomere number for each fascicle was estimated by dividing the fascicle length by the average sarcomere length for the fascicle (Simpson et al. 1995, Tabary et al. 1976). Sarcomere number was averaged across fascicles to provide a representative value for superficial and deep fascicles for each muscle. Most individual fibers in the rabbit

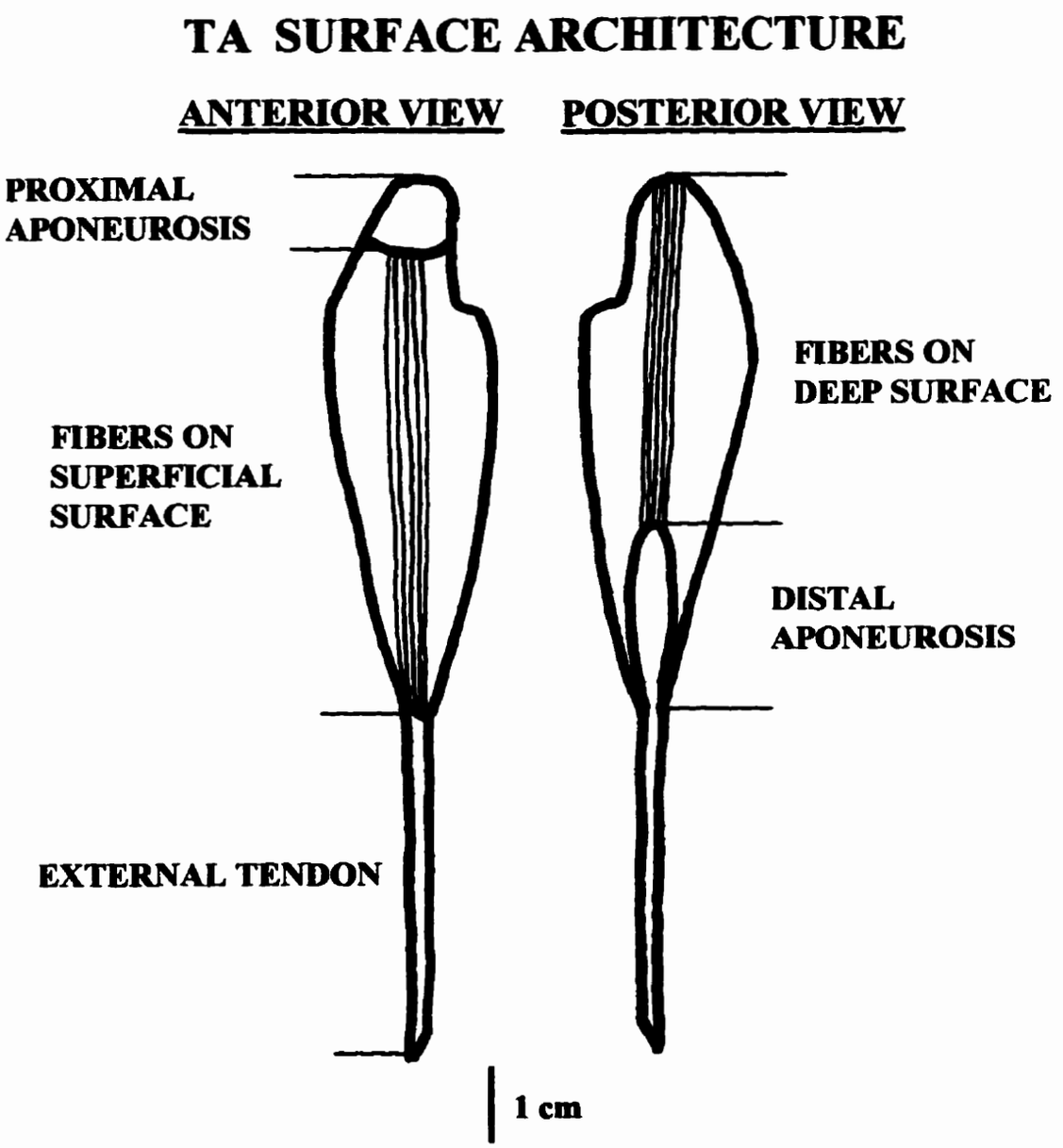

Figure 3.3. Anterior and posterior views of the surface architecture of the rabbit tibialis anterior muscle-tendon complex.

TA do not span the distance from proximal to distal aponeuroses (Mackay and Harrop 1969; Crawford 1973); they are arranged in serial, overlapping, fashion such that up to three fibers are required to span the distance between aponeuroses. The measurements in the present study thus indicate sarcomere number for fascicles that span the distance from proximal to distal aponeuroses, which seems functionally more important than sarcomere number for individual fibers. Previous studies of sarcomere number adaptation in the rabbit TA, and in the cat TA (which also has series-fibered architecture), also reported fascicle sarcomere number (Simpson et al. 1995, Tabary et al. 1976).

Muscle physiological cross-sectional area (PCSA) was estimated from the equation: PCSA (cm^2) = muscle mass (g) / fiber length(cm) * muscle density(1.0564 g/cm^3; Mendez and Keys 1960), where fiber length was calculated by multiplying sarcomere number (averaged over superficial and deep regions for each muscle) by 2.2 μm (Lieber and Blevins 1989). Muscle mass was corrected for the loss of mass due to fixation (measured by comparing mass before and after fixation in a pilot study; Table 3.1) before calculating cross-sectional area.

Muscle architecture measurements were also performed for the matching soleus muscle for each hindlimb similar to those performed for the TA. Architecture of the soleus muscle is highly sensitive to changes in its mechanical environment (Lieber, 1992). Thus, if TA adaptations are due to altered ankle joint kinematics or hindlimb loading, then it is expected that the soleus muscle will show adaptive changes as well.

Data analysis. For the primary hypothesis, mean values were compared between control, sham-operated, and release groups using one-way analysis of variance (ANOVA). The Scheffe test was used for post-hoc multiple comparisons. For the secondary hypothesis, mean values were compared between release and release plus EDL ablation groups using two-sample t-tests. For all statistical tests, the 0.05 level was taken to indicate statistical significance.

Table 3.1. Muscle mass before and after formalin fixation for experimental (left) and contralateral (right) TAs from control and released rabbits.

	Experimental			Contralateral		
	Before (g)	**After (g)**	**% Diff**	**Before (g)**	**After (g)**	**% Diff**
Control						
Rabbit 1	3.05	2.77	10	2.97	2.75	8
Rabbit 2	3.20	2.92	10	3.17	2.97	7
Rabbit 3	3.39	3.04	12	3.42	3.14	9
mean			**11**			**8**
Released						
Rabbit 1	2.88	2.68	7	2.96	2.80	6
Rabbit 2	2.72	2.49	9	3.08	2.92	5
Rabbit 3	2.51	2.43	3	3.32	3.06	8
mean			**6**			**6**

NB: mass measured after muscles were blotted dry; % Diff = difference between masses relative to mass after fixation x 100%.

Results

TA muscle excursion increased with release. The primary hypothesis that increasing muscle excursion results in increased serial sarcomere addition following TA release in growing animals was tested by measuring muscle excursion and sarcomere number as well as other possible influences on sarcomere number adaptation. For an ankle joint motion from 160 (full plantarflexion) to 40 (full dorsiflexion) degrees, muscle excursion for the released TA was significantly larger (40%) than that for the control or sham-operated TAs (Figure 3.4). Muscle excursion for the control and sham-operated TAs were not significantly different, and nearly identical. Thus the TA release procedure had the desired effect of increasing the muscle excursion of the TA.

TA sarcomere number increased with release. Sarcomere numbers for the released TA were significantly larger for the released TA than those for the control and sham-operated TAs (Figure 3.4). In comparison with the control TA, sarcomere numbers for the released TA were 14% and 22% larger for superficial and deep fascicles, respectively. In comparison with the sham-operated TA, sarcomere numbers for the released TA were 11% and 17% larger for superficial and deep fascicles, respectively. Sarcomere numbers were not significantly different between control and sham-operated TAs, in either superficial or deep regions. In addition, sarcomere number for the contralateral (right) TA showed no significant differences between groups in either the superficial or deep regions. Thus the TA release procedure resulted in increased serial sarcomere addition in the experimental TA but did not appreciably affect serial sarcomere addition in the contralateral TA.

To provide an indication of the reliability of the procedure for sarcomere number estimation, the entire procedure (fascicle sampling, fascicle length measurement, sarcomere length measurement, sarcomere number calculation) was repeated for one muscle. On average, sarcomere number varied between repeated measurements by 2%, suggesting that the procedure was reliable.

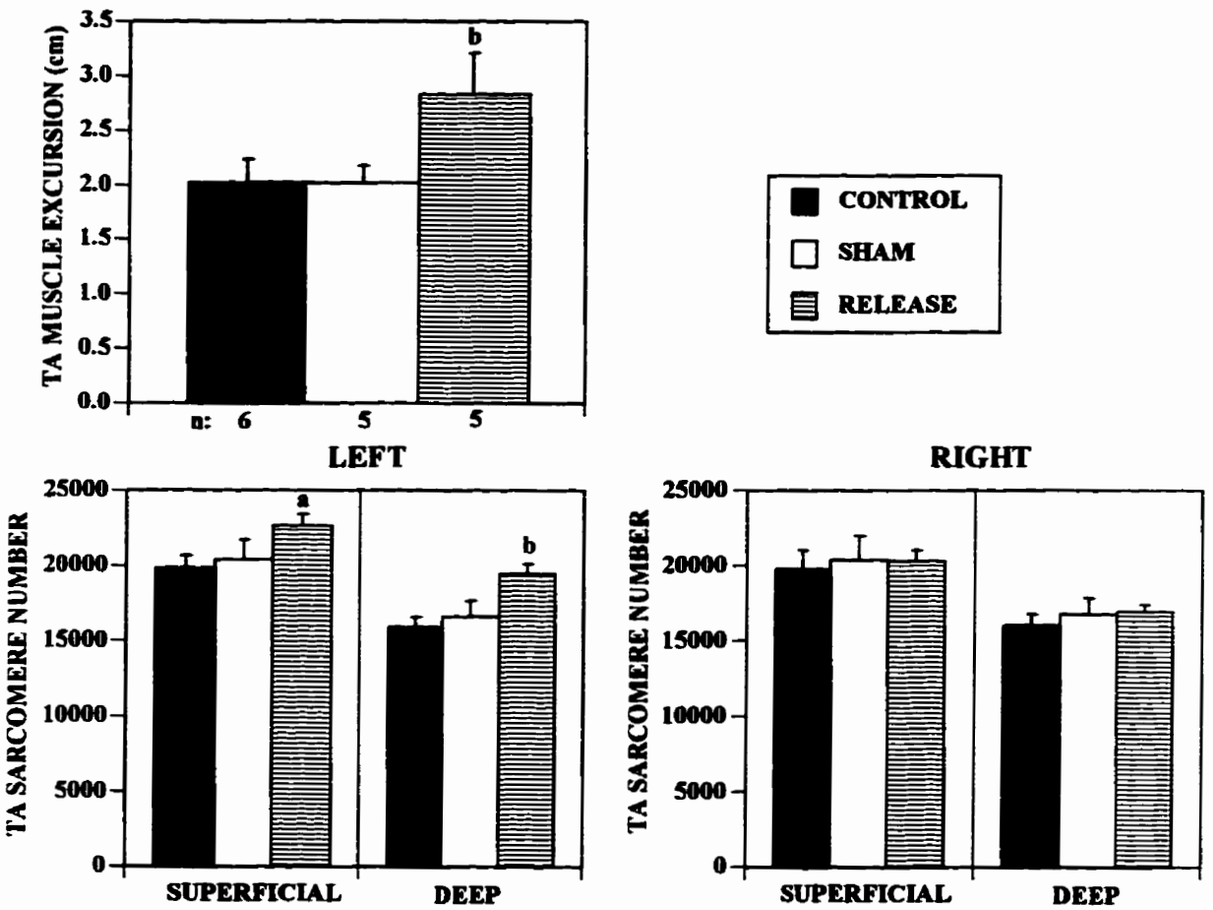

Figure 3.4. Tibialis anterior (TA) muscle excursion and sarcomere number for control, sham-operated and release groups. Muscle excursion measured for the left (experimental) TA, sarcomere number measured for both left and right TAs. Values shown are means with S.D. bars. Muscle excursion data lost for 1 rabbit in sham-operated and release groups because of technical problems with fluoroscopy. For sarcomere number data, n = 6 for each mean value.
[a,b] $p < 0.01$, $p < 0.001$, respectively; release group significantly different from control and sham-operated groups.

Rabbit growth not changed with release. The body mass of the rabbits at sacrifice was not significantly different for control (3.3 $\pm$ 0.4 kg), sham-operated (3.3 $\pm$0.3 kg), and release (3.2 $\pm$ 0.4 kg) groups. Tibia length was also not significantly different between control (10.6 $\pm$ 0.3 cm), sham-operated (11.0 $\pm$ 0.4 cm), and release (10.6 $\pm$ 0.2 cm) groups. Thus the release procedure did not appreciably affect the mass or tibial growth of the rabbits.

Cage activity not changed with release. The average amount of time spent moving in the cage over a 24 hour period was not significantly different between control (39.3 $\pm$ 14.8 minutes), sham-operated (41.0 $\pm$ 14.9 minutes), and release (44.2 $\pm$ 5.7 minutes) groups. Hence the release procedure did not appreciably affect the cage activity levels of the rabbits.

In vivo ankle joint kinematics not changed with release. Parameters of ankle joint kinematics for the swing phase of treadmill hopping showed no significant differences between groups for the experimental (left) leg (Table 3.2). For the contralateral (right) leg, the maximum ankle joint angle achieved during hopping was statistically smaller (by 8%) for the release compared to the control and sham-operated groups. No other parameters of ankle joint kinematics were significantly different between groups for the contralateral leg. Thus the release procedure did not appreciably affect ankle joint kinematics of the experimental leg, and minimally influenced those of the contralateral leg.

To provide an indication of the reliability of measuring ankle joint kinematics from the video record, the entire group of 20 hops for 1 rabbit were digitized twice. The mean values for all parameters of ankle joint kinematics varied by less than 3% between repeated digitizations, suggesting that the digitizing procedures were reliable. To evaluate the influence of the force transducer implant on ankle joint kinematics, treadmill hopping was recorded for three rabbits before and after the implant. The mean values for swing time, minimum angle, and maximum angle varied by an average of 4% between pre- and post-implant measurements, and joint excursion and maximum dorsiflexion velocity varied an average of 7%. The differences observed across rabbits between pre- and post-implant

Table 3.2. Ankle joint kinematics for experimental (left) and contralateral (right) limbs for each group during the swing phase of hopping on a treadmill at 0.3 m/s.

Group	Swing time (s)	Maximum angle (°)	Minimum angle (°)	Excursion (°)	Peak velocity (°/s)
Left leg					
Control	0.15 (0.01)	118 (12)	50 (3)	68 (11)	1210 (85)
Sham	0.15 (0.01)	119 (6)	46 (9)	72 (8)	1262 (200)
Release	0.14 (0.01)	109 (10)	47 (9)	62 (10)	1128 (121)
Right leg					
Control	0.15 (0.02)	122 (8)	54 (5)	68 (10)	1128 (74)
Sham	0.16 (0.01)	120 (4)	50 (11)	70 (7)	1140 (202)
Release	0.14 (0.01)	107 (6)[a]	45 (5)	62 (7)	1139 (106)

[a] release group significantly different from control and sham-operated groups ($p < 0.01$).

