A BIOMECHANICAL ANALYSIS OF THE ROLE OF THE CRURAL FASCIA IN THE CAT HINDLIMB

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**A BIOMECHANICAL ANALYSIS OF THE ROLE OF THE CRURAL FASCIA IN THE
CAT HINDLIMB**

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"Nature created nothing without purpose"
(Artistotle)

This dissertation is respectfully dedicated to the great gift of all animals that made this work possible.

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LIST OF ABBREVIATIONS

Muscle Abbreviation	Muscle Name
aBF	Anterior Biceps Femoris
FHL	Flexor Hallicus Longus
G	Gracilis
LG	Lateral Gastrocnemius
MG	Medial Gastrocnemius
pBF	Posterior Biceps Femoris
Plant	Plantaris
Sol	Soleus
SM	Semimembranosus
ST	Semitendonosus
TA	Tibialis Anterior

SUMMARY

The potential of the crural fascia to increase the articulation of the posterior thigh muscles through the in series connection of the structures, suggests that the crural fascia may influence the endpoint force direction of the muscles by partially redirecting the muscular force output. Furthermore, not only the in series connections should be considered but also how the parallel alignment of the crural fascia and the triceps surae may influence the force direction from the muscles. A redirection in force may, in turn, affect the intra-limb coordination or contribute to the selection of a task variable muscle activation pattern. The *central objective* was to evaluate the role of the synergistically located, posterior, distal musculature and connective tissue during locomotion. The *central hypothesis* was that the crural fascia would redirect the force output from the posterior thigh muscles to the endpoint and consequently increase propulsion within the limb.

We selected to perform our studies in the spontaneously locomoting decerebrate cat, which allows us to investigate acute treatments applied to the hindlimb. The overall objective was accomplished by: (1) evaluating the role of the crural fascia during level walking; (2) determine the acute effect of denervating the triceps surae muscles and disrupting the crural fascia during level walking; and (3) evaluating the change in force direction output of selective stimulation of muscles in different limb configurations before and after complete fasciotomy. Our findings demonstrated that the crural fascia not only assists in propulsion but also acts to stabilize the distal limb. Furthermore, the acute denervation of the triceps surae resulted in a decrease in leg length and an increase

in ankle yield during the weight acceptance phase of stance. This suggests that the

conservation of the limb length as a task level variable is an adaptation rather than an

immediate response.

CHAPTER 1

INTRODUCTION

There are nearly 1.7 million people living in the US with limb loss. Most of these people can return to full, productive lives as functional members of society with the assistance of prosthetics (America 2010). This number grows each year as people continue to suffer from musculoskeletal disease and injuries. Furthermore, some diseases and injuries do not require full limb amputation but may lead to functional deficits requiring assistance. By understanding the functionality of the anatomy within a healthy limb and how the components are controlled we will be able to prevent injuries in healthy individuals and design new assistive devices for those with disabilities.

The movement of different limb segments requires force produced by the appropriate muscles in the correct plane. The functions of muscles are often described according to their action in the sagittal plane at the joints that they cross, whether they are mono-articular like soleus, an ankle plantarflexor, or bi-articular like Semitendonosus (ST), a hip extensor and knee flexor. The higher articulated muscles have been suggested to couple movements between joints (van Ingen Schenau 1994). For example, the medial head of the gastrocnemius muscle flexes the knee and plantarflexes the ankle, but also has a strong abduction torque at the ankle due to the twist of the calcaneal tendon (Lawrence et al. 1993). Therefore consideration that muscle functionality should not be constrained to the sagittal plane only, as many muscles are also multi-axial in action. Musculotendinous units, comprised of muscles and their corresponding tendons, are often

considered to be bone-to-bone attachments, but many muscles are attached to fascia

sheets or planes, which could change the endpoint of the muscle and therefore the

articulation and potentially the function of the muscles within the limb. For example, the

cheetah hindlimb has strong tendon-like connections from the hamstrings to the ankle

joint (Figure 1.1) potentially coupling the hip extension, knee flexion action of the

posterior thigh muscles to ankle plantarflexion resulting in a strong propulsive force (van

Ingen Schenau 1994). This augmentation of action is not limited to the hindlimbs of

quadrepeds, as it has been suggested that structures such as the fascia cruris in the foreleg

functions like an epimuscular aponeurosis, extending the leg extension capabilities of the

ventral extensor muscles (van der Wal 2009).
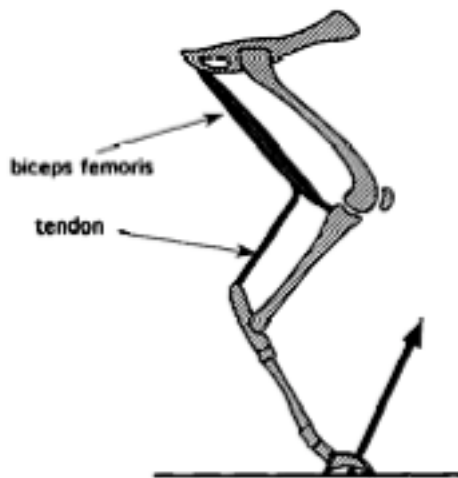
Figure 1.1. Figure from van Ingen Schenau depicting the tendinous connection from the biceps femoris muscle to the calcaneus in the cheetah and how this supports strong propulsion in the limb.

Fascia Anatomy

To understand the functional role that fascia could be playing during movement it is

important to introduce some ideas regarding fascia anatomy. The term fascia is derived

from the Latin term for band or bandage (Bejamin, 2009) and fascia is often described as the bag that holds the body together due to its pervasive nature. Although fascia is generally poorly classified, it is generally defined as being composed of two subtypes, superficial (or subcutaneous) fascia and deep fascia. While both are connective tissues composed of collagen and elastin fibers, the deep fascia typically has a large number of these fibers densely packed together (Benjamin 2009). The superficial fascia's function is typically ascribed to allowing for sliding movements (van der Wal 2009) and is often discussed in regards to allowing for movement between the skin and underlying muscle and returning the skins position after movement. The deep fascia is typically attributed to forming compartments surrounding muscles. Although the medial and lateral boundaries of these compartments often originate from bone the proximal and distal boundaries may arise from muscle and tendons. The structure of the compartment and muscle-fascia attachments has suggested that fascia can act as an ectoskeleton (Wood Jones 1944), an external skeleton serving as attachment points for muscles. In turn, this soft tissue skeleton's stiffness can be modified and therefore its function augmented by the activation of muscles attached to it (Huijing et al. 2003).