NB: Values shown are means and (S.D.). Swing time: duration of swing phase; Maximum angle: maximum included angle between leg and foot during swing phase; Minimum angle: minimum included angle during swing phase; Excursion: difference between maximum and minimum angles; Peak velocity: peak velocity of dorsiflexion during swing phase; n = 6 for both sham-operated and release groups, n = 5 for control group; data from one rabbit in control group discarded because of technical problems.

measurements were not systematic. Thus, the transducer implant did not appreciably affect ankle joint kinematics.

Soleus architecture not changed with release. Neither soleus sarcomere number nor PCSA showed significant differences between groups, for either the experimental or contralateral legs (Figure 3.5). Thus the release procedure did not appreciably affect soleus architecture.

In vivo TA force production decreased with release. For hopping on the treadmill, peak *in vivo* force for the experimental (left) TA of the release group was significantly smaller than that for the control and sham-operated groups (Figure 3.6). The impulse (the integral of force over time during the swing phase) for the released group was also significantly smaller than that for the sham-operated group. For hopping on the walkway, peak force and impulse were significantly smaller for the released group compared to the control and sham-operated groups. For the large force-producing flicking motion, peak force for the released group was significantly smaller than that for the sham-operated group, while impulse was significantly smaller than that for both control and sham-operated groups. *In vivo* force production of the experimental TA of control and sham-operated groups showed no significant differences for any of the activities. *In vivo* force production of the contralateral (right) TA also showed no significant differences between control, sham-operated and release groups for any of the activities. In short, the release procedure resulted in decreased TA force production for a variety of activities requiring both small and large force magnitudes.

To evaluate the possibility that *in vivo* force measurements were influenced by tissues surrounding the tendon force transducer (especially via impingement on the tibia), maximum elicited TA forces were measured for one muscle under two conditions: (1) *in situ* and (2) with the tendon held by a clamp away from the tibia and other tissues. When the muscle was held isometric at identical lengths between conditions (corresponding to those at which peak *in vivo* force usually occurred), this measured maximum elicited force varied by 4% between conditions.

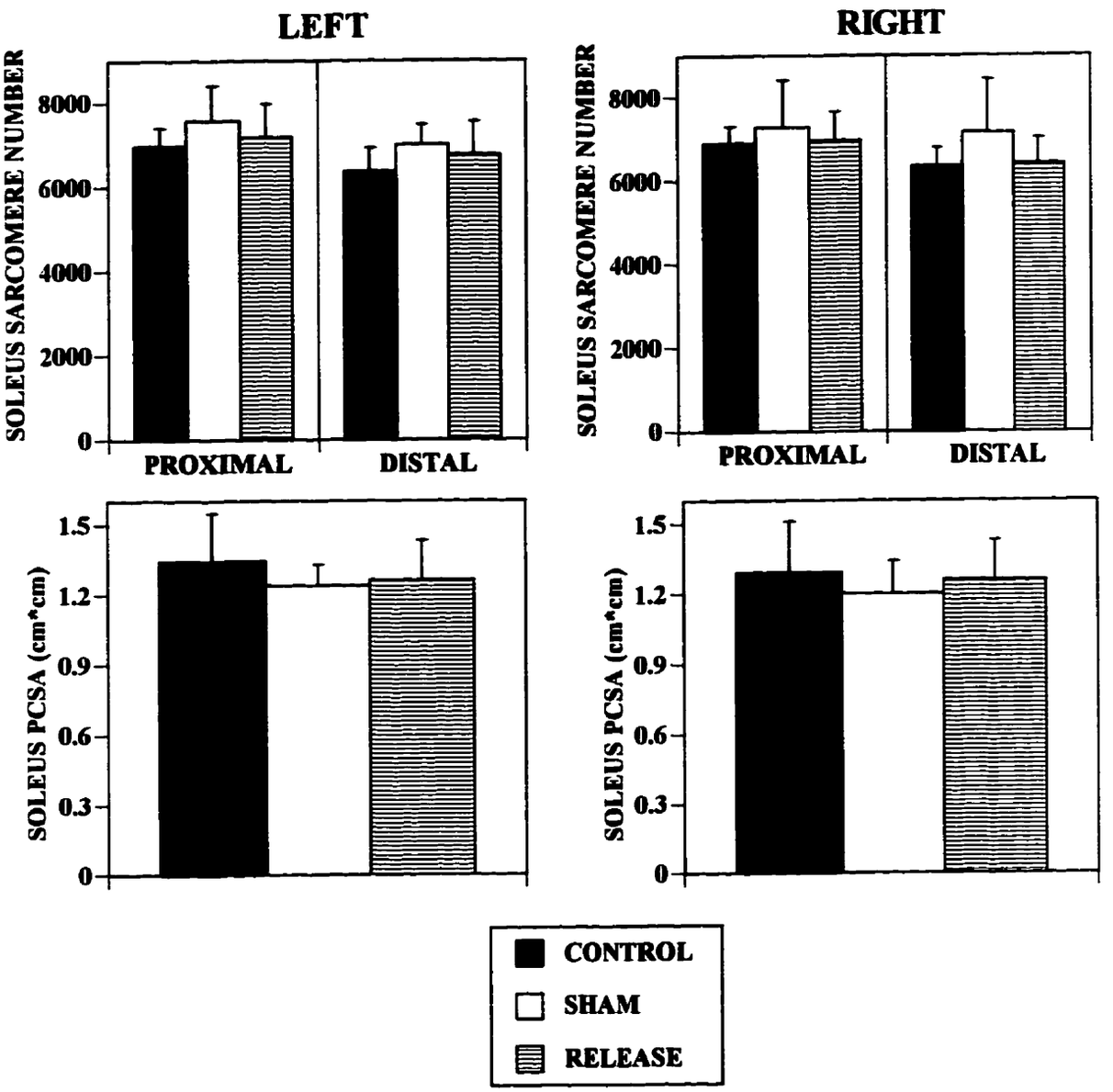

Figure 3.5. Architecture of the left and right soleus muscles for control, sham-operated and release groups. Values shown are means with S.D. bars. n = 6 for each mean value. PCSA: physiological cross-sectional area.

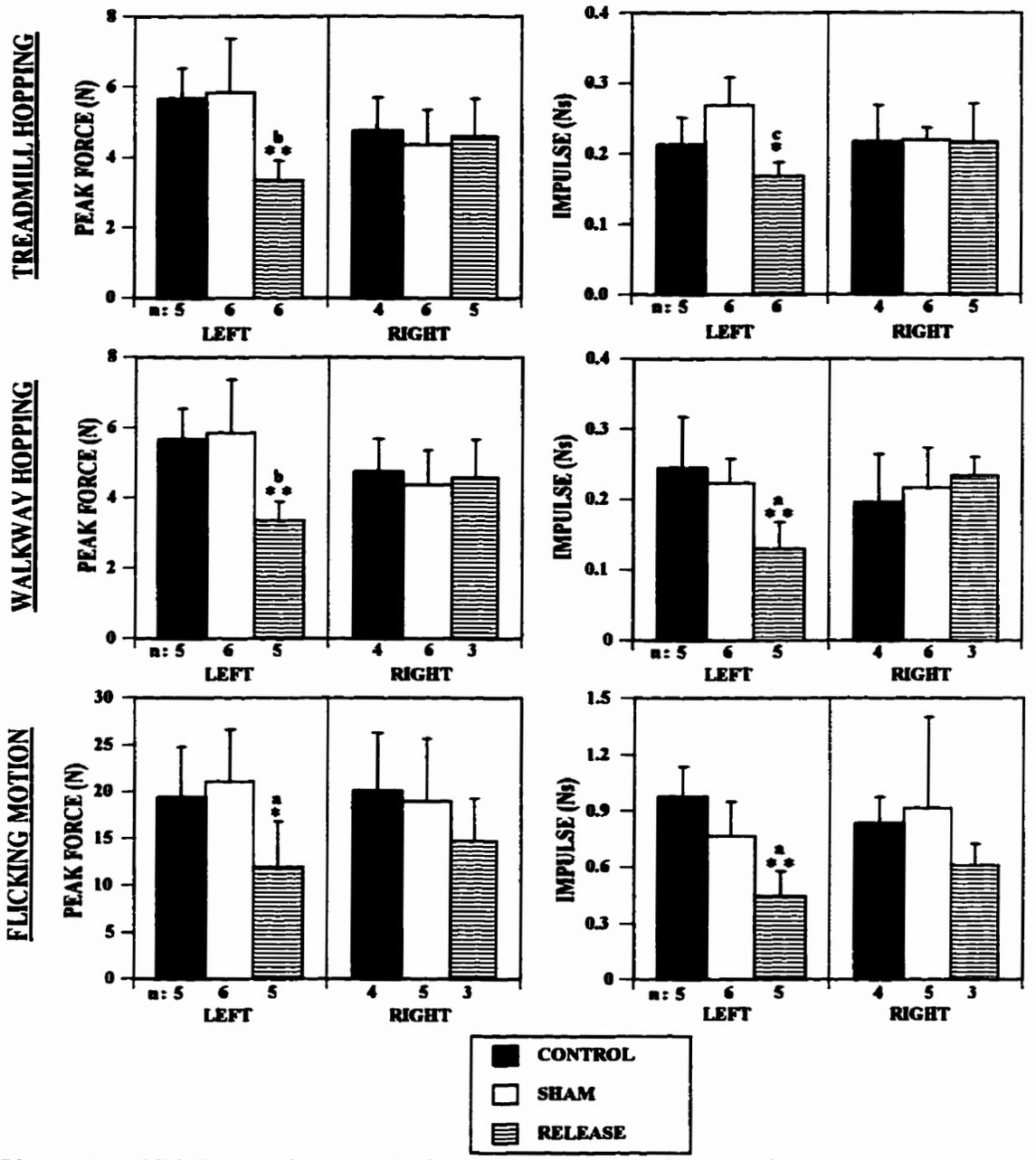

Figure 3.6. Tibialis anterior muscle force production during hopping on a motor-driven treadmill, during hopping on a walkway, and during a large force-producing flicking motion for control, sham, and release groups. Peak force and impulse (integral of force over time) measured for both left (experimental) and right (contralateral) muscles. Values shown are means with S.D. bars. n (shown below each bar) sometimes less than 6 because of technical difficulties with force transducers and recording equipment.

*,** release group significantly different from control group, or control and sham-operated groups, respectively.

[a,b,c] $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively.

In situ TA force production and PCSA decreased with release. The maximum *in situ* isometric force was significantly smaller for the experimental TA of the release group than for that of the control and sham-operated groups (Figure 3.7). Maximum force for control and sham-operated groups was not significantly different. Maximum force of the contralateral TA also showed no significant differences between groups. PCSA for the experimental TA was significantly smaller for the release group than for the control and sham-operated groups. PCSA for the experimental TA was not significantly different between control and sham-operated groups, and PCSA for the contralateral TA showed no significant differences between any groups. The decreased maximum *in situ* isometric force and PCSA for the released TA support the argument that force production was chronically decreased in this muscle.

TA tendon length decreased with release. External tendon length was significantly shorter for the released TA compared with the control and sham-operated TAs (12% and 16%, respectively; Figure 3.8). External tendon length was not significantly different between control and sham-operated TAs. Proximal aponeurosis length was significantly shorter for the released TA compared with the control and sham-operated TAs (21% and 24%, respectively), as was distal aponeurosis length (23% and 25%, respectively), whereas control and sham-operated TA aponeurosis lengths were not significantly different. Thus release of the TA resulted in shorter lengths of all tendon components.

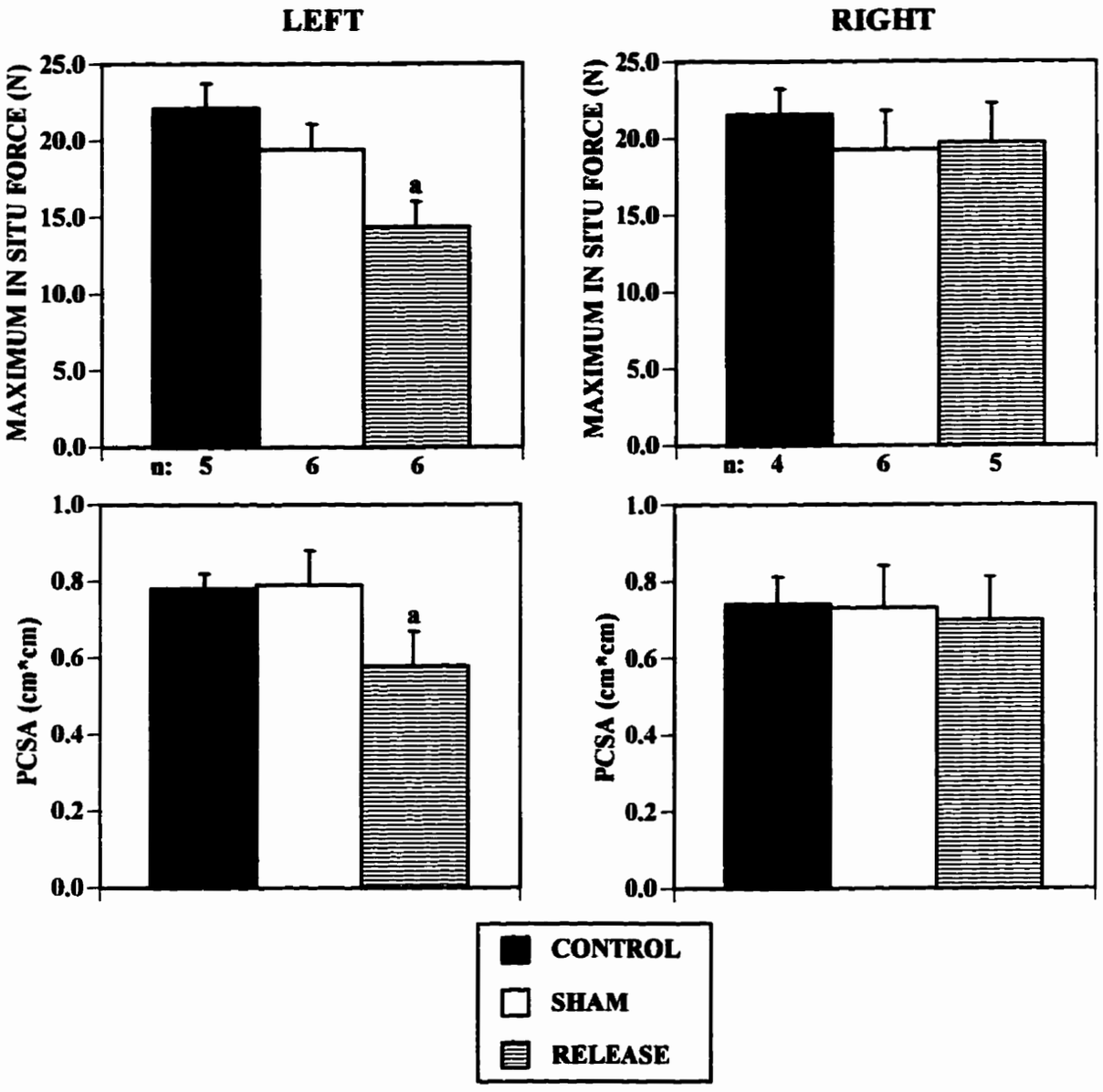

Figure 3.7. Maximum *in situ* isometric force and physiological cross-sectional area (PCSA) for the left (experimental) and right (contralateral) tibialis anterior for control, sham, and release groups. Values shown are means with S.D. bars. For *in situ* force data, n (shown below each bar) sometimes less than 6 because of technical difficulties with force transducers and recording equipment. For PCSA data, n = 6 for each mean value.

[a] $p < 0.001$; release group significantly different from control and sham-operated groups.

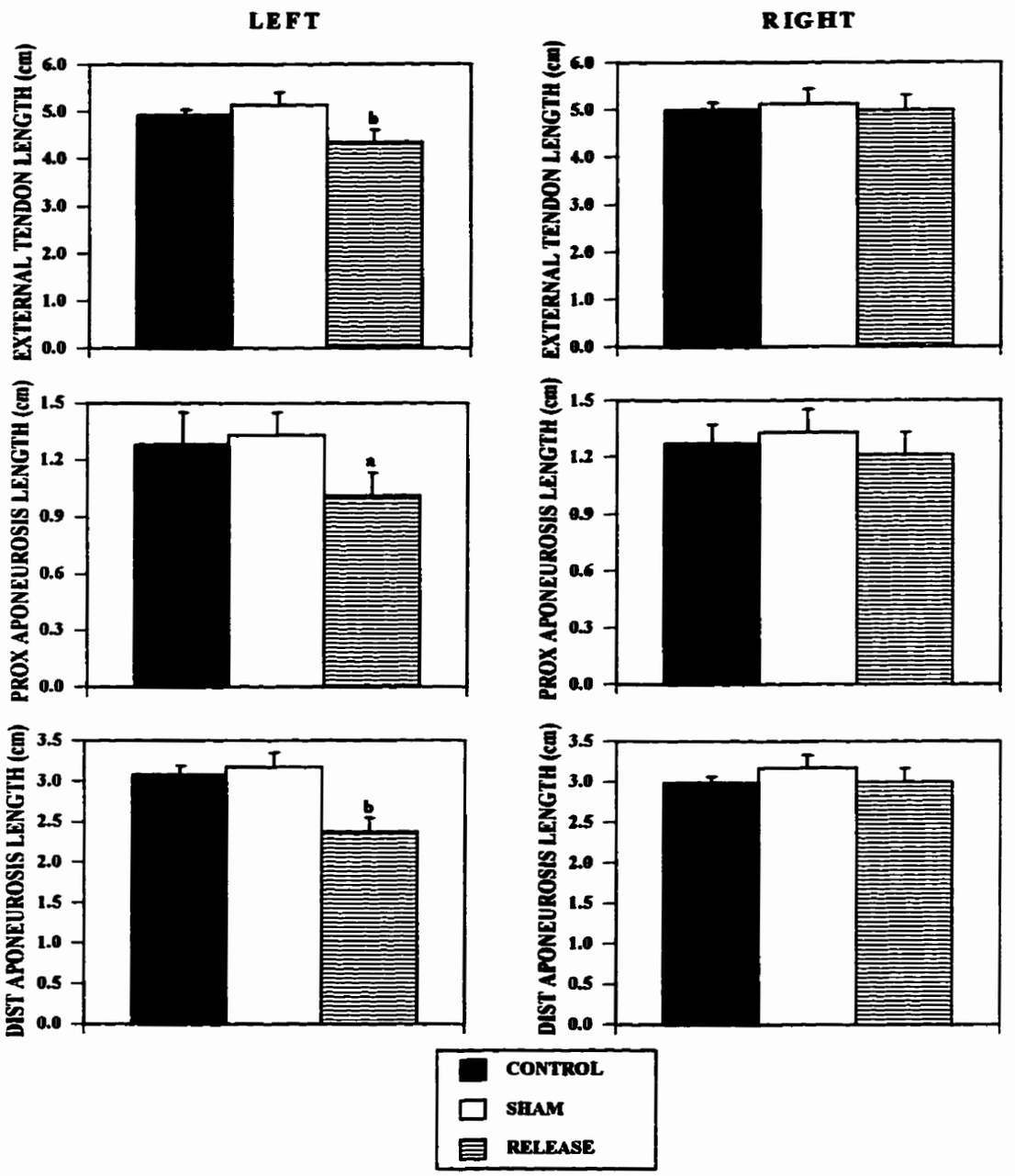

Figure 3.8. External tendon length, proximal aponeurosis length and distal aponeurosis length for control, sham, and release groups. Values shown are means with S.D. bars. n = 6 for each mean value.
[a,b] $p < 0.01$, $p < 0.001$, respectively; release group significantly different from control and sham-operated groups.

In vivo TA force production increased by EDL ablation. The secondary hypothesis, that decreased TA force production results in increased serial sarcomere addition following TA release (via decreased longitudinal tendon growth), was tested by attempting to increase force production of the released TA by partial ablation of the EDL. Peak *in vivo* force production for the experimental TA of the release plus ablation group was significantly greater than that for the release group for hopping on the treadmill, for hopping on the walkway, and for the large force-producing flicking motion (Figure 3.9). Impulse during the swing phase was also greater for the release plus ablation group than for the release group. In short, EDL ablation had the desired effect of increasing force production of the released TA.

TA muscle excursion and sarcomere number not changed by EDL ablation. Muscle excursion of the experimental TA for the release plus EDL ablation group was not significantly different from that for the release only group (Figure 3.10), suggesting that ablation did not affect the increase in muscle excursion associated with release. Sarcomere number was also not significantly different for the experimental TA between release plus ablation and release only groups, for either the superficial or deep regions (Figure 3.10). Thus, increasing the force production of the released TA via EDL ablation did not inhibit the increase in serial sarcomere addition associated with TA release.

TA tendon length not increased by EDL ablation. External tendon length for the experimental TA was significantly shorter ($p < 0.05$) for the release plus EDL ablation group (4.00 ± 0.24 cm) than for the release only group (4.33 ± 0.28 cm). Proximal aponeurosis length (1.00 ± 0.09 cm versus 1.01 ± 0.12 cm) and distal aponeurosis length (2.63 ± 0.33 cm versus 2.37 ± 0.18 cm) were not significantly different between release plus ablation and release only groups, although there was a trend of longer distal aponeurosis length in the release plus ablation group ($p = 0.09$). Thus, longitudinal tendon growth of the released TA was not increased by EDL ablation as predicted by the secondary hypothesis; however, tendon did appear to adapt differently to release plus ablation than to release only.

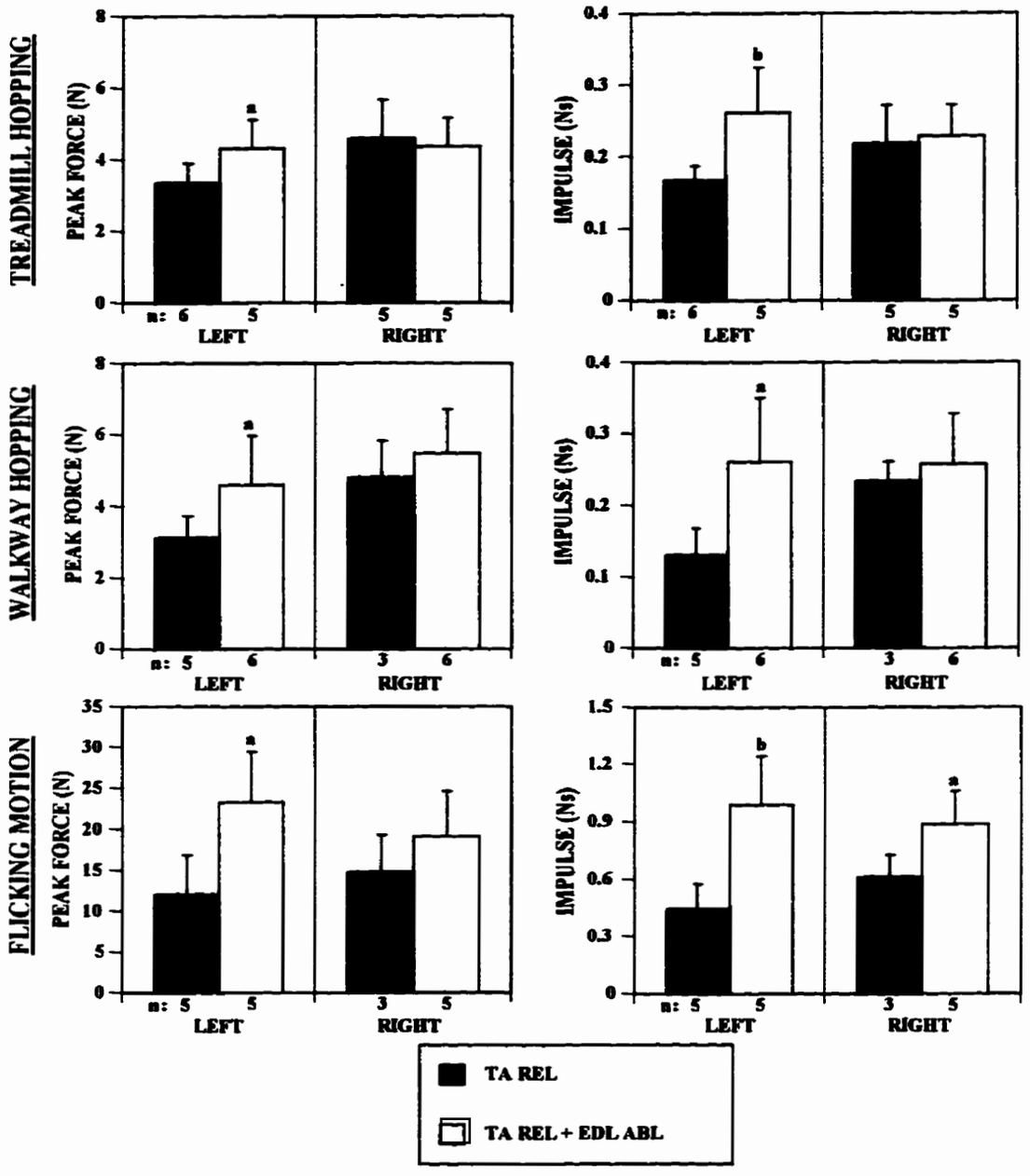

Figure 3.9. Tibialis anterior muscle force production during hopping on a motor-driven treadmill, during hopping on a walkway, and during a large force-producing flicking motion for release (TA REL) and release plus ablation (TA REL + EDL ABL) groups. Peak force and impulse (force x time) were measured for both left (experimental) and right (contralateral) muscles. Values shown are means with S.D. bars. n (shown below each bar) sometimes less than 6 because of technical difficulties with force transducers and recording equipment.
[a,b] $p < 0.05$, $p < 0.01$, respectively; release plus ablation group significantly different from release only group.

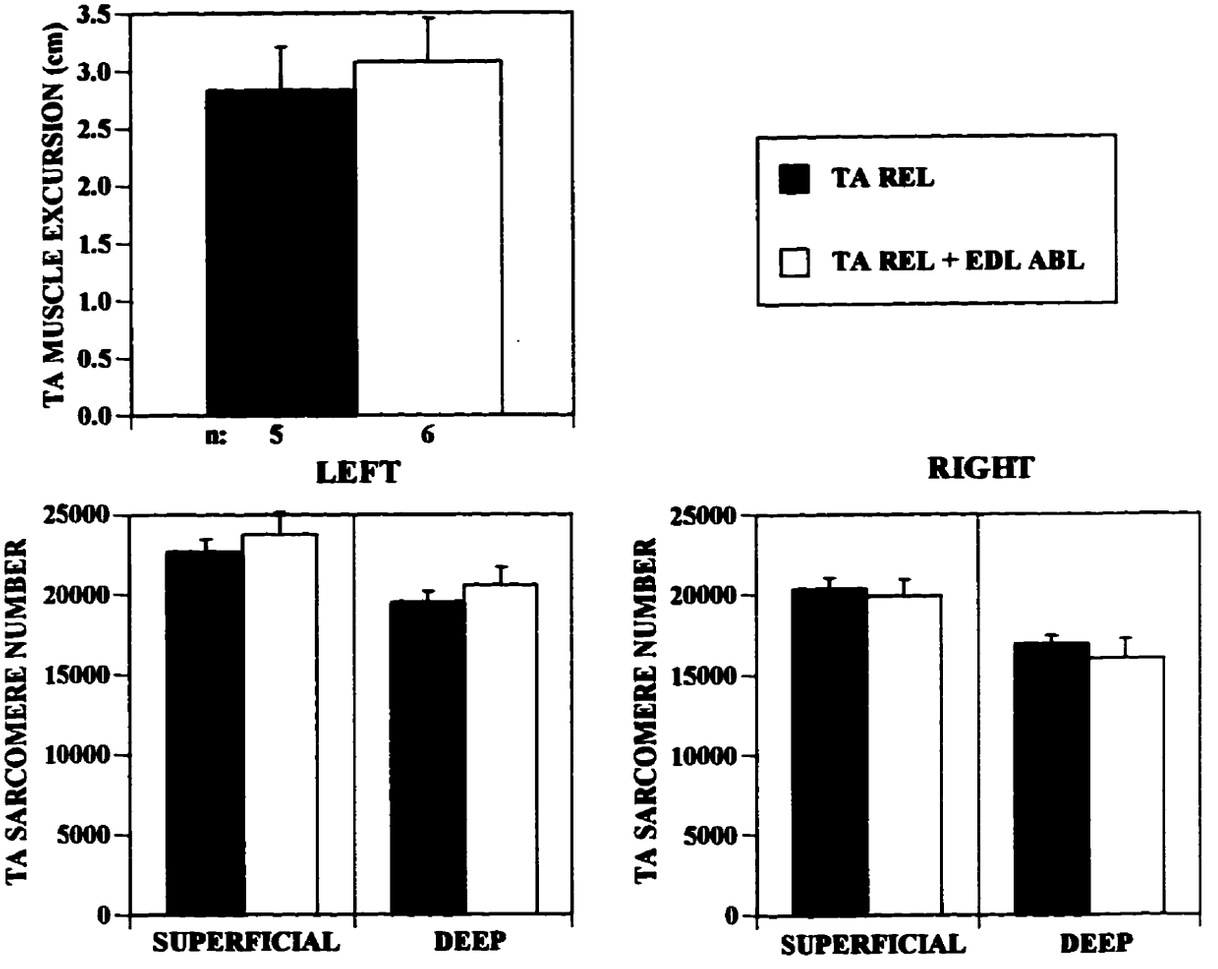

Figure 3.10. Tibialis anterior (TA) muscle excursion and sarcomere number for release (TA REL) and release plus ablation (TA REL + EDL ABL) groups. Values shown are means with S.D. bars. Muscle excursion data was lost for 1 rabbit in release group because of technical problems with fluoroscopy. For sarcomere number data, n = 6 for each mean value.

Rabbit growth and activity not changed by EDL ablation. The mass of the rabbits at sacrifice (3.4 ± 0.3 kg versus 3.2 ± 0.4 kg), tibia length (10.7 ± 0.2 cm versus 10.6 ± 0.2 cm), and cage activity (46.2 ± 7.9 minutes versus 44.2 ± 5.7 minutes) were not significantly different for the release plus ablation group versus the release only group, suggesting that EDL ablation did not appreciably affect the mass or tibial growth, or cage activity levels of the animals.

In vivo ankle joint kinematics changed by EDL ablation. For the experimental leg, swing time (0.15 ± 0.01 seconds versus 0.14 ± 0.01 seconds), the maximum ankle joint angle (122 ± 14 degrees versus 109 ± 10 degrees), ankle joint excursion (55 ± 13 degrees versus 62 ± 10 degrees), and maximum velocity of dorsiflexion (970 ± 197 degrees/second versus 1128 ± 120 degrees/second) for hopping on the treadmill were not significantly different for the release plus ablation versus the release only group. The minimum ankle joint angle during hopping was significantly larger for the release plus ablation group (68 ± 3 degrees) than for the release only group (47 ± 9 degrees). Thus, ablation of the EDL appeared to reduce maximum dorsiflexion of the ankle during the swing phase of treadmill hopping.