The crural fascia is a deep fascia forming a compartment around the triceps surae muscles in the posterior, distal limb. The crural fascia is composed of a large sheet that covers the caudal surface of the gastrocnemius muscles. Preliminary investigations (in our lab) into the structure of the sheet in cats, with a dissecting microscope, reveal a pinnate cross-alignment of fibers within the sheet suggesting a non-uniform directing of force within the tissue, which has also been reported in human crural fascia (Stecco et al. 2009). This sheet originates from the distal surface of the posterior thigh muscles: Gracilis (G),

Semitendonosus (ST), Semimembranosus (SM) and posterior Biceps Femoris (pBF)

(Figure 1.2) arising near the muscle-tendon interface for these muscle (Crouch 1969;

English and Weeks 1987). These muscles are bi-articular muscles that typically act to

extend the hip and flex the knee, with the exception of G, which adducts the limb and

flexes the knee. Within the sheet are two thickened bands that run through the medial

and lateral sides of the sheet. The medial band begins as a broadened fan of connective

tissue near the tendons of ST and G and narrows to a thickened band terminating on the

medial calcaneus. The lateral band begins on the underside of the pBF muscle,

perpendicular the muscle fiber arrangement with the limb in a natural stance. This band

continues down the lateral side until the calcaneal tendon. The band then follows the

twist of the tendon and inserts medially onto the calcaneus. The sheet of crural fascia

remains continuous between the bands and forms a tube around the tendons inserting

onto or crossing over the calcaneus. The anatomical connection of the crural fascia from

the bi-articular posterior thigh muscles to the calcaneus suggests that these muscles could

be considered to be tri-articular.
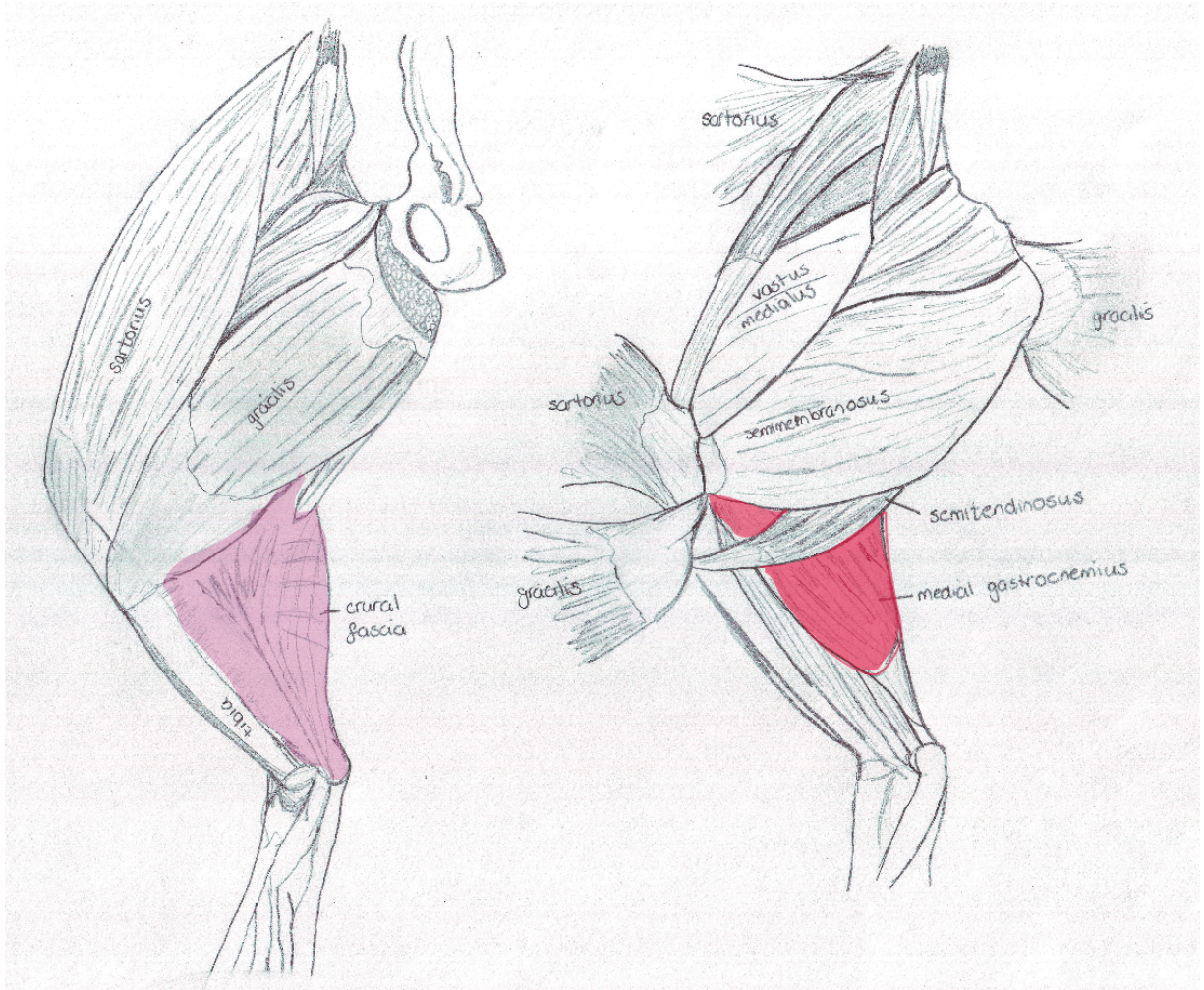
Figure 1.2 A sketch of the medial view of the hindlimb (modified from original sketch by Crouch). The left figure is a superficial view of the hindlimb with the crural fascia highlighted in pink. Note the direct attachment to the gracilis and semitendonosus muscles. The right figure shows the superficial layer of muscle and fascia removed. The medial gastrocnemius muscle is shown in red directly below the crural fascia.

The two alignments of fibers, uniform or parallel within the bands and multi-directional within the sheet, suggest different properties and functions within the crural fascia. Although deep fascia has been shown to have viscoelastic and non-linear properties following the typical viscoelastic rule of collagenous tissue (Wang et al. 2009) the directions or alignment of the fibers may influence the values associated with the sample of tissue tested. The fascia lata has even been shown to have comparable properties to tendons suggesting that the longitudinal fibers of the fascia predominate in loading,

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similar to the parallel arrays of collagen fibers in tendons (Butler et al. 2004).   Our

cursory examination of alignment in the crural fascia suggests that the fibers in the bands

are organized longitudinally, similar to tendons and may therefore be important in

loading. However, the sheet appears to have a cross structure of organization with fibers

running in a pinnate form around 45° to each other (personal observation). The

advantage of the sheet lacking unidirectional organization it is that is able to respond to

more than one direction, but reduces the efficiency of the response to any single direction

(Stecco et al. 2009; Wainwright et al. 1976). However it does allow for the flexibility to

respond to changes in leg orientation, internal pressure and the force of multiple insertion

muscles. Furthermore, studies of various retinaculae at the ankle have shown to have a

cross alignment of collagen fibers (likened to the laces of a shoe) for stability (Abu-

Hijleh and Harris 2007). Therefore one can infer that the tendinous bands in the crural

fascia may be designed more for unidirectional loading (similar to tendons) while the

sheet is able to respond to multi-directional loading and potentially stabilize the limb.

Therefore, in order to truly determine the role of the crural fascia a sequential disruption

of the tissue should be performed instead of a complete fasciotomy to allow for the

discerning of the different sections. In order to determine if the sheet potentially allows

for cross transmission of force it should be split longitudinally to stop the cross

transmission from occurring. Then by cutting each band we would be able to determine

if there is a loss of propulsion to the distal limb as well as if the bands play a role in

stabilizing the limb during locomotion.