Linear relationship between TA muscle excursion and sarcomere number. Plots of average TA sarcomere number (for both superficial and deep fascicles) versus average muscle excursion for each group of this study (plus an EDL ablation only group) showed significant linear relationships (Figure 3.11). This supports the working hypothesis that muscle excursion is important in regulating sarcomere number in these growing rabbits.

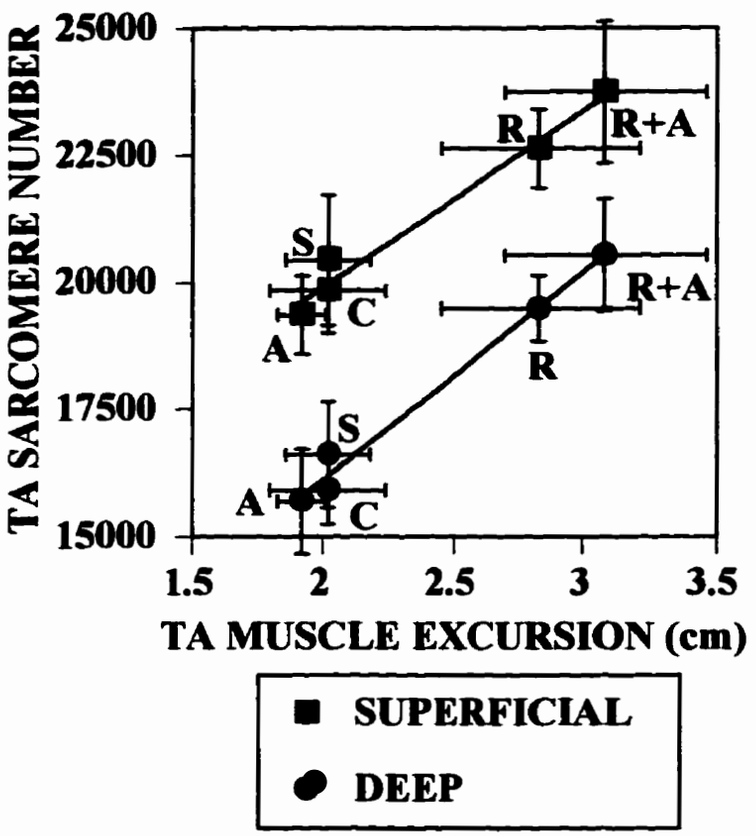

Figure 3.11. Plot of tibialis anterior (TA) sarcomere number (for superficial and deep fascicles) versus TA muscle excursion. Data for control (C), sham-operated(S), release only (R), release plus ablation (R+A), and ablation only (A) groups included (each data point represents mean value with S.D. bars for 6 rabbits). Least-squares linear regression yielded significant ($p < 0.001$) fits for both superficial ($y = 3470x + 12970$; $r^2 = 0.98$) and deep ($y = 4080x + 7960$; $r^2 = 0.99$) fascicles.

Discussion

Previous studies have suggested that muscle excursion may be important in regulating sarcomere number in growing animals (Crawford 1954, 1961, Williams and Goldspink 1971, 1978). However, changes in the *in vivo* mechanical environment of the muscle (excursion, force production, joint kinematics, animal activity) were not measured in these studies; thus this hypothesis could not be evaluated.

Primary hypothesis. The primary hypothesis of this study was that increasing muscle excursion results in increased serial sarcomere addition following TA release in growing animals. TA release resulted in increased muscle excursion, and in increased sarcomere number in both superficial and deep fascicles of the TA. TA release did not appreciably affect rabbit mass nor tibia length at sacrifice, nor cage activity over a 24 hour period. These results suggest that increased serial sarcomere addition in the released TA was not the result of changes in animal growth or activity. In addition, TA release did not appreciably affect ankle joint kinematics of the experimental leg during hopping on a treadmill, suggesting that the increased serial sarcomere addition was not due to a chronic change in ankle joint kinematics. Architecture of the soleus muscle (both sarcomere number and PCSA) is highly sensitive to changes in the mechanical environment (Lieber 1992); the absence of an effect of TA release on soleus architecture provides further evidence that chronic changes in joint kinematics or hindlimb loading were not responsible for the increased serial sarcomere addition in the released TA. Finally, average sarcomere number and average muscle excursion for all of the experimental groups of this study showed a linear relationship. Taken together, these results support the hypothesis that increased muscle excursion results in increased serial sarcomere addition following TA release in growing animals.

The percentage increase in muscle excursion was larger than the percentage increase in sarcomere number for the released TA. This may simply indicate that muscle excursion does not regulate sarcomere number in a one-to-one manner. Another explanation for the discrepancy between the increases in muscle excursion and sarcomere number is that the muscle excursion over which the TA is normally active may not be increased by release as

much as the total TA muscle excursion. However, the estimated TA muscle excursion during force production in treadmill hopping for the release group (1.74 $\pm$ 0.30 cm) was 40% greater than that for the control group (1.24 $\pm$ 0.11 cm). Thus, the muscle excursion during force production in hopping was increased by the same percentage as total muscle excursion.

Two further possible explanations for the greater increase in muscle excursion than in sarcomere number are: (1) that muscle excursion does not accurately reflect excursion at the sarcomere level (the latter is likely more important for mechanical signal transduction in the muscle cell), possibly because of slippage of in-series fibers, and (2) that muscle excursion is not increased as much immediately post-release in 4-week-old rabbits as after 12 weeks of growth. To examine these possibilities, the increase in sarcomere excursion with release was estimated in a separate group of 4-week-old rabbits (following Muhl et al. 1978). For 6 rabbits, the left TA was fixed in formalin in full plantarflexion and the right TA was fixed in full dorsiflexion; 3 rabbits had bilateral TA releases, and the other 3 served as controls. Released muscle excursion (1.59 $\pm$ 0.10 cm) was greater than control muscle excursion (1.23 $\pm$ 0.10 cm) and sarcomere excursion for the released muscle (1.24 $\pm$ 0.03 μm) was greater than that for control muscle (0.98 $\pm$ 0.08 μm). The similar percentage increase in muscle (29%) and sarcomere (27%) excursion with release suggests that muscle excursion reflects sarcomere excursion fairly well, and that in-series fiber slippage does not appreciably affect passive length changes. These measurements in fixed muscles from 4-week-old rabbits showed a smaller percentage increase in muscle excursion with TA release than the fluoroscopic measurements after 12 weeks of growth (29% versus 40%). If these measurements represent a real increase with age in the effect of release on muscle excursion, the stimulus for serial sarcomere addition may not be as great as indicated by the increase in muscle excursion after the 12 week growth period (40%), but would still be greater than the percentage increase in sarcomere number.

Secondary hypothesis. The secondary hypothesis of this study was that decreased TA force production results in increased serial sarcomere addition following TA release. TA release resulted in decreased *in vivo* force production of the TA for a variety of activities

that required both small and large force magnitudes. Decreased maximum *in situ* isometric force and PCSA for the released TA support the argument that force production was chronically decreased in the released TA.

Chronically decreased force production of the released TA may not have provided a sufficient stimulus for normal longitudinal tendon growth, as external tendon, proximal aponeurosis, and distal aponeurosis lengths were shorter than control. A link between tension and longitudinal tendon growth is supported by previous observations that longitudinal tendon growth is increased when growing muscle is immobilized in a stretched position, and that this increase can be attenuated by denervating the immobilized muscle (Blanchard et al. 1985). In addition, decreased tendon growth has been associated with surgical shortening of bone; a chronic decrease in tension may be important in this model as well (Tardieu et al. 1983). Decreased longitudinal tendon growth would chronically increase the length of the released TA. Since chronically increased muscle length has been shown to be a stimulus for sarcomere number addition (Tabary et al. 1972, Tardieu et al. 1977), this sequence of events could be responsible for the increase in serial sarcomere addition observed.

Although partial ablation of the EDL increased *in vivo* force production in the released TA in the present study, increasing the force production of the released TA did not inhibit the increased growth in sarcomere number associated with release. Thus the secondary hypothesis that decreased TA force production results in increased serial sarcomere addition was not supported by these results.

The increased minimum angle of the ankle joint during hopping for the release plus ablation group compared to the release only group may have been due to a decreased ability to dorsiflex the foot when EDL force production is removed. The increased force production of the TA may not have been enough to compensate for EDL ablation (the contribution of the EDL to dorsiflexion is unknown, as EDL forces were not measured). However, the change in kinematics appeared not to affect TA serial sarcomere addition, as TA sarcomere number was not different for release plus ablation and release only groups.

Partial ablation of the EDL did not increase longitudinal tendon growth as predicted by the secondary hypothesis. Ablation did appear to decrease external tendon length (by 3 mm) and showed a trend of increasing distal aponeurosis length (by 3 mm). Thus, ablation seemed to alter the relative distribution of internal and external tendon lengths. In summary, upon examination of all the groups of this study, no clear relationship between TA force production and longitudinal tendon growth could be discerned.

Other considerations. The series-fibered architecture of the TA provides interesting possibilities for increasing sarcomere number in fascicles spanning the distance between proximal and distal aponeuroses. Fascicle sarcomere number could be increased by (1) sliding of fibers past each other without sarcomere addition, (2) addition of sarcomeres only to the ends of the fibers at the proximal and distal aponeurosis without fiber sliding, or (3) addition of sarcomeres to all fibers with concomitant sliding of fibers past each other. These possibilities remain unexplored and would be intriguing issues for future study.

Satellite cell proliferation and fusion may be important in adaptive longitudinal growth of muscle fascicles. Moss and Leblond (1971) have demonstrated that satellite cells are the source of myonuclei during growth. Williams and Goldspink (1971) presented electron microscopic evidence for satellite cell fusion at the end of growing muscle fibers. Cyclic stretch of muscle cells has been shown to release insulin-like growth factor 1 (IGF-1; Perrone et al. 1995), which, in turn, stimulates proliferation and differentiation of satellite cells (Allen and Rankin 1990). Thus, increased muscle excursion following release could increase the rate of proliferation and differentiation of satellite cells, which could be involved in increased serial sarcomere addition.

Cyclic strain (which could be considered excursion) has been shown to increase DNA, RNA and protein synthesis and accumulation in cultured cells of different types (e.g. muscle: Vandenburgh et al. 1989, tendon: Banes et al. 1995, endothelial: Awolesi et al. 1995). For muscle cells, cyclic strain increases the growth in length and diameter of cultured myotubes (Vandenburgh et al. 1989); however, the influence of cyclic strain on sarcomere number in myotubes has not been investigated. Although controlled comparisons of static versus cyclic strain appear to not have been made for muscle cells, cyclic strain

appears to be better than static strain for preventing bone loss and producing new bone formation *in vivo* (Lanyon and Rubin 1984).

The mechanisms by which muscle transduces information from cyclic strain into changes in protein synthesis and accumulation have not been elucidated. This transduction mechanism could involve stretch-activated or -inactivated ion channels (Morris and Sigurdson 1989, Vandenburgh and Kaufman 1981), mechanically-induced changes in the integrin-associated cytoskeleton (Ingber 1991), and/or mechanically-induced release of growth factors (e.g. IGF-1; Perrone et al. 1995), which could work in an autocrine/paracrine manner. A putative mechanotransducer for sarcomere addition ideally would (1) be located at sites of sarcomere addition (e.g. muscle-tendon junction; Williams and Goldspink, 1971), (2) be responsive to mechanical signals deemed important for sarcomere addition (e.g. excursion), (3) stimulate release of terminal sarcomeres from the sarcolemma to allow sarcomere addition (Epstein and Fischman, 1991), and (4) stimulate protein synthesis needed for sarcomere addition.

The interpretation of the data for this study is limited by the lack of information about the mechanical environment at time points other than immediately prior to sacrifice. The possibility exists that differences in the mechanical environment between groups changed over the growth period. For example, muscle excursion was increased less in the measurements immediately after release (29%) than in the measurements immediately prior to sacrifice (40%). However, in both measurements muscle excursion was significantly increased, and the difference between measurements does not substantially affect the interpretation of the data. In addition, visual observation of the rabbits suggested that joint kinematics did not change appreciably over time (each rabbit was observed at least once per week). The observation that changes in cross-sectional area of the muscle, as well as the maximum *in situ* isometric muscle force, paralleled changes in *in vivo* force production support the argument that force production was chronically decreased in the released TA. Hence the possibility that a change in the mechanical environment over time would affect the interpretation of the results is considered unlikely.

The interpretation of the data for this study is also limited by the lack of knowledge of unmeasured factors that could influence sarcomere number regulation. For example, the release surgical procedure could have produced unknown systemic factors that may have influenced serial sarcomere addition. The age-matched control and sham-operated animals should control for the effects of growth and the surgical procedures (apart from TA release). However, the results may have been influenced by unknown interactions between muscle growth and TA release. Nonetheless, the results are pertinent to understanding regulation of sarcomere number in growing animals.

In conclusion, the results of this study support the working hypothesis that muscle excursion is important in regulating sarcomere number following TA release in growing animals. Characterization of the *in vivo* mechanical environment of the TA allowed direct correlation of muscle excursion and sarcomere number, and allowed exclusion of altered cage activity, ankle joint kinematics and TA force production as possible factors contributing to altered serial sarcomere addition. The cellular mechanisms that regulate sarcomere number remain an exciting area for future study.

CHAPTER 4:

Increasing the Moment Arm of the Tibialis Anterior Induces Structural and Functional Adaptation: Implications for Tendon Transfer

Introduction

Tendon transfer is performed to restore joint function in patients with peripheral nerve damage, spinal cord injury, brain damage, and neuromuscular disease (e.g. cerebral palsy). Recent studies have emphasized the importance of muscle architecture when selecting donor muscles (Lieber et al. 1992, Zajac 1992). For example, physiological cross-sectional area (PCSA) strongly influences the maximum isometric force-producing capability of muscle (Powell et al. 1984). In addition, the number of sarcomeres in series in muscle fibers or fascicles (sarcomere number) influences muscle force-length and force-velocity properties (Sacks and Roy 1982, Williams and Goldspink 1978). Mathematical models of tendon transfer have also shown that muscle architecture is an important determinant of the functional outcome of the procedure (Giat et al. 1994, Loren et al. 1995).

Tendon transfer likely alters the mechanical environment of the transferred muscle. Although few experimental data exist on the topic, Lieber et al. (1996) showed that, for transfer of the flexor carpi ulnaris into the tendons of the extensor digitorum communis, sarcomere excursion (the change in sarcomere length required to produce the full joint range of motion) is increased by 40%. The increased sarcomere excursion resulted from an increased moment arm of the transferred muscle. Transfer of the tibialis posterior into the tendon of the tibialis anterior (Carayon et al. 1967) likely also increases the moment arm and excursion of the transferred muscle, as the moment arm and excursion of the tibialis anterior are larger than those of the tibialis posterior (Spoor et al. 1990). In addition, *in vivo* force production of transferred muscles may be altered, as the function of the muscle is altered.

However, a review of the literature revealed no measurements of *in vivo* force production of transferred muscles during normal activities.