Although the crural fascia arises from the distal end of the posterior thigh muscles it also surrounds the triceps surae muscles. The triceps surae is composed of the medial and lateral gastrocnemius muscles and the soleus muscle. The gastrocnemius muscles originate from the medial and lateral sesamoid bones of the femur. The two heads merge about one third of the way down the tibia and the muscle rapidly narrows as it inserts into the calcaneal tendon, which inserts into the proximal end of the calcaneus (Crouch 1969). The gastrocnemius muscles are bi-articular muscles acting to flex the knee and extend the ankle. The soleus muscle originates from the lateral surface of the head of the fibula and terminates in the calcaneal tendon and is therefore a mono-articular muscle.

Muscle force affected by fascia

Studies investigating the role of myofascia in force transmission between muscles have shown that the contraction of a muscle on one side of the fascia affects the force output in the synergistic muscle (Huijing et al. 2003; Maas and Huijing 2005; Maas et al. 2005a; Maas et al. 2005b). This force transference was disrupted after the physical separation of the muscles by blunt dissecting the myofascia between the muscles. Furthermore, this suggests that muscular force does not only get transmitted linearly to an endpoint but also perpendicular to the muscle and that the gastrocnemius muscle could transmit force to the crural fascia.

Additional studies wherein the force output of muscles is recorded with the surrounding fascia compartment intact or disrupted showed a decrease in muscle force output and intra-compartmental pressure after the fasciotomy (Garfin et al. 1981; Huijing et al.

2003). Therefore supporting that the compartmental fascia may affect the force output of the muscles it surrounds, particularly when the muscle is contracted. Furthermore, although this has not been investigated, the compartment fascia may also limit the medial and lateral torques produced by the muscles by limiting their range of motion.

The step cycle

During locomotion, the limbs muscles are activated for retraction and protraction of the limb during stance and swing respectively. During early stance or E2 the limb goes from paw placement to under the center of mass. While the hip extends through this time period the knee and ankle flex as the center of mass (COM) passes over the joints. The hip is out of phase with the knee and ankle during this period of the step cycle suggesting that the COM has a large effect on the limb, however the joints still reflect that the limb is being retracted during the period. After the COM has passed over the limb, all three joints extend in order to propel the COM forward through E3 to paw off. The in-phase activity of the three joints during E3 could partially be attributed to the propulsive action of the proximal, posterior muscles in the limb that act to extend the hip and couple the knee extension and ankle plantarflexion through the connection with the crural fascia. After paw off and through early swing (F) the hip, knee and ankle flex to swing the paw forward. Once the paw has passed under the COM the knee and ankle extend to reach forward for paw placement (E1).

Stability

A stable limb is often defined in non-linear dynamics terms as being able to return to the original trajectory of the limb after a perturbation has occurred. An unstable limb would not be able to recover the original trajectory. We define a limb could be considered stable during locomotion if it followed a consistent trajectory from step to step with low inter-step variability. Consequently, the limb will be considered to be less stable if the trajectory between the steps becomes more variable.

In human walking, the sagittal plane is considered to be mechanically stable while the frontal plane required neural control (Bauby 2000, Winter 1995). This was further supported by the fact that spinal cats require an external mechanical clamp on the tail to maintain mediolateral stability (Grillener and Warren, 1985). However there are passive mechanical structures within the limb that are considered to act as mechanical stabilizers such as the retinaculae at the ankle (Abu-Hijleh and Harris 2007). The crural fascia could be acting in a similar manner as a passive mechanical stabilizer in the distal limb.

Coordination

Coordination is the efficient movement of a combination of body parts to achieve a certain task, whether this is as simple as taking a step or complex as opening a jar. Often the task requires both power output from the muscles as well as stabilization of the segments involved in the movement. In walking, the limb would need to provide propulsion to move the center of mass forward as well as have stable trajectories of the segments for efficient force transmission. Given the synergistic location of the crural

fascia and the triceps surae muscles in the leg, it can be supposed that they have a synergistic role within the limb in the sagittal plane. Given their anatomical locations and the connection of the crural fascia to the hamstrings, both the gastrocnemius and the hamstring muscles can be considered to be knee flexors and ankle plantarflexors thereby affecting intralimb coordination. In particular, it is known that during the weight acceptance stage of stance (E2) the hip is out of phase with the knee and the ankle while in the propulsive stage of stance (E3) the three joints are in phase (Goslow et al. 1973). Given that the bi-articulator movement of the triceps surae is between the knee and the ankle, while the hamstrings could be considered to be tri-articular between the hip, knee and ankle, it could be suggested that the triceps surae contributes more to counter the weight acceptance yield of the knee and ankle while the hamstrings and crural fascia retract the leg during E2. During E3 the hamstrings and crural fascia may contribute more to the coordination the hip, knee and ankle.

Due to the limb being comprised of multiple segments and each segment has multiple degrees of freedom the limb has a high number of redundant degrees of freedom. Bernstein (1967) described motor coordination as the control of redundant degrees of freedom of the limb. Although this high number of degrees of freedom allows for great flexibility it also increases the complexity of the control. The crural fascia could help to reduce the complexity of the control by linking multiple segments together given its anatomical connection from the thigh to the leg segment therefore reducing the need to control the knee joint. Furthermore, it has been argued that the nervous system places more weight on the limb variables, such as limb length and limb orientation, than the

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joint variables, such as the hip, knee and ankle included angles (Chang et al. 2009). Structures in the limb that link segments and across joints could provide proprioceptive information to control the limb variables and reduce the need for consideration of control of the joint variables.

Control of joint and limb variables

Chang et al (2009) showed that although there was an increase in ankle dorsiflexion during the paralytic stage after reinnervation of the triceps surae muscles, while the limb variables, limb length and limb orientation, were conserved. This suggests a preference for the conservation, either biomechancially or neurologically, of global task variables. It has been previously suggested that neural encoding in motor control is task dependent (Loeb 1985) whereby motor units are organized and selected dependent on the task. Furthermore, the limb is built of more than just muscles and the consideration of the architectural units of muscle and linking connective tissue is more consistent with the consideration of the whole limb than the consideration of individual muscles acting at joints (van der Wal 2009). The question therefore arises as to whether the conservation of task level variables is a consequence of biomechanics or a neural adaptation. This can be investigated by performing a sequential denervation of the triceps surae and complete fasciotomy to determine if the global variables are conserved in the acute preparation and if the crural fascia plays a role in providing feedback to conserve the limb variables while the triceps surae only effects the knee and ankle joints.

Summary

The considerations of the influences of connective tissues on the articulation (van der Wal 2009; van Ingen Schenau 1994) and forces of the muscles associated with them (Garfin et al. 1981; Huijing et al. 2003; Maas and Huijing 2005), the role in coordination between joints (Goslow et al. 1973; van Ingen Schenau 1994), and the support of task level variables (Chang et al. 2009) suggests that structures such as the crural fascia may play an important role during functional tasks. Therefore, we wish to examine the functional role of the crural fascia during walking in the hindlimb of the cat. In particular, we wish to investigate how the structure of the fascia as well as its parallel and series attachments to different muscles contributes to control of the endpoint of the limb.