Muscle architecture adapts to an altered mechanical environment. Sarcomere number adapts in response to bone lengthening (Simpson et al. 1995), tendon lengthening (Tardieu et al. 1979), and immobilization (Williams and Goldspink 1978). Mechanical factors are likely important in these perturbations; excursion and/or tension are thought to be important in regulating sarcomere number (cf. Chapter 3, Goldspink 1985, Herring et al. 1984). In addition, increased or decreased loading produces muscle hypertrophy or atrophy, respectively, (Booth and Thomason 1991, Roy et al. 1991). Although the potential for post-transfer muscle adaptation has been recognized (Lieber et al. 1996), a review of the literature revealed no experimental data on the subject. This is surprising because of the well known capability of muscle to adapt. Post-transfer adaptation would affect the outcome of tendon transfer procedures by changing the passive and active properties of the transferred muscle.

An experimental model in which tendon path is altered without actual transfer of the tendon should provide insight into the capability of muscle to adapt without the complications associated with transferring the tendon (e.g. tendon transection, healing, inflammation, scar formation). Releasing the tibialis anterior (TA) from its retinacular restraint at the ankle joint was presumed by Crawford (1954, 1961) to change the path of the TA tendon and increase its moment arm (Figure 4.1). An increased TA moment arm would increase ankle joint torque production, and alter joint kinematics, if the force production of either the TA or the extensor digitorum longus (EDL; the other major ankle dorsiflexor) were not decreased. Four months after TA release had been performed on growing rabbits, the maximum isometric force-length relation of the released TA, measured *in situ*, showed a smaller peak force and a wider plateau than the contralateral TA (Crawford 1961). These results are consistent with a smaller PCSA and larger sarcomere number in the released TA than in the contralateral TA. These results suggest that *in vivo* released TA force production could be chronically decreased in a manner that compensates for the increased moment arm, and thus achieves near normal ankle joint torque.

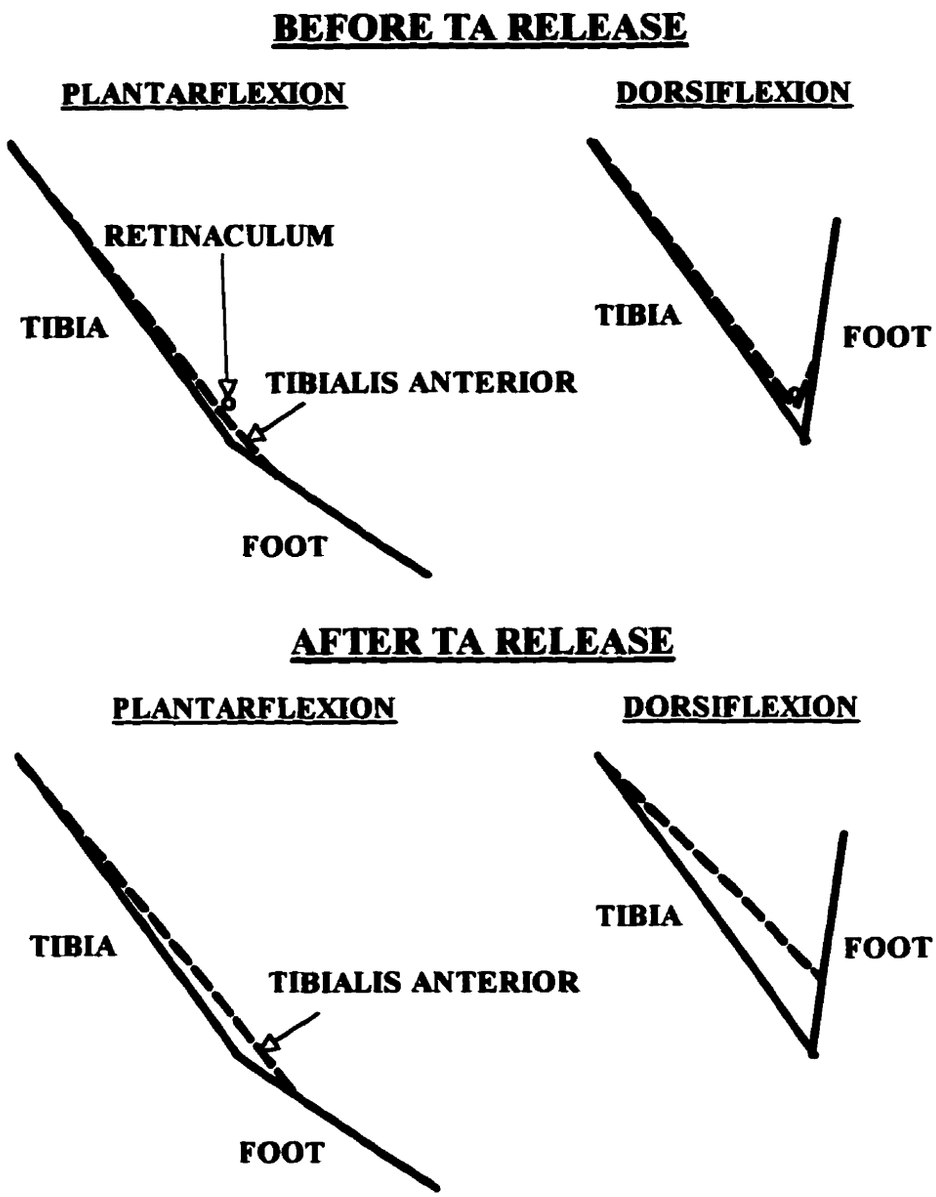

Figure 4.1. Releasing the TA from its retinacular restraint increases TA moment arm about the ankle joint. The retinaculum normally constrains the TA to follow a path close to the tibia and foot (before release). When the retinaculum is surgically removed, the TA is allowed to move away from the tibia, or "bowstring", thus increasing TA moment arm (after release; the illustration exaggerates the effect of release because fascia and skin act to restrain the TA). TA moment arm is increased more in dorsiflexed positions than in plantarflexed positions because of the geometry of the system.

The purpose of this study was to test the working hypothesis that the TA adapts following release in a manner that helps to achieve near normal TA torque at the ankle joint. The working hypothesis was divided into two specific hypotheses: (1) *in vivo* TA force is smaller in released compared with control TAs such that TA torque at the ankle joint is not significantly different between control and release rabbits and (2) differences in TA architecture between control and released TAs following release are consistent with decreased force production in released compared with control TAs. These hypotheses were tested by measuring *in vivo* force and torque production during hopping, *in situ* maximum isometric force and torque during elicited contractions, and architectural parameters (PCSA and sarcomere number), for control and released TAs. This appears to be the first published report of structural and functional muscle adaptation in a model of tendon transfer in growing animals.

Methods

Animals. Female New Zealand White rabbits (4-week-old) were obtained from a single supplier (Vandermeer, Edmonton). Twelve rabbits each were placed into control, sham-operated, and TA release groups. All procedures associated with this study followed the guidelines of the Canadian Council on Animal Care.

Sham and TA release procedures. Animals undergoing sham and TA release procedures were anesthetized with halothane. Surgeries were performed under strictly sterile conditions. For animals in the release group, the proximal extensor retinaculum was removed, and the fascia overlying the TA muscle belly and tendon was divided 1 cm proximal and distal to the ankle (Crawford 1954). For sham-operated animals, identical procedures were used, except the retinaculum was not removed.

In vivo TA muscle force. *In vivo* TA muscle force production was measured for 6 rabbits in each group 11-12 weeks post-surgery. Muscle force was measured using custom made E-shaped tendon force transducers (Herzog et al. 1993). Under strictly sterile conditions and halothane anesthesia, transducers were implanted on the left and right TA. The transducer leads were passed subcutaneously to the back of the animal where they were sutured to fascia for strain relief.

Muscle forces were recorded 5-7 days after the transducers were implanted. The animal was briefly anesthetized with isoflurane (< 1 minute), the leads were exteriorized and linked by a cable to a strain gage amplifier (Intertechnology, 2100 System). After the animal recovered from anesthesia, muscle force was recorded at 500 Hz via microcomputer while the rabbit was hopping on a motor-driven treadmill at 0.3 m/s. Force data were low-pass filtered at 50 Hz (2nd-order recursive Butterworth filter), and peak force and force impulse (the integral of force over time) for each hop were determined. Peak force and impulse were averaged over 20 hops to provide representative values for each rabbit.

In vivo ankle joint kinematics. Bilateral saggital plane ankle joint kinematics were also measured for the rabbits during treadmill hopping. Two high-speed (60 Hz) video cameras (Motion Analysis) were used to record rabbits hopping on the treadmill; one camera each was focused on the lateral surface of the left and right legs. The 20 hops selected for

TA force analysis (cf. previous paragraph) were also used for kinematic analysis. Video data were synchronized with force data using a time code generator (Datum, Model 9550).

The swing phase was identified for each hop (separately for each leg). Every frame showing the swing phase, plus 10 frames prior to, and 10 frames after, the swing phase, were analyzed. In each frame, two reflective markers placed on the lateral side of each foot (one at the lateral malleolus, one at the metatarsal-phalangeal joint), and two points on the anterior surface of the leg (one near the knee and one near the ankle) were digitized manually (Motion Analysis, Manual Input System). These points were selected to minimize artifact from soft tissue movement and they defined the long axes of the foot and leg. Ankle joint angles were defined as the included angle between the long axes of the leg and foot, and these angles were low-pass filtered (2nd-order recursive Butterworth) at 10 Hz. For each hop, the duration of the swing phase (swing time), the maximum and minimum angles achieved in each hop (typically at takeoff and mid-swing), the difference between these values (joint excursion) and the maximum velocity of dorsiflexion were calculated. Each parameter was averaged over the 20 hops to provide representative values for each rabbit.

In situ TA muscle force. After the *in vivo* force and kinematic measurements, maximum *in situ* TA muscle force was assessed. Each animal was anesthetized with isoflurane, and nerve cuff stimulating electrodes were implanted on the left and right common peroneal nerves. The animal was then placed in a device designed to secure the lower limb with the knee joint fixed at 90 degrees. The foot was strapped to a footplate, which could be fixed at any desired ankle joint angle. The nerve cuff was connected to an isolated stimulator (Grass, Model S88) and supramaximal stimulation intensity was determined (pulse duration = 0.1 ms, frequency = 150 Hz, train duration = 400 ms, intensity = 1-2 Volts). Peak developed isometric TA force was measured using the tendon force transducer for ankle joint angles from 60 to 160 degrees in 20 degree increments (full plantarflexion = 160 degrees). Following this protocol, the animal was euthanized with an overdose of pentobarbital, and tendon force transducers were immediately calibrated by hanging a series of known weights on the tendon.

TA excursion, moment arm, and torque production. TA muscle excursion and moment arm were determined for the left leg of the remaining 6 rabbits in each group at 12 weeks post-surgery. Each rabbit was anesthetized with isoflurane, and a nerve stimulating cuff was implanted on the left common peroneal nerve. Radiopaque wire markers were implanted (1) at the tibial tubercle and (2) on the TA tendon near the muscle-tendon junction. The rabbit was placed on its side on an x-ray table. A fluoroscope was focused on the left leg, and the displacement of the markers was recorded on fluoroscopic video during three trials of dorsiflexion. The foot was first held manually in a fully plantarflexed position, the TA was submaximally stimulated (pulse duration = 0.1 ms, frequency = 40 Hz, train duration = 2 seconds, intensity = 1-2 Volts), the foot was allowed to dorsiflex slowly, and the movement of the markers was recorded. A scale was placed in the field of view of the fluoroscope, and in the plane of the leg, to calibrate the distances measured on the fluoroscopy images.

For each trial, every frame of the fluoroscopy video record from the onset of stimulation with the foot plantarflexed until the foot reached full dorsiflexion was analyzed. Eight points were manually digitized in each frame: the two ends of the scale, the two wire markers, two points defining the long axis of the tibia and two points defining the long axis of the foot. The ankle joint angle and the distance between the wire markers were calculated for each frame. Data were then combined over the three trials. For each muscle, stepwise least-squares polynomial regression was used to fit the data. The moment arm of the TA was then calculated by differentiating the resulting polynomial equation.

In vivo and *in situ* TA torque production were estimated by multiplying force production for a given animal by the appropriate moment arm. Moment arm was calculated by linear interpolation from averaged moment arm-joint angle relations for the appropriate group.

TA muscle architecture. After measurement of TA moment arm, rabbits were euthanized by an overdose of pentobarbital. Both lower limbs were removed, skinned, and immersed and stored (>1 month) in 10% neutral buffered formalin with the TA left attached to the bones and the ankle joint positioned at 90°.

After fixation, each TA was carefully isolated, and the mass of the muscle was measured. The muscle belly was divided into thirds with longitudinal cuts. The central third of each muscle was then placed in 30% HNO_3 (Loeb and Gans 1986) for approximately 10 hours to allow teasing of small fascicles of fibers (approximately 20 fibers in diameter) that spanned the distance between proximal and distal aponeuroses of the muscle. Twelve fascicles were teased from each muscle (6 each from the superficial and deep surfaces of the muscle). Fascicles were mounted in glycerol jelly on microscope slides, and fascicle length was measured using a video analysis system (Media Cybernetics, ImagePro Plus). Sarcomere length was measured at five points along the length of each fascicle using a laser diffraction system (beam diameter = 0.8 mm; Allinger, 1995). Sarcomere number for each fascicle was calculated by dividing the fascicle length by the average sarcomere length for the fascicle. Sarcomere number was averaged across fascicles to provide a representative value for superficial and deep fascicles for each muscle.

Muscle physiological cross-sectional area (PCSA) was estimated from the equation: PCSA (cm^2) = muscle mass (g) / fiber length(cm) * muscle density(1.0564 g/cm^3; Mendez and Keys 1960), where fiber length was calculated by multiplying sarcomere number (averaged over superficial and deep regions for each muscle) by 2.2 μm (Lieber and Blevins 1989). Muscle mass was corrected for the change in mass due to fixation (measured by comparing muscle mass before and after fixation in pilot studies; cf. Table 3.1) before calculating cross-sectional area.

Identical architectural measurements were made for each EDL to determine whether TA release produced adaptation in EDL architecture.

Data analysis. Mean values were compared between control, sham-operated, and release groups using one-way analysis of variance (ANOVA). The Scheffe test was used for post-hoc comparisons. For all statistical tests, the 0.05 level was taken to indicate statistical significance.

Results

For the full range of ankle joint motion (40 to 160 degrees), excursion of the released TA (2.83 $\pm$ 0.38 cm) was increased compared to control (2.02 $\pm$ 0.22 cm) and sham (2.02 $\pm$ 0.16 cm) TAs. Moment arm for the released TA was increased compared to control and sham TAs at dorsiflexed angles, but not at plantarflexed angles (Figure 4.2).

Peak *in vivo* TA force during hopping was significantly smaller for the released TA compared to the control and sham TAs (Figures 4.3 and 4.4). Force impulse for the released TA was also significantly smaller compared to the sham group. Peak *in vivo* TA torque and torque impulse were not significantly different for control, sham and released TAs. Parameters of ankle joint kinematics also did not show significant differences between control, sham, and release groups (Table 4.1).

The *in situ* maximum isometric force-ankle joint angle relation for the released TA showed smaller forces at all joint angles than those for the control and sham TAs (Figure 4.5). The calculated isometric torque-ankle joint angle relation for the released TA was similar to those of the control and sham TAs, especially for the joint angles used during hopping (60-120 degrees).

PCSA was significantly smaller in released compared with control and sham TAs (Figure 4.6). In addition, sarcomere number was significantly larger in released compared with control and sham TAs. These architectural changes were associated with smaller mass of the released TA compared with the sham TA, but not compared with the control TA.

Specific tension, calculated by dividing the maximum isometric force at optimal length (averaged over animals for each group) by PCSA (averaged over animals for each group), was 28 N/cm^2 for the control TA, 25 N/cm^2 for the sham TA, and 25 N/cm^2 for the released TA.