1.1   OBJECTIVES AND HYPOTHESIS

The potential of the crural fascia to increase the articulation of the posterior thigh muscles through the in series connection of the structures, suggests that the crural fascia may influence the endpoint force direction of the muscles by partially redirecting the muscular force output. Furthermore, the manner in which the in series connections as well as the parallel alignment of the crural fascia and the triceps surae might influence the force direction from the muscles should be considered. A redirection in force may, in turn, affect the intra-limb coordination or contribute to the selection of a task variable muscle activation pattern. In order to address the aforementioned considerations, we elected to perform our studies in the spontaneously locomoting decerebrate cat, which allows us to investigate acute treatments applied to the hindlimb. We selected this preparation as it

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allowed us to examine the acute effects of a treatment without having to wait for the

animal to recover from surgery or provide drugs to counter any nocioceptive stimulation.

Furthermore, this preparation has been utilized extensively in the field of motor control

from central pattern generator studies (Baev et al. 1991; Donelan and Pearson 2004; Mori

1987), to vestibular feedback (Gottschall and Nichols 2007) to postural control

(Honeycutt et al. 2009; Honeycutt and Nichols). In the following chapters we will

discuss the role of different distal limb elements in the force transmission in the hindlimb

of the decerebrate cat.

In chapter 2 we describe the kinematic changes and EMG responses of the decerebrate

cat during level walking the sequential disruption of the sheet and each band of the crural

fascia. We *hypothesize* that the crural fascia will play a role in propulsive force

transmission through the limb as well as stabilization of the distal limb during level

walking. Our objectives are to: 1) elicit the roles of the different anatomical components,

the sheet and tendinous bands, through the sequential disruption of the crural fascia; 2)

determine if the crural fascia plays a role in neural feedback during level walking.

In chapter 3 we investigate the acute response of the locomoting cat to the denervation of

the triceps surae muscles.   We *hypothesize* that the kinematic response will be equivalent

to the result of increased ankle yield during stance as reported in the chronic awake,

behaving animal. Furthermore, we *hypothesize* that the triceps surae and crural fascia

have synergist actions in the distal limb given their co-location. Our *objectives* are to 1)

determine if the acute denervation of the triceps surae results in a change in the joint and,

or the limb level variables; 2) determine if the triceps surae and crural fascia synergistically influence propulsion.

Finally, in chapter 4 we investigate how muscle activation is directed functionally at the endpoint and whether the crural fascia influences that direction. We *hypothesize* that muscles with direct connections to the crural fascia will have a change in output direction after the complete fasciotomy. Our *objective* is to quantify the muscle output directions in different static kinematic configurations and determine how the crural fascia affects the force transmission.

CHAPTER 2

DISRUPTION OF THE CRURAL FASCIA INCREASES MEDIAL-LATERAL VARIABILITY DURING LOCOMOTION

2.1 INTRODUCTION

Previous studies of the crural fascia, a connective tissue surrounding the posterior, distal portion of the limb, have provided evidence for force transmission from the hamstrings to the ankle through the crural fascia under non-locomotor conditions (English and Weeks 1987; Hyde et al. 1999; Wicke and Zajac 1981). These studies suggest that the crural fascia could play a role in the motor function of the limb during locomotion by mechanically linking different limb segments. The crural fascia, in particular, links the thigh and leg segments due to its anatomical location, initiating proximally from the distal hamstrings and terminating on the calcaneus, with the body of the tissue surrounding the gastrocnemius muscles. This large sheet of tissue contains two thickened, tendinous bands that run vertically through the medial and lateral sides and are likened to the tendinous bands in the hindlimb of the cheetah as described by (van Ingen Schenau 1994). It was proposed that the tendinous bands in the hindlimb of the cheetah allow for distal propulsive force transmission from the proximal hamstrings to the distal calcaneus and therefore allowed for the coupling of plantarflexion of the ankle, flexion of the knee and extension of the hip thereby combining the actions of limb segments. Although the intersegmental linking and force transmission have been proposed it has not been experimentally shown during locomotion.

Given that the tendious bands may play a role in force transmission, little consideration has been given to the sheet that envelops the gastrocnemius muscles and the tendinous bands. The sheet of tissue is comparable to the extramuscular connective tissue investigated by Maas et al. (2005b). This study showed that after a full longitudinal compartment fasciotomy the force transmission capabilities of synergistic muscles were reduced. Furthermore Garfin et al. (1981) showed that compartment fasciotomy resulted in a decrease in muscle force produced under twitch and titanic contractions. These studies were conducted by investigating the total force magnitude of selected muscles pre and post fasciotomy. They did not account for the directions of muscle actions within the limb and how the crural fascia could be limiting or enhancing the range of output force direction. Given that the gastrocnemius muscles have been shown to have non-sagittal force outputs (Lawrence et al. 1993), it could be suggested that the sheet of the crural fascia surrounding the gastrocnemius muscles could play a role in limiting the limb forces in the non-sagittal plane, when muscles at the crural fascia origin are tensed, such as during uphill climbing or during movements with higher velocities. This could be particularly pertinent during tasks such as walking where increased sagittal force output is more efficient. Stability can be considered to be describing the trajectory as free from change or variability. Therefore, we define a stable limb during locomotion has having low variance between step trajectories and the limb is considered to be less stable if the variance increases. Therefore, we suggest that the crural fascia could be acting to increase stability in the frontal plane during locomotion by reducing variability in the trajectory of the limb.

In order to address the functional implications of the ability of the crural fascia in conducting force transmission and controlling muscle output direction we evaluated the kinematics of the limb during level walking in both the sagittal and non-sagittal planes. Furthermore we attempted to elicit the roles of the different anatomical components, the sheet and the tendinous bands, through sequential disruption. We *hypothesized* that the sheet would provide for medial-lateral stability by limiting the output directions and the bands would contribute to propulsive force. Therefore we predicted that the crural fascia would play a role in propulsive force transmission through the limb as well as stabilization of the distal limb during level walking.

2.2   METHODS

2.2.1   Surgical preparation

The effects of the sequential disruption of crural fascia were investigated in six decerebrate cats walking at 0.7m/s atop a treadmill. All experimental protocols were approved by the Emory University and Georgia Institute of Technology Institutional Animal Care and Use Committee.

For each experiment, the animal was initially anesthetized using isoflurane gas. An intravenous line of Ringers solution was inserted and sutured into the external jugular in order to administer fluids throughout the experiment. While under anesthetic the skin

over the gastrocnemius muscle in the right leg was longitudinally split from the popliteal fat pad behind the knee to within 1cm of the calcaneus. The skin was very carefully blunt dissected off the crural fascia and resealed using a flat-edged alligator clip. Bipolar electromyography electrodes were inserted into select muscles (to be described later).

The animal was then supported atop a variable speed treadmill in a natural stance by affixing the head to a stereotaxic frame and supporting the animal using a clamp applied to the base of the tail. A premamillary decerebration was performed where the brainstem was transected at a 45° angle beginning rostral to the superior colliculi and ending rostral to the mammillary bodies allowing for the preservation of the sub thalamic nucleus. All brain matter rostral and lateral to the transection was removed. Kinematic markers were taped to the left and right hindlimbs (further described in the kinematics section). The anesthetic was then slowly reduced until eliminated. Stepping was then evoked by either running the treadmill or occasionally with manual stimulation around the base of the tail. The tail height was then adjusted to ensure continuous paw contact with the treadmill during stance and clearance of the toe during swing.

Recordings were made under four, consecutive fascia disruption conditions: 1) intact – no manipulations to the crural fascia; 2) split – longitudinally split over the gastrocnemius muscles; 3) medial band cut – a horizontal cut through the fascia to medial side of the medial band; 4) lateral band cut – a horizontal cut through the fascia to the lateral side of the lateral band completing the fasciotomy (Figure 2.1C). After each disruption the skin was resealed with the alligator clip. A minimum of three trials were recorded under each

fascia condition; a trial is defined as a consistent, spontaneous period of walking for at

least 20 seconds. At the end of the experiment the animal was euthanized with 1ml

euthasol administered through the intravenous line.
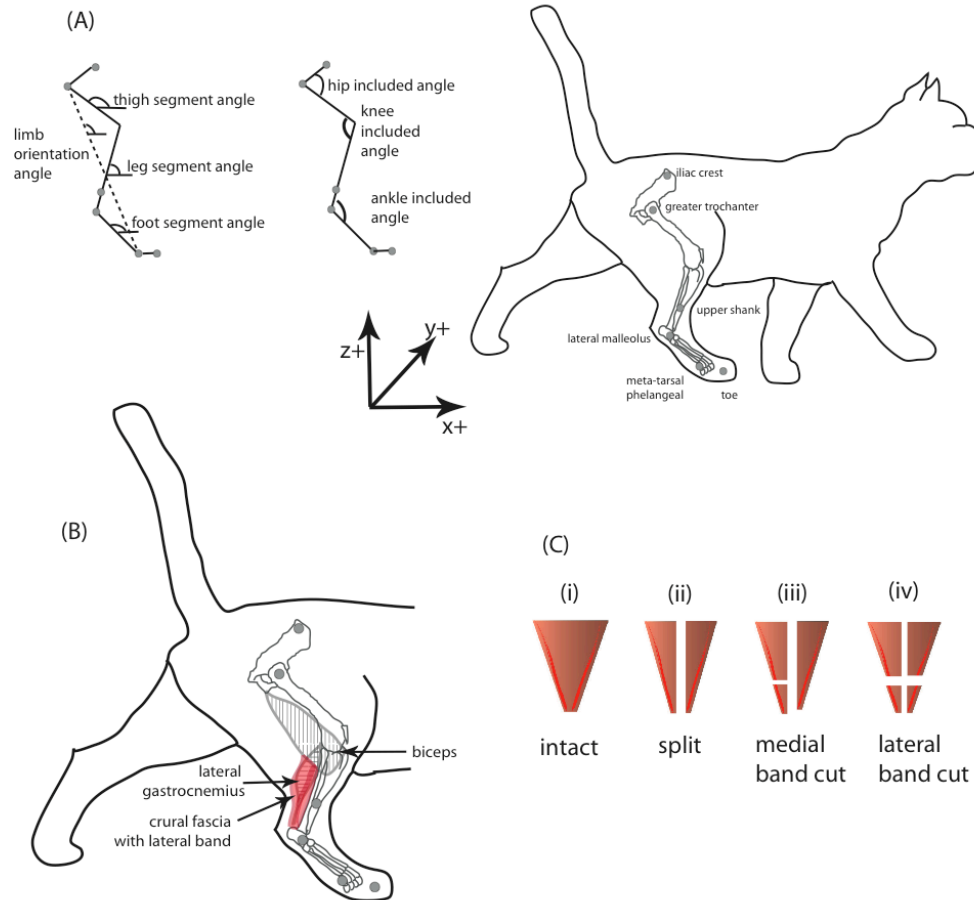
Figure 2.1. (A) Demarks the location of the kinematic markers on the right hindlimb of the cat. The positive direction of the axes and the sagittal plane segment angles are illustrated. The segment and sagittal angle representations are shown. Although the sagittal segment angles are depicted the frontal plane segments use the same demarkings. (B) The lateral view of the crural fascia originating from the biceps femoris muscle, surrounding the triceps surae and terminating on the calcaneous. (C) The sequential crural fascia disruption steps as seen from the posterior of the cat hindlimb. The darkened stripes represent the medial and lateral bands. The four disruption steps are: i) intact crural fascia; ii) longitudinally split from the fat pad to the calcaneus; iii) medial band cut over medial gastrocnemius muscle; iv) lateral band cut over lateral gastrocnemius muscle.

2.2.2 Kinematics

The three-dimensional trajectories of reflective markers on the right hindlimb were used to describe the mechanical changes observed under different fascia disruption conditions. Kinematic markers were placed on the right limb at the iliac crest, greater trochanter, upper shank (to calculate the virtual knee), lateral malleolus, meta-tarsal phelangeal joint (MTP) and toe (Figure 2.1A). The virtual knee was calculated as a vector projection from the lateral malleolus marker though the upper shank. The magnitude of the vector was the measured length of the leg from the lateral malleolus to the center of the knee joint. Three-dimensional kinematics were recorded from the spontaneously stepping, premammillary cat using either six Vicon cameras (cat 1, 2, 3) or two cameras and Peak Motus software (cat 4, 5, 6) at 125 Hz. When the Vicon cameras were used, reflective markers were placed on both the left and the right hindlimbs. Paw contact and paw off were demarked post-hoc by video analysis of the animal walking. The timings of paw contact and paw off were defined as when the toes initially make and lose contact with the treadmill respectively. This did not always coincide with maximal retraction and protraction of the limb. The data was analyzed using custom Matlab scripts and each marker was low pass filtered at 6Hz. The resulting 3D marker traces were then used for all analysis presented in this paper.

In order to fully visualize the net effect of complete fasciotomy of the crural fascia, stick figure reconstructions of the limb in the sagittal, horizontal and frontal planes were created for swing and stance. Each plane is defined in a global coordinate system that is consistent across all cats. The stick figures were reconstructed as three line segments

(thigh, leg and foot) from the filtered positions of the kinematic markers. The thigh segment was defined as a line from the greater trochanter to virtual knee; while the leg is defined as a line from virtual knee to lateral malleolus; and the foot is defined as a line from the lateral malleolus to MTP in the plane of interest. Each figure represents the overlaying of nine steps from one trial under the intact and complete fasciotomy conditions for stance and swing individually for all three planes.

In order to elicit the interlimb effects of the complete fasciotomy of the crural fascia we examined the resultant change in trajectory of the intertrochanteric line. The intertrochanteric line was reconstructed from the greater trochanter markers on the right and left limbs as viewed from the front of the cat. Black and grey lines denoted stance and swing for the right hindlimb respectively (Figure 2.3). Only the intact and complete fasciotomy are depicted to visualize the resultant role of the crural fascia in interlimb effects.