EDL architecture (PCSA, sarcomere number) was not significantly different between release, control and sham groups (Figure 4.7).

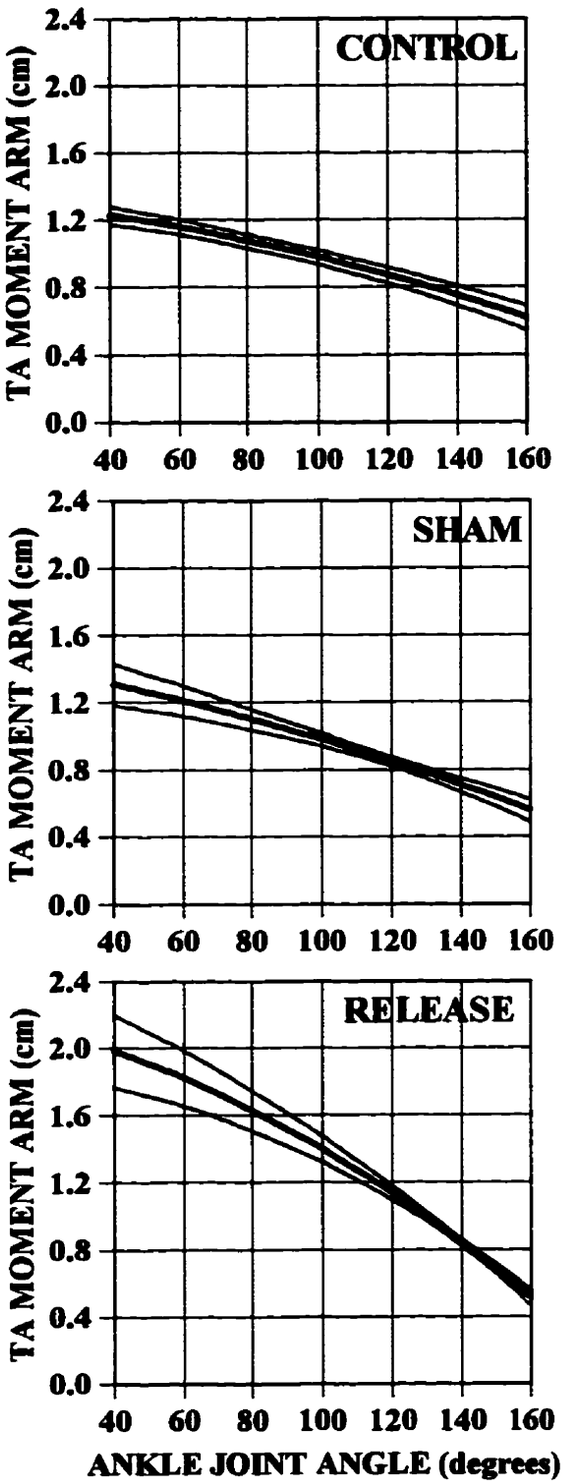

Figure 4.2. TA moment arm-ankle joint angle relations for control (n = 6), sham-operated (n = 5), and release groups (n = 5). Thick line represents ensemble average for each group, thin lines represent ± S.D. n less than 6 for sham and release groups because of technical problems with fluoroscopy.

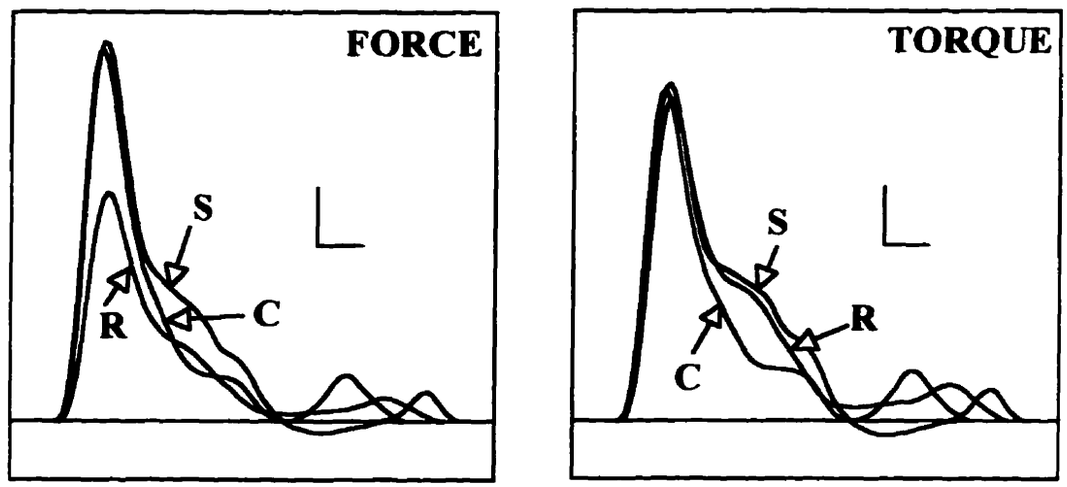

Figure 4.3. Representative raw data for *in vivo* force and torque for one hop each of control (C), sham (S), and release (R) rabbits. Vertical scale represents 1 N and 1 N-cm, for force and torque, respectively. Horizontal scale represents 0.02 seconds.

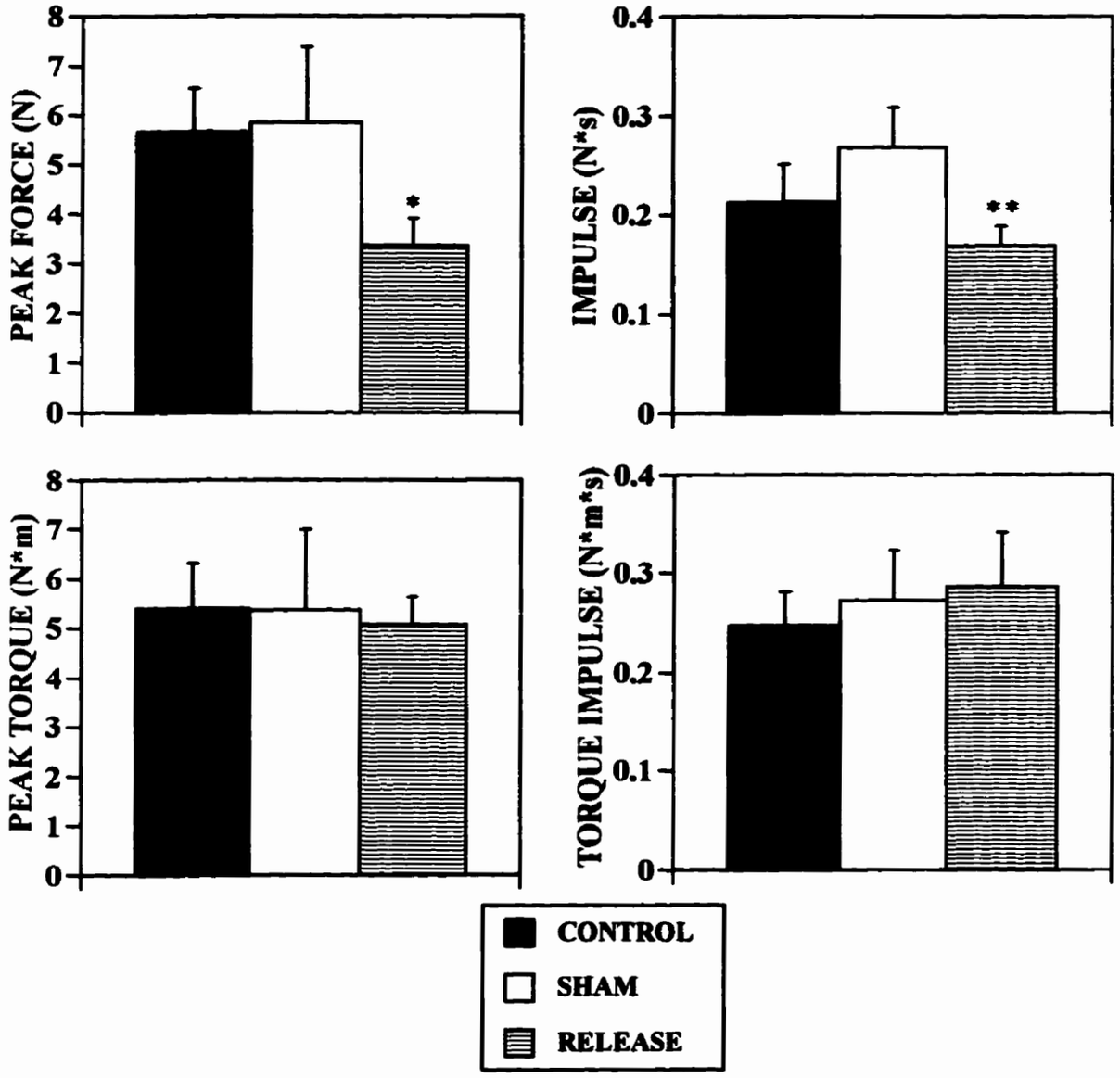

Figure 4.4. *In vivo* force and torque production during hopping for control (n = 5), sham (n = 6), and release (n = 6) groups. Values shown are means with S.D. bars. n less than 6 for control group because of technical problems with a force transducer.
* $p < 0.01$; release group significantly different from control and sham groups.
** $p < 0.001$; release group significantly different from sham group.

Table 4.1. Ankle joint kinematics for the experimental (left) limb during the swing phase of hopping on a treadmill.

Group	Swing time (s)	Maximum angle (°)	Minimum angle (°)	Excursion (°)	Peak velocity (°/s)
Control	0.15 (0.01)	118 (12)	50 (3)	68 (11)	1210 (85)
Sham	0.15 (0.01)	119 (6)	46 (9)	72 (8)	1262 (200)
Release	0.14 (0.01)	109 (10)	47 (9)	62 (10)	1128 (121)

NB: Values shown are means and (S.D.). Swing time: duration of swing phase; Maximum angle: maximum included angle between leg and foot during swing phase; Minimum angle: minimum included angle during swing phase; Excursion: difference between maximum and minimum angles; Peak velocity: peak velocity of dorsiflexion during swing phase; n = 6 for both sham-operated and release groups, n = 5 for control group; data from one rabbit in control group discarded because of technical problems.

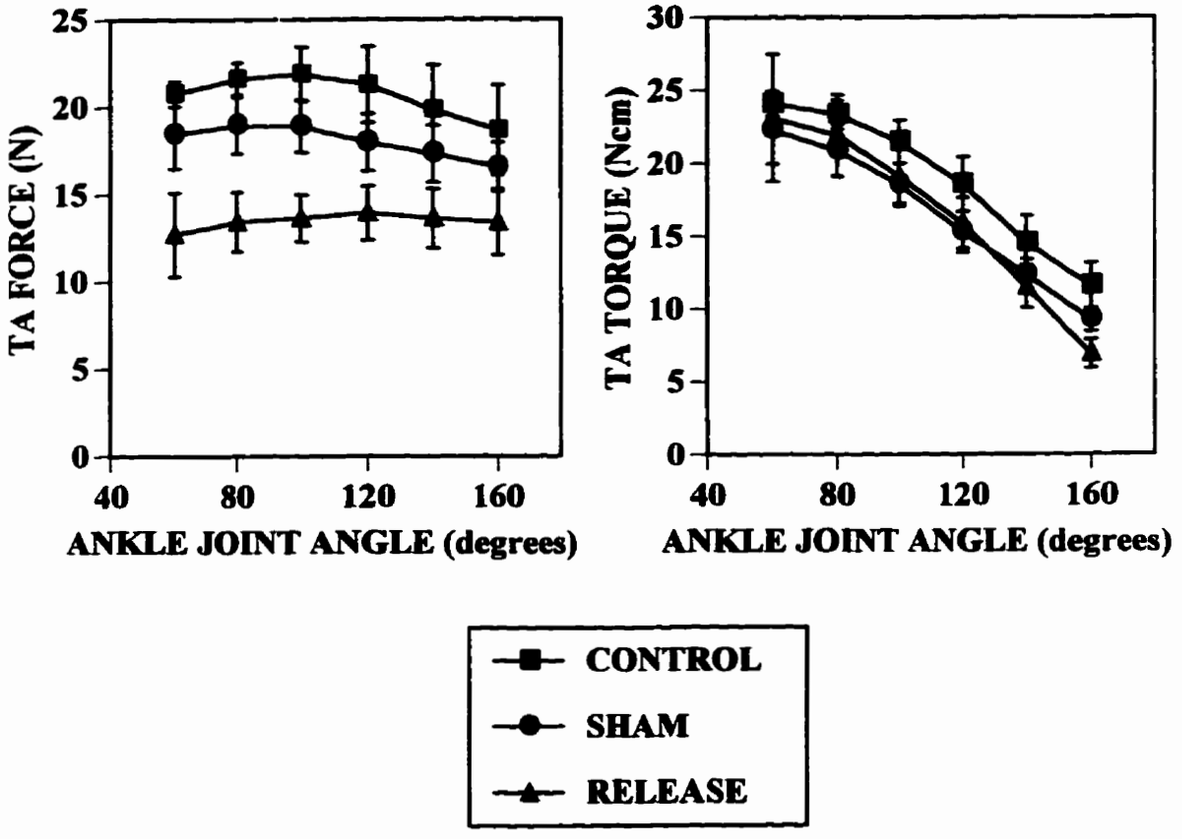

Figure 4.5. Maximum isometric *in situ* TA force- and torque-ankle joint angle relations for control (n = 5), sham (n = 6), and release (n = 6) groups. Values shown are means with S.D. bars. n less than 6 for control group because of technical problems with a force transducer.

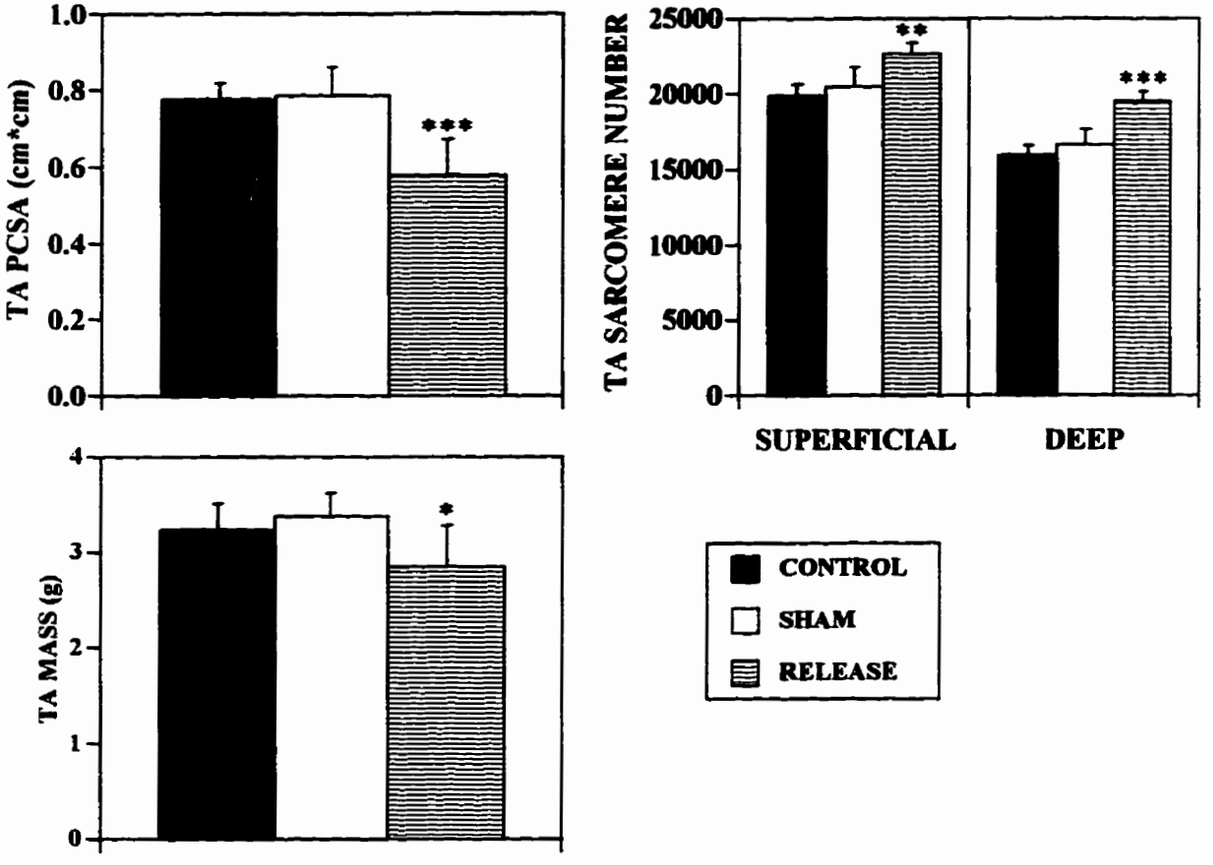

Figure 4.6. TA physiological cross-sectional area (PCSA), sarcomere number and mass for control, sham and release groups (n = 6 for each). Values shown are means with S.D. bars.