During swing, the foot often showed modest circumduction during movement. At this time in the step cycle, the foot is not grounded by the treadmill and can therefore move freely in the transverse direction. In order to evaluate the change in circumduction of the paw during swing we calculated the circumduction area. This area is defined as horizontal movement of the MTP marker during swing for the duration of the step length. Due to slight medial-lateral variations in paw placement at the initiation and termination of swing we baseline adjusted each step so that the paw contact and paw off were set to 0 in the medial-lateral direction and the linear offset was used to adjust all additional swing

points (example shown in Figure 2.4). The circumduction area was then calculated as the average under the curve for each step from the baseline-adjusted trajectory of the MTP during swing. The circumduction area from nine steps in each trial was evaluated and averaged together under each condition for each animal. The mean area and variance was then determined across all steps in a condition. A one-way ANOVA was performed on the circumduction area for each cat with an alpha value of 0.05. In order to compare the results between cats all results were converted to a percentage change from the intact crural fascia condition.

Furthermore, the MTP kinematic marker allowed for the calculation of the duty factor and contact length for each cat. The contact length was determined as the distance between the MTP marker at paw contact and paw off. The duty factor was computed as the percent time in stance of each step. A one-way ANOVA was performed in order to elicit any statistical difference between the sequential fascia disruption conditions.

Calculation of the segment angles provided a quantification of the orientation of the thigh (virtual knee to greater trochanter), leg (lateral malleolus to virtual knee) and foot (MTP to lateral malleolus) throughout the step cycle in the frontal and sagittal planes. The segment angles were calculated for five of the six cats as Cat 6 lost the lateral malleolus marker during the split condition trials. The sagittal and frontal plane angles were calculated with the distal marker at the origin and the angle computed following Cartesian coordinates (Figure 2.1). The frontal plane segment angles were calculated as though looking at the cat from the front of the cat while the sagittal plane segment angles

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were calculated as though looking at the cat from the lateral side with the head towards the 0 degree. Nine consecutive steps from each trial were then normalized to 4% bins and averaged. Due to slight differences in base angle of trajectory of the segments in each trial in the same condition, all trajectories in a fasciotomy condition were averaged and the offset for each trial was then calculated. Each step in a trial was then adjusted by the corresponding offset value. This resulted in mean adjusted trajectories for each condition for each cat, thereby removing any bias in the variance calculation that could be attributed the trials in a condition not having the same mean. The variance was calculated for each bin in the step cycle and then summed across the step cycle.

The included angles of the hip, knee and ankle were calculated in order to investigate the relationship between the segments. Furthermore by differentiating the results twice we are able to calculate the angular accelerations of the joints. In order to provide a more global view of the role of the crural fascia only the intact and complete fasciotomy conditions were quantified and evaluated. The resulting angular and acceleration trajectories were then normalized to 4% bins in order to average nine steps from three trials under each condition together. A t-test was performed on the acceleration at paw contact, paw off and peak acceleration during stance for each cat to determine statistical difference in results.

An important methodological issue was the possibility that the effects we observed might have been due to deterioration of the preparation. This possibility was difficult to test due to the fact that it was not possible to reverse the fasciotomy, despite numerous

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attempts to do so. In order to preclude this possibility, all trials were collected within an

hour of the onset of locomotion even though most cats continued to walk for upwards of

four hours.

2.2.3 EMG electrodes

Pairs of bipolar electromyographic wires were inserted into semitendinosis (ST, 4 cats),

posterior biceps femoris (pBF, 2 cats), semimembranosus (SM, 4 cats), gracilis (G, 2

cats), medial gastrocnemius (MG, 1 cat), and tibialis anterior (TA, 2 cats) and sutured

into place with surgical silk. Not every cat had the same combination of muscles.

Mineral oil was applied to the muscles and the skin incision closed with staples. The

EMG recordings were high pass fourth order Butterworth filtered and rectified post-hoc

for each trial. The first 10 consistent steps of each trial were compared to determine if

there was a change in EMG activity profiles. However only the first three steps will be

shown in the paper.

<div align="center">

2.3   RESULTS

</div>

2.3.1   Stick figures

In the sagittal plane (Figure 2.2A) the pre and post fasciotomy plots for swing and stance

look very similar suggesting that the crural fascia has little effect on the sagittal plane

kinematics during level walking. Upon close comparison of the two conditions it is

<div align="center">

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</div>

observed that after complete fasciotomy in swing and stance there is a mild increase in variability of the greater trochanter marker. Furthermore during swing the foot is placed more anterior to the intact crural fascia and the ankle appears to be more flexed (although this was not observed in every cat).

The stick figure representations (Figure 2.2A) clearly show an increase in variance in the medial-lateral direction post complete fasciotomy and represent one of the major findings presented in this paper. During stance in the non-sagittal planes (Figure 2.2Bi and 2.2Ci) the paw is initially abducted and then adducted towards paw off therefore slipping on the belt. At the initiation of swing (Figure 2.2Bii) the limb initially abducts to mid swing and then adducts to paw contact. This trajectory of the paw through swing will be referred to as limb circumduction. Although this overall trend in limb configuration through the step cycle was observed, two cats showed an exaggerated adduction of the paw during stance. We suspect that these cats were lower weight bearing due to the ability of the paw to slip during stance. Furthermore they also showed a large adduction of the limb at paw off (see chapter appendix for further details). Post fasciotomy all cats showed a similar trend although there were some individual differences. The adduction of the limb decreased during stance and the foot overall followed a more linear trajectory. However, there was a noticeable increase in the variability of the limb segments (also observable in the frontal plane Figure 2.2C iv). Although the paw trajectories were more linear during stance the consistency of the paw off location decreased and occasionally showed wide medial-lateral fluctuations in position. This variability continues into swing when the limb would initially vary in medial-lateral excursions (Figure 2.2Biv and 2.2Civ).

Intriguingly, on occasion it was noted that the right limb would initially swing so medially that it would interfere with the left limb (see chapter appendix). Furthermore, the toe and greater trochanter both showed a greater range of medial-lateral movement post-fasciotomy and in two out of five cats the limb was placed more laterally to the greater trochanter post fascia disruption (not depicted in the example shown in Figure 2.2) which resulted in a wider stance. This greater medial-lateral movement of the greater trochanter could be attributed to the increased abduction and adduction of the limb through the step cycle.
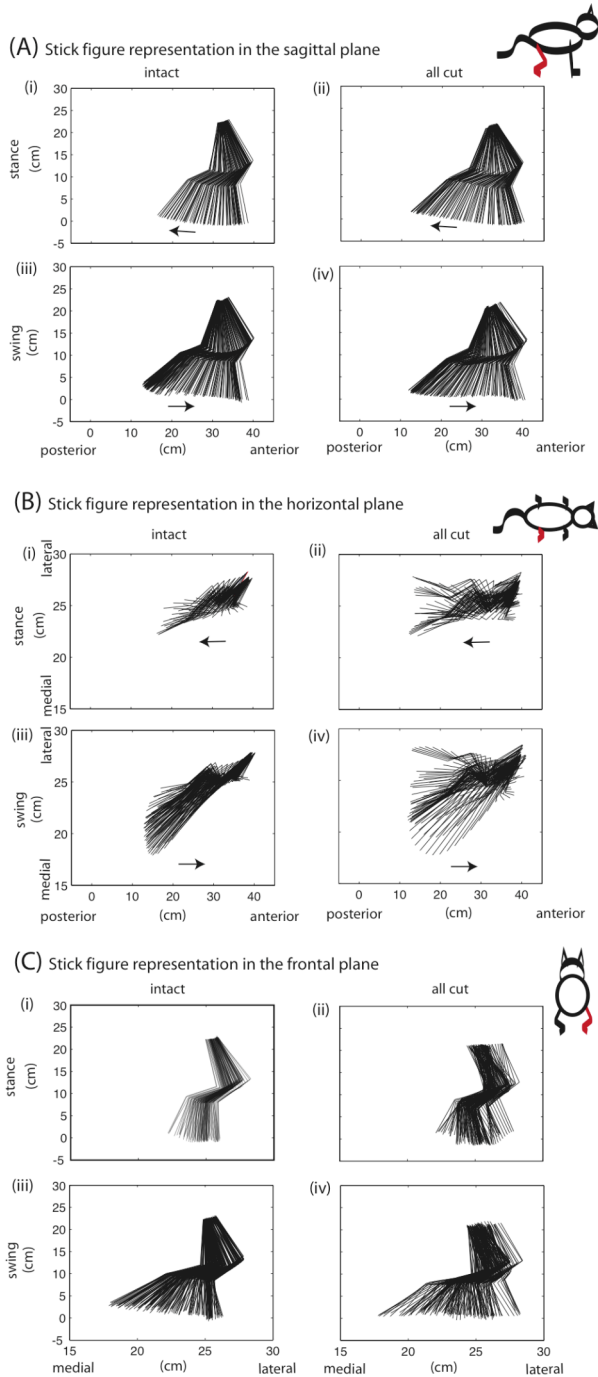
Figure 2.2. The stick figures represent the right hindlimb during swing and stance in the (A) sagittal (with front of cat to the right), (B) horizontal plane (looking down on the cat with the head to the right) and (C) frontal plane (medial to the right, as though looking at the cat from the front) under intact and fascia disrupted conditions: (i) intact stance (ii) all fascia disrupted stance (iii) intact swing (iv) all fascia disrupted swing. The arrows represent the direction in which time would be traveling in the horizontal and frontal planes. The segments represent the thigh, leg and foot in descending order. Notice the increased variance in segments in the horizontal and frontal planes when the fascia has been disrupted. Less of a difference is discernable in the sagittal plane.

2.3.2 Intertrochanteric line

The intertrochanteric line was examined in the frontal plane to describe the interlimb effects after complete fasciotomy. The intertrochanteric line is represented in the frontal plane as a line from the greater trochanter of the right limb to the greater trochanter of the left limb as shown in Figure 2.3 as viewed from the front of the cat. This reconstruction was performed on three cats and cat 5 is illustrated in the figure. The intertrochanteric line in the intact case depicts an oscillation between each limb as they in turn rise during swing and fall during stance. Upon complete crural fascia fasciotomy of the right limb a tilting of the intertrochanteric line is observed with the right side being lifted higher during swing and stance while the left side is lower. Both sides appear to have shifted by approximately 0.5 cm. The simultaneous shifting may be due to the mechanical linking of the greater trochanter on each limb by the connection to the pelvis. The center of the pelvis is somewhat restricted by an elastic restraint in medial-lateral and vertical movement by the clamp at the base of the tail. Therefore the clamping may contribute to the tilting resulting in the increase and decrease in the height of the right and left greater trochanter respectively. Furthermore the clamping may potentially limit the movement of the pelvis and we surmise maybe the pelvis as a whole would rise, with less tilt in the non-clamped preparation. Additionally, the right hindlimb greater trochanter marker depicts an increase in lateral excursion during swing while the left greater trochanter marker does not show a compensatory change. This increase in excursion was also observed in all cats.
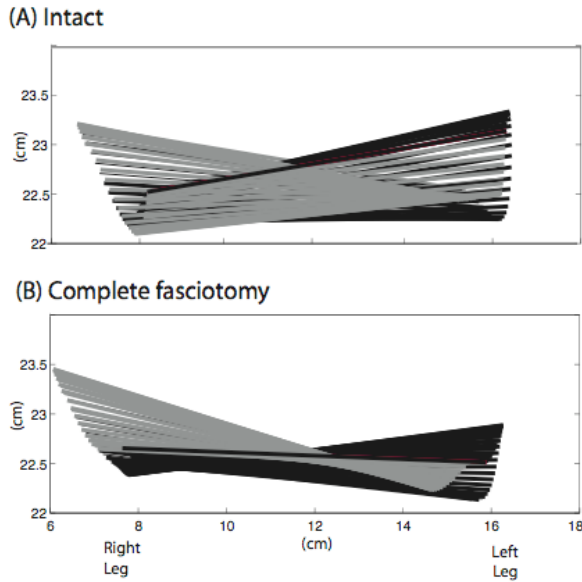
Figure 2.3. The intratochanteric line is plotted as a line from the right limb to the left limb as viewed from the front of cat 5. One step from each condition is represented in this figure. Stance for the right limb is in black and swing is in grey. Post fasciotomy (B) there is a decreased height span as the right limb greater trochanter is in a higher position in both stance and swing resulting in the left limb not rising as high during swing. Additionally the right limb shows an increased lateral excursion during swing.

2.3.3 Effects on limb endpoint

The effect of the fasciotomy at the limb endpoint (MTP trajectory) in the horizontal plane was examined in order to elucidate the cumulative effect on the limb. Having already observed an increase in variability along the limb segment as shown in Figure 2.2 stick figures as well as a change in the trajectory of the intertochanteric line as shown in Figure 2.3 during swing, we examined the centered overlaying trajectories of the MTP marker and quantified the circumduction area and its corresponding variance during swing. The results depicted in Figure 2.4A of the centered MTP trajectory show an increasing trend across the fascia disruption conditions with respect to both circumduction area and variance. Stance was also evaluated but did not show a change in area due to the linear

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paw contact with the treadmill, which grounded and limited the movement of the end

point of the limb. The MTP trajectory during swing is shown in Figure 2.4A where the

centered MTP trajectories from cat 6 are plotted on unequal axes (the medial-lateral axis

has been amplified).   When the fascia is intact the trajectories line up consistently except

for two outliers in this example and was found to have a mean circumduction area of 21

cm^2. Upon the longitudinal split of the crural fascia the circumduction area increased

from 21 cm^2 to 35 cm^2 (Figure 2.4Ab). This increasing trend in circumduction area is

observed in four out of six cats and when averaged across cats the circumduction area

increased by 11% from the intact condition. After the split the medial band was

transected and the circumduction area increased in five out of six cats by 20% from the

intact circumduction area (Figure 2.4B). Two of the cats (cat 3 and cat 4) had the highest

variance with one band disrupted, which suggests that the asymmetry created by having

one intact band may cause an imbalance in force or control at the point of fascia

insertion. Upon complete fasciotomy the average circumduction area increased to 48 cm^2

for cat 6 (Figure 2.4Ad) and increased by an average of 34% across cats. The trend to

increase in circumduction area is shown in Figure 2.4B from intact to all cut is observed

in five out of six cats with one cat (cat 3) initially having a larger initial circumduction

area that decreased after the split.