* $p < 0.05$; release group significantly different from sham group

** $p < 0.01$; release group significantly different from control and sham groups

*** $p < 0.001$; release group significantly different from control and sham groups

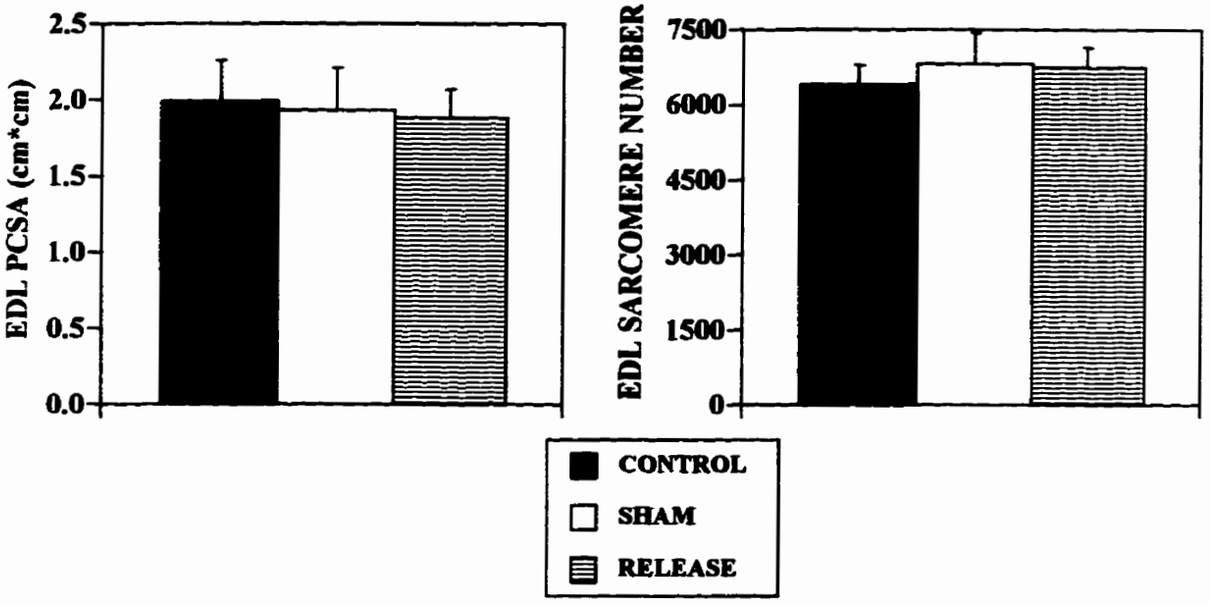

Figure 4.7. Extensor digitorum longus (EDL) PCSA and sarcomere number for control, sham, and release groups (n = 6 for each). Values shown are means with S.D. bars. There were no significant differences between groups.

Discussion

Since (1) tendon transfer likely changes the moment arm, excursion (Lieber et al. 1996), and force production (i.e. the mechanical environment) of the transferred muscle, and (2) changes in the mechanical environment of muscle induces adaptation in its architecture (Booth and Thomason 1991, Goldspink 1985, Roy et al. 1991), tendon transfer is likely to induce adaptation in muscle architecture. However, a review of the literature revealed no experimental data on how muscle adapts after tendon transfer. The present study was performed to test the hypotheses that, following tendon path alteration and increased moment arm and excursion (via TA release), (1) *in vivo* TA force is smaller in released compared with control TAs such that TA torque at the ankle joint is not significantly different between control and release rabbits and (2) differences in TA architecture between control and released TAs following release are consistent with decreased force production in released compared with control TAs.

Hypothesis 1. TA moment arm was larger, and *in vivo* TA force was smaller, for the released TA compared with control and sham TAs, such that *in vivo* TA torque at the ankle joint was similar during hopping for all groups. Thus, the first hypothesis was supported by these data. Maximum isometric *in situ* TA force was also smaller for the released TA compared with the control and sham TAs, whereas *in situ* TA torque was similar between groups. Thus, the *in situ* measurements supported the notion that *in vivo* force production was chronically decreased to help achieve near normal TA torque production at the ankle joint.

Hypothesis 2. PCSA was smaller (26%) for the released TA compared with the control TA. The smaller PCSA of the released TA may have been the result of chronically smaller *in vivo* force production (Roy et al. 1991). There was also an indication of a smaller specific tension (11%) for the released TA than for the control TA. The decreased PCSA and specific tension would both act to decrease the force-producing capability of the released TA.

Sarcomere number was larger for the released TA compared with the control TA (14% and 22% in superficial and deep regions of the muscle, respectively). The larger

sarcomere number may have been the result of the larger excursion for the released TA (cf. Chapter 3). However, TA muscle excursion was increased more than sarcomere number (40% versus 14% and 22%). If sarcomere excursion was also increased more than sarcomere number (cf. Chapter 3, Discussion), this would suggest that each sarcomere would have to shorten at a faster rate in the released TA compared with the control TA to achieve the same ankle dorsiflexion velocity. Based on the force-velocity relationship (Hill 1938, Edman 1979), the larger increase in excursion than in sarcomere number would act to reduce *in vivo* force production during hopping for a given level of muscle activation.

A simple Hill muscle model (Hill, 1938) was used to predict the maximum force capability of the released and control TAs at the angle and velocity at which peak force occurred during hopping. The equation used was: $F = [(F_o b - aV)/(b+V)]\, l(x)$, where F is the force produced at muscle shortening velocity V (calculated from ankle joint kinematics and muscle moment arms), F_o is maximum isometric force at optimal length, l(x) is the fraction of F_o produced at length x, $a = 0.25F_o$ and $b = 0.25V_o$, where V_o is the maximum velocity of shortening (taken to be 13 optimal fiber lengths per second; Spector et al. 1980). The maximum force capabilities calculated were 11.1 N and 6.0 N for control and released TAs, respectively. The peak *in vivo* force for control and released TAs were 51% and 55%, respectively, of the calculated maximum capability, suggesting that each TA was activated to a similar degree for peak force during treadmill hopping. Thus, the second hypothesis of this study was supported by the differences in muscle architecture between groups and the results from the Hill model; the differences in muscle properties were consistent with the decreased *in vivo* force production.

Other considerations. Measurements of neural activation of the TA were not made for this study. Immediately after release, it is possible that neural activation was altered as ankle joint kinematics did not appear different between groups (qualitative visual observations), despite the increase in moment arm. However, it appears that the architectural adaptations observed may have been sufficient to account for the decreased force production and for the maintenance of normal TA torque at the ankle joint by 12 weeks post-release. Further study using electromyographic measurements, especially at

early time points, may allow identification of neural adaptations/compensation to increased moment arm and excursion with release. Such measurements may also be used to determine whether the smaller PCSA in the released compared with the control TA may have resulted from initially decreased neural activation and decreased force production.

It is possible that ankle joint torque following TA release could have been maintained near normal levels by altered neural activation and force production of the EDL. However, the EDL showed no evidence of architectural adaptation, providing evidence against a chronic change in EDL force production following TA release.

The experiments in this study were performed on growing animals. Use of growing animals complicates the interpretation of the data; the results may be influenced by unknown interactions between growth and TA release. However, since many tendon transfer procedures are performed on growing children (Miller et al. 1982), use of a growth model such as in the present study is relevant. A pilot study using skeletally mature animals showed smaller adaptations 4 months post-release (9% decrease in PCSA, 3% increase in sarcomere number); these results suggest that, as might be expected, post-release adaptation may be slower in skeletally mature than in growing animals. The reasons for these differences are unknown and provide opportunity for future study.

The results of the present study suggest that the TA is capable of adapting to increased moment arm and excursion in a manner that helps to achieve near normal TA torque at the ankle joint. Adaptation after actual tendon transfer may differ from that after TA release, and should be examined in future investigations. However, Lieber et al. (1996) showed that, in humans, transfer of the flexor carpi ulnaris (FCU) into the tendons of the extensor digitorum communis (EDC) increased sarcomere excursion by 40% immediately post-transfer due to an increased moment arm, similar to the effects of TA release. Sarcomere number and PCSA adaptation in response to the increased excursion and moment arm after the FCU to EDC tendon transfer would affect the predictions of functional outcome made by Lieber et al. (1996) based on their sarcomere length measurements. Such adaptation would also affect the use of intraoperative laser diffraction measurements (Fleeter et al. 1985, Lieber et al. 1996) and muscle force measurements (Freehafer et al. 1979) to

guide the placement of transferred muscles. For example, if sarcomere length was used to guide the placement of a transferred muscle to produce optimum force (or torque) at a specified joint angle, post-transfer adaptation could disturb the carefully planned outcome. Clearly, knowledge of post-transfer adaptation is needed when considering the functional outcome of tendon transfer procedures.

CHAPTER 5:

Summary

Adaptations in the number of sarcomeres in series in muscle fibers or fascicles (sarcomere number) may be important in pathologic conditions (e.g. cerebral palsy) and clinical procedures (e.g. tendon transfer, bone lengthening), as well as normal muscle function. Despite the importance of sarcomere number adaptations, the signal(s) responsible for sarcomere number adaptation remain to be elucidated. In addition, the capability of muscle to adapt after tendon transfer has not been examined. This chapter contains brief summaries of the results of the dissertation, speculation on issues that may not be appropriate for the manuscript chapters, and suggestions for future research.

There were two main purposes for this dissertation. The first purpose was to test the hypothesis that increasing muscle excursion via TA release results in increased serial sarcomere addition in growing animals. The second purpose was to use the TA release model as an initial examination of the capability of a muscle to adapt following tendon transfer. The hypothesis tested for the second purpose was that increasing the moment arm and excursion of the TA (via TA release) results in adaptation of muscle architecture that helps to achieve near normal TA torque at the ankle joint. For the first purpose, the specific aims were:(a) to determine whether TA release is associated with increased excursion and increased serial sarcomere addition in young rabbits, (b) to determine whether altered animal or joint activity following TA release is associated with altered serial sarcomere addition, (c) to determine whether TA release is associated with decreased *in vivo* TA force production, and if so, (d) to determine whether increasing force production of the released TA inhibits the increase in serial sarcomere addition associated with release. For the second purpose, the specific aims were: (a) to determine whether decreased force production compensates for the increased moment arm of the released TA in a manner that helps to achieve near normal TA torque at the ankle joint, and (b) to determine whether TA architectural adaptations are consistent with decreased TA force production.

Excursion is important in regulating TA sarcomere number following TA release

TA release was performed in 4-week-old rabbits, which were then allowed to grow for 12 weeks. Age- and sex-matched control and sham-operated rabbits served as controls. Muscle excursion (measured using fluoroscopy) and sarcomere number (measured in small fascicles of muscle fibers) were increased in the TAs of released compared to control and sham-operated rabbits. Comparisons of cage activity (measured over 24 hours with a motion detector) and ankle joint kinematics (measured during hopping on a treadmill with a motion analysis system) between groups suggested that altered animal and joint activity could be eliminated as an explanation for the increase in sarcomere number. These results support the primary hypothesis that increasing muscle excursion results in increased serial sarcomere addition in growing animals. However, *in vivo* TA muscle force production (measured with tendon force transducers) was decreased for released versus control and sham-operated rabbits.

A secondary hypothesis to explain the increase in sarcomere number with release was then developed: decreased *in vivo* force production in the released TA may have inhibited tendon growth, which in turn, would have increased the working length of the muscle which may have promoted the increase in sarcomere number. In another group of rabbits, partial ablation of the EDL was performed in conjunction with TA release in an attempt to increase force production of the released TA. *In vivo* measurements verified an increase in TA force production for release + ablation rabbits compared with release only rabbits, but the increase in sarcomere number was not different between release + ablation and release only rabbits. These results suggested that the decreased force production associated with release was not responsible for the increase in sarcomere number. Combining data from all the groups in these experiments, sarcomere number and muscle excursion showed a significant linear relationship.

In summary, the results of this study support the hypothesis that increasing excursion results in increased serial sarcomere addition in growing animals. Previous studies have indicated that decreasing excursion results in decreased serial sarcomere addition (Tardieu et al. 1977, Williams and Goldspink 1978). The present and previous studies, when considered

together, support the working hypothesis that excursion is important in regulating sarcomere number in growing animals.

Muscle adaptation helps maintain normal TA torque production

TA release was performed in 4 week-old rabbits, which were then allowed to grow for 12 weeks. Age- and sex-matched control and sham-operated rabbits served as controls. Twelve weeks post-release, TA moment arm and excursion were increased in released compared with control rabbits. Decreased *in vivo* TA force production compensated for the increased moment arm in released rabbits such that *in vivo* TA ankle joint torque was similar between all groups. The *in situ* maximum isometric force-ankle joint angle relation for the released TAs also showed differences from control that compensated for the increased moment arm such that *in situ* TA torque-ankle angle relations were similar between all groups. Physiological cross-sectional area was smaller and the number of sarcomeres in series was larger, in the released TA compared with the control TA. These architectural adaptations were consistent with the observed functional adaptations. Thus, the TA adapted to an increased moment arm and excursion in a manner that helped to achieve near normal ankle joint torque.

TA release may provide a model to investigate the architectural and functional adaptations associated with tendon transfer without the complications associated with transferring the tendon (e.g. tendon transection, healing, inflammation, scar formation). Although the potential for post-tendon transfer muscle adaptation has been recognized (Lieber et al. 1996), a review of the literature revealed no experimental data, and models of tendon transfer have not included this possibility (Giat et al. 1994, Loren et al. 1995). Tendon transfer likely changes the moment arm, excursion and function of the donor muscle (Lieber et al. 1996, Carayon et al. 1967). The results of this study suggest that the TA is capable of adapting to increased moment arm and excursion in a manner that helps to achieve near normal TA torque at the ankle joint. Such adaptation after tendon transfer procedures would affect the use of intraoperative laser diffraction measurements (Fleeter et al. 1985, Lieber et al. 1996) and muscle force measurements (Freehafer et al. 1979) to guide

the placement of transferred muscles. For example, if a muscle was transferred at a given sarcomere length to produce optimum force (or torque) at a specified joint angle, post-transfer adaptation could disturb the carefully planned outcome. Clearly, knowledge of post-transfer adaptation is needed when considering the functional outcome of tendon transfer procedures.

Static versus cyclic strain

Excursion, or cyclic strain, appears to be important for serial sarcomere addition in growing animals. Reducing cyclic strain (or excursion) by immobilizing growing muscle in a stretched position results in reduced serial sarcomere addition (Tardieu et al. 1977, Williams and Goldspink 1971). Increasing cyclic strain (or excursion) via TA release results in increased serial sarcomere addition.

Cyclic strain has been shown to increase DNA, RNA and protein synthesis and accumulation in cultured cells of different types (e.g. muscle: Vandenburgh et al. 1989; tendon: Banes et al. 1995; endothelial: Awolesi et al. 1995). For muscle cells, cyclic strain increases the growth in length and diameter of cultured myotubes (Vandenburgh et al., 1989); however, the influence of cyclic stretch on sarcomere number in cultured muscle cells has not been investigated.

Cyclic strain appears to be better than static strain at inducing responses from a variety of cells. In bone, cyclic strain appears to be better than static strain for preventing bone loss and producing new bone formation *in vivo* (Lanyon and Rubin, 1984). In cartilage, cyclic strain increased proteoglycan synthesis while static strain decreased proteoglycan synthesis (Sah et al. 1992). In endothelial cells, pulsatile fluid flow increased nitric oxide production more than constant fluid flow (Noris et al. 1995). Although controlled comparisons of cyclic versus static strain appear to not have been made for muscle cells, it would not be surprising if cyclic strain was better than static strain at producing responses in muscle cells.

Growing animals

Complications and relevance. The use of growing animals complicates the interpretation of results; it is difficult to attribute adaptation specifically to an experimental perturbation when the perturbation is superimposed on a growing organism. The use of age-matched control and sham-operated animals in the dissertation should control for the effects of growth and the surgical procedures (apart from TA release). However, the results may be influenced by unknown interactions between growth and TA release. This is a limitation of these studies, yet it is relevant to study these hypotheses in growing animals.

The use of growing animals is practically relevant. Cerebral palsy, a prevalent neuromuscular disorder originating in childhood, is associated with muscle contractures, equinovarus deformity, toe-walking, and crouch gait (Tardieu et al. 1989, Thometz et al. 1989). These pathologies are thought to be associated with impaired longitudinal growth of muscle (O'Dwyer et al. 1989, Tardieu et a. 1982). In addition, tendon transfer, tendon lengthening, and bone lengthening are often performed on children (Miller et al. 1982, Paley 1990, Thometz et al. 1989). Adaptation of muscle in response to these surgical procedures is important in determining their outcome. Reappearance of muscle contractures after tendon lengthening (Truscelli et al. 1979) may result from post-surgical decreases in sarcomere number. Development of muscle contracture during bone lengthening may result from an inability of muscle to lengthen as quickly as bone (Lehman et al. 1991, Paley 1990). In summary, studying sarcomere number adaptations in growing animals may provide information critical to improving treatments for cerebral palsy, and for improving the outcome of tendon transfer, tendon lengthening and bone lengthening in children.

Differences in adaptations between young versus adult animals. Studying sarcomere number adaptation in growing animals is also important because the long-term response of sarcomere number to experimental perturbation depends on the age of the animal. Long-term immobilization (weeks) increases sarcomere number in adult animals but decreases serial sarcomere addition in growing animals (Tardieu et al. 1977, Williams and Goldspink 1978). In addition, TA release increases serial sarcomere addition in growing rabbits by an average of 18% after 12 weeks, but data from pilot experiments suggest that TA release in

skeletally mature (12 months old) animals increases sarcomere number by an average of only 3% after 16 weeks.

The different responses in adult and growing animals may be related to differing effects of the perturbations on tendon. Longitudinal tendon growth can be altered in growing animals, whereas changes in tendon length appear to be difficult to induce in adult animals (Tabary et al. 1972, Tardieu et al. 1977, Williams and Goldspink 1978). Immature (reducible) crosslinks in the tendon decrease with age, and are replaced with mature (non-reducible) crosslinks (Bailey et al. 1974). This change in the type of cross-link with age may be related to the different susceptibility of tendon to experimental perturbation. For example, Davison (1989) has shown that the mechanical properties (tensile strength, creep) of rat tail tendon are related to the ratio of non-reducible to reducible crosslinks present. Although tension has been proposed as a possible stimulus for tendon growth (Blanchard et al. 1985; Davison, 1992; Tardieu et al. 1983), no evidence supporting this idea was found in the present study. Although muscle and tendon growth appear to be coordinated (increased longitudinal muscle growth is associated with decreased longitudinal tendon growth and vice versa), the coordinating mechanisms remain to be elucidated.

The different responses in adult and growing animals may also be related to different levels of circulating growth factors between age groups (e.g. Florini and Roberts 1980). Growth factors may interact with mechanical load to produce a cellular response. In cultured muscle cells, cyclic strain stimulated cell growth in medium supplemented with serum and embryo extract, but cyclic strain appeared to have less of an effect in supplement-free medium (Vandenburgh et al. 1989). In cultured tendon cells, platelet-derived growth factor and insulin-like growth factor are required for cyclic strain-induced stimulation of cell division (Banes et al. 1995). Thus, the level of circulating growth factors present *in vivo* could affect the relative responses of muscle and tendon to immobilization and/or TA release.

A final possibility is that the different responses in adult and growing animals may be related the greater number of satellite cells present in growing versus adult muscle (Schultz 1976). Satellite cells are the source of nuclei added to muscle fibers during post-natal

growth (Moss and Leblond 1971). Satellite cells have been located at the muscle tendon-junction in growing animals (Williams and Goldspink 1971), and could be important in elongation of muscle fibers during growth. Satellite cells may be regulated by growth factors produced and released by muscle cells (fibroblast growth factor, insulin-like growth factors, platelet-derived growth factor; Schultz and McCormick 1994). The greater number of satellite cells in growing muscle could make muscle in growing animals more sensitive to an experimental perturbation (e.g. TA release) than in adult animals. The role of satellite cells in longitudinal muscle growth remains to be explored.

Series-fibered architecture and longitudinal muscle growth

Most individual fibers in the rabbit TA do not span the distance from proximal to distal aponeuroses (Mackay and Harrop 1969; Crawford 1973); they are arranged serially such that up to three fibers are required to span the distance between aponeuroses. In short, the TA has "series-fibered architecture". Such series-fibered architecture is common in mammalian muscle and particularly in avian muscle (Trotter et al. 1995). Speculation of evolutionary advantage for series-fibered architecture has focused on the difficulties associated with the time required for spreading of action potentials over long continuous fibers (Gans et al. 1989).

The number of in-series fibers spanning the distance between aponeuroses is determined before birth, and longitudinal growth appears to take place throughout the length of the muscle (Crawford 1954, Gaunt and Gans 1990). These observations suggest that longitudinal muscle growth may occur by sarcomere addition within individual fibers and concomitant slippage of fibers past one another. Individual fibers could lengthen either by addition of sarcomeres at the ends of the fibers (Williams and Goldspink 1971) or by interstitial addition of sarcomeres in the fibers (Jakubiec-Puka 1985).

The effect of series-fibered architecture on muscle function has not been elucidated. Trotter et al. (1995) have suggested that force generated by in-series fibers is transmitted to the tendon by shear forces borne by the endomysium. If the endomysium is responsible for force transmission of in-series fibers, the possibility exists that compliance of series-fibered

muscles would be greater than that of continuous fibered muscles. However, Trotter et al. (1995) estimated the increased strain associated with series-fibered architecture during maximum muscle contraction, and concluded that the value obtained (0.0036%) was negligible. In addition, Chanaud et al. (1991) reported that passive percentage fascicle length changes were similar to, but slightly larger than, percentage sarcomere length changes in the cat biceps femoris for the full *in vivo* excursion of the muscle (34% versus 29%; n = 2). Similar findings were presented in this dissertation; TA release produced a similar percentage increase in muscle (29%) and sarcomere (27%) excursion, suggesting that muscle excursion reflects sarcomere excursion fairly well, and that in-series fiber slippage does not appreciably affect passive length changes. The available data thus suggest that the series-fibered architecture of the TA does not appreciably influence the transmission of increased muscle excursion to individual fibers and sarcomeres.

Continuous-fibered and series-fibered muscles appear to adapt similarly to experimental perturbations. For example, immobilization of adult rabbit muscle in a stretched position increases sarcomere number for fascicles that span the distance from proximal to distal aponeuroses both in the continuous-fibered soleus and in the series fibered TA (Tardieu et al. 1977, Williams et al. 1986). In addition, immobilization of growing rabbit muscle in a stretched position decreases fascicle length both in the continuous-fibered soleus and in the series fibered TA (Tardieu et al. 1977, Crawford 1973). In summary, the series-fibered architecture of the TA does not seem to affect the nature of adaptation of sarcomere number or fascicle length in fascicles that span the distance from proximal to distal aponeuroses.

Suggestions for future study

Neural adaptation/compensation with TA release. Neural adaptation/compensation with TA release would be of interest because joint kinematics did not appear to be altered immediately after release (qualitative observations) despite the increased moment arm and excursion of the TA. Such adaptation would also be of interest as an initial investigation of the ability of the nervous system to adapt its control of muscle after changing the function of

the muscle; such a change in muscle function is commonly associated with tendon transfer procedures. To test the hypothesis that, after TA release, there is a short-term decrease in neural activation of the TA to help maintain normal ankle joint torque production and kinematics, measurements could be made of TA electromyographic (EMG) activity, TA muscle force, TA ankle joint torque, and ankle joint kinematics immediately before and after TA release. It would also be of interest to test the hypothesis that, as predicted by the results of this dissertation, TA muscle activation returns to near normal levels 12 weeks post-release. Positive results for both hypotheses would suggest that the smaller PCSA of the released TA was the result of initially decreased muscle activation and force production and that the resulting smaller PCSA allowed activation of the TA near normal levels by 12 weeks post-release.

Cellular response to TA release. The involvement of satellite cells, insulin-like growth factors, and immediate early genes in the response of muscle to TA release may be fruitful avenues for future study. Satellite cells are the source of nuclei added to muscle fibers during post-natal growth, as nuclei in differentiated muscle fibers are not capable of dividing (Moss and Leblond 1971). Satellite cells have been located at the muscle tendon-junction in growing animals (Williams and Goldspink 1971), and could be important in elongation of muscle fibers during growth. To test the hypothesis that TA release increases satellite cell proliferation and fusion, ^{3}H-thymidine could be injected into animals at different time points after release, and the incorporation of ^{3}H-thymidine into satellite cell nuclei and muscle cell nuclei at the ends of muscle fibers could be followed at different time points after injection by autoradiography and electron microscopy (Moss and Leblond 1971). TA release would be expected to be associated with increased ^{3}H incorporation into satellite and muscle cell nuclei at the ends of muscle fibers.

Increased insulin-like growth factor I (IGF-I) protein and mRNA levels have been associated with increased muscle mass induced by stretch-immobilization of the rabbit TA (Goldspink et al. 1995), with increased protein synthesis and content induced by cyclic strain (Perrone et al. 1995), and with proliferation and differentiation of satellite cells (Allen and Rankin 1990). If IGF-I participates in the increased sarcomere number with TA release,

increased IGF-I expression may be expected at the ends of muscle fibers. To test the hypothesis that IGF-I expression is increased at the ends of muscle fibers after TA release, immunohistochemistry and *in situ* hybridization could be used to locate IGF-I protein and mRNA in muscle cells, and to determine whether TA release increases IGF-I protein and mRNA levels at the ends of muscle fibers.

Increased immediate early gene expression has been associated with stretch-immobilization of the rabbit TA (c-fos; Goldspink et al. 1995) and with compensatory hypertrophy of the rat plantaris and soleus (c-myc; Whitelaw and Hesketh 1992). If c-fos and c-myc are involved in the signal transduction pathway for increasing sarcomere number with TA release, increased c-fos and c-myc expression may be expected at the ends of muscle fibers. To test the hypothesis that c-fos and/or c-myc expression is increased at the ends of muscle fibers after TA release, immunohistochemistry and *in situ* hybridization could be used to locate c-fos and c-myc protein and mRNA in muscle cells, and to determine whether TA release increases c-fos and c-myc protein and mRNA levels at the ends of muscle fibers.

Modeling TA release *in vitro*. To determine whether sarcomere number is regulated by excursion in muscle cells, the effects of TA release could be modeled *in vitro*. Vandenburgh and Karlisch (1988) have described a device that can be used to uniaxially stretch muscle cells attached to an elastic substratum. A unidirectional (ramp) stretch could be used to simulate elongation of the tibia and the associated increase in the distance between origin and insertion of the TA. Excursions of different magnitudes could be superimposed on top of the ramp stretch to simulate TA excursion associated with ankle joint motion with and without release. To best simulate TA release, maximum strain would be the same in experimental and control cells because the maximum distance between origin and insertion is the same in released and normal TAs. Increased excursion in experimental cells would be achieved by decreasing the minimum stretch of the cells.

To test the hypothesis that excursion regulates sarcomere number in muscle cells, sarcomere number would be measured in cells exposed to differing excursions. In addition, the contribution of cell proliferation and fusion to elongation of myotubes could be

measured by following incorporation of ^{3}H-thymidine into nuclei. Immediate early gene and IGF-I expression could also be measured to determine whether excursion regulates expression of these factors at the ends of myotubes. To test whether autocrine/paracrine action of IGF-I is important in elongation of myotubes, an antibody to the IGF-I receptor could be used to block the receptor and inhibit the action of IGF-I on the muscle cell (Quinn et al. 1994).

Sarcomere number adaptation to bone lengthening. Although not directly related to the TA release model used in this dissertation, bone lengthening is another clinically relevant circumstance in which to study changes in sarcomere number. Development of muscle contracture during bone lengthening may result from an inability of muscle to lengthen as quickly as bone (Lehman et al. 1991, Paley 1990). In fact, Paley (1990) has stated that "muscle is the single largest limiting factor in limb lengthening today". Knowledge of the mechanisms that regulate sarcomere number addition will be important for reducing the problems associated with muscle contracture during bone lengthening. For example, stretching the affected muscle by splinting or by pin-immobilization is often used to prevent muscle contracture associated with bone lengthening (Lehman et al. 1991). If immobilization is used on growing children, the result may be decreased serial sarcomere addition (Tardieu et al. 1977, Williams and Goldspink 1971) and impaired muscle function, despite prevention (at least initially) of the contracture. Based on the results of this dissertation, ensuring normal muscle excursion may be a better approach, possibly by continuous passive motion (Frank et al. 1984). The hypothesis that maintaining normal muscle excursion is important for improving serial sarcomere addition associated with bone lengthening could be tested in a rabbit model for bone lengthening (Simpson et al. 1995, Sun et al. 1995). Chronic electrical stimulation has been shown to increase the rate at which sarcomeres are added to the rabbit TA during stretch immobilization (Williams et al. 1986). Experiments could be performed to determine whether electrical stimulation increases the rate of longitudinal growth of muscle during bone lengthening. Finally, experiments could be designed to examine the efficacy of growth factors (e.g. growth hormone, IGF-1)

administered pharmaceutically or by gene transfer (Dhawan et al. 1991, Huard et al. 1997, Trippel et al. 1996) in improving longitudinal muscle growth during bone lengthening.

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