It was noted that upon the sequential fascia disruptions the centered MTP trajectories

became more variable. When quantifying the variance of the circumduction areas across

steps we found that the variance increased from 289 to 1388 cm^2 in the depicted example

from intact to complete fasciotomy respectively. The circumduction area variance

increased for five out of six cats when splitting the crural fascia with an average 150%

across all cats from the intact variance. When the medial band was transected the

variance across cats increased by 163% from the intact condition. Upon complete

fasciotomy the variance increased by 405% from the intact condition. The increase in

variance from intact (Figure 2.4Bb) is observed in five out of six cats with cat 2 initially

showing a large variance that decreased after the split but subsequently followed the

trend to increase with the three remained fasciotomy stages. Overall the effect on the

circumduction area variance was larger than the circumduction area upon the sequential

disruption of the crural fascia during swing.

Although the above measures on the horizontal plane projection showed a large change in

variability the contact length and duty factor did not change when evaluated in the

sagittal plane. The contact length percentage of control averaged across cats was: 94%

when split, 96% with the medial band cut and 102% with all disruptions. The

corresponding duty factor percentage change is 100% when split, 97% with the medial

band cut and 105% with all cut. No statistical difference was discerned when tested with

an ANOVA.
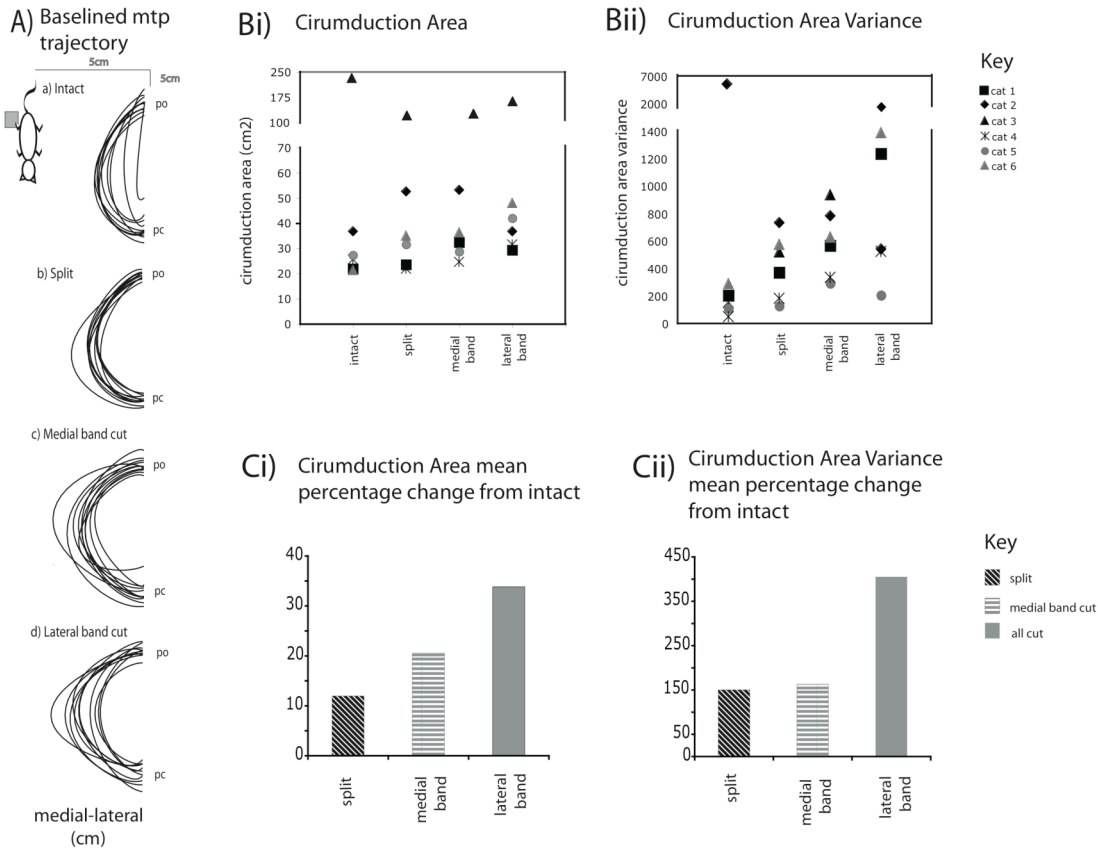
Figure 2.4. (A) depicts the nine steps from a trial under the four fascia conditions in one cat. This figure shows the increase in circumduction area as well as highlights the increased variance of the swing under the different fascia cut conditions. **(Bi)** Shows the circumduction area for all six cats under the four fascia disruption conditions. Overall there is a general trend to increase the area with each sequential disruption. One cat had a particularly high area and is therefore demarked on a segmented axis. **(Bii)** The circumduction area variance for all cats shows an increasing trend of increasing variance upon the sequential disruptions of the crural fascia. **(Ci)** The percentage change from intact for the mean of all cats depicts a small increase in circumduction area as you further disrupt the crural fascia. **(Cii)** The variance shows a dramatic percentage increase compared to the area with each of the fascia disruption steps.

2.3.4 Sagittal segment angles

The mean sagittal plane segment and limb angles did not show a significant difference in trajectory (Figure 2.5Ai) after sequential fasciotomy. The sagittal plane angles describe the limb segment and overall limbs' orientation with the distal end assumed to be at the origin of the vectors on Cartesian coordinates. The orientation can be visualized by stick

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figure representation over the step cycle in Figure 2.5Aii. The limb is initially protracted at paw contact; becomes vertical by mid stance; retracts to paw off; and protracts through swing to paw contact. During limb protraction to paw contact the thigh and foot were protracting while the leg is in a near vertical configuration. As stance progresses the limb orientation angle and the thigh and foot segment angles become more vertical while the leg is retracted to a near horizontal position. During limb retraction to paw off the foot and thigh also retract while the leg remains in the near horizontal retracted position. In swing, the limb, thigh and foot protract to paw contact while the leg only begins to protract in mid swing returning to a near vertical configuration. These configurations do not change upon the sequential fascia manipulations.

Although the mean angles do not show a significant change, the variance of the segment angles shows a marked change between the segments and crural fascia manipulations (Figure 2.5B), with the highest change in variance for most angles being after the medial band is cut. The foot segment angle has the highest overall variance followed by the thigh, limb and leg angles in descending order. The percentage change in variance across cats in the sagittal plane (Figure 2.7A) shows that the highest change in variance occurs when the medial band is disrupted for the foot, leg and limb angles suggesting an asymmetry in the system with one remaining band potentially increasing non-sagittal movements. The change in variance decreases again once the lateral band has been disrupted suggesting that the symmetry has been restored, however the variance is still higher than the intact or split condition. The thigh segment angle has the highest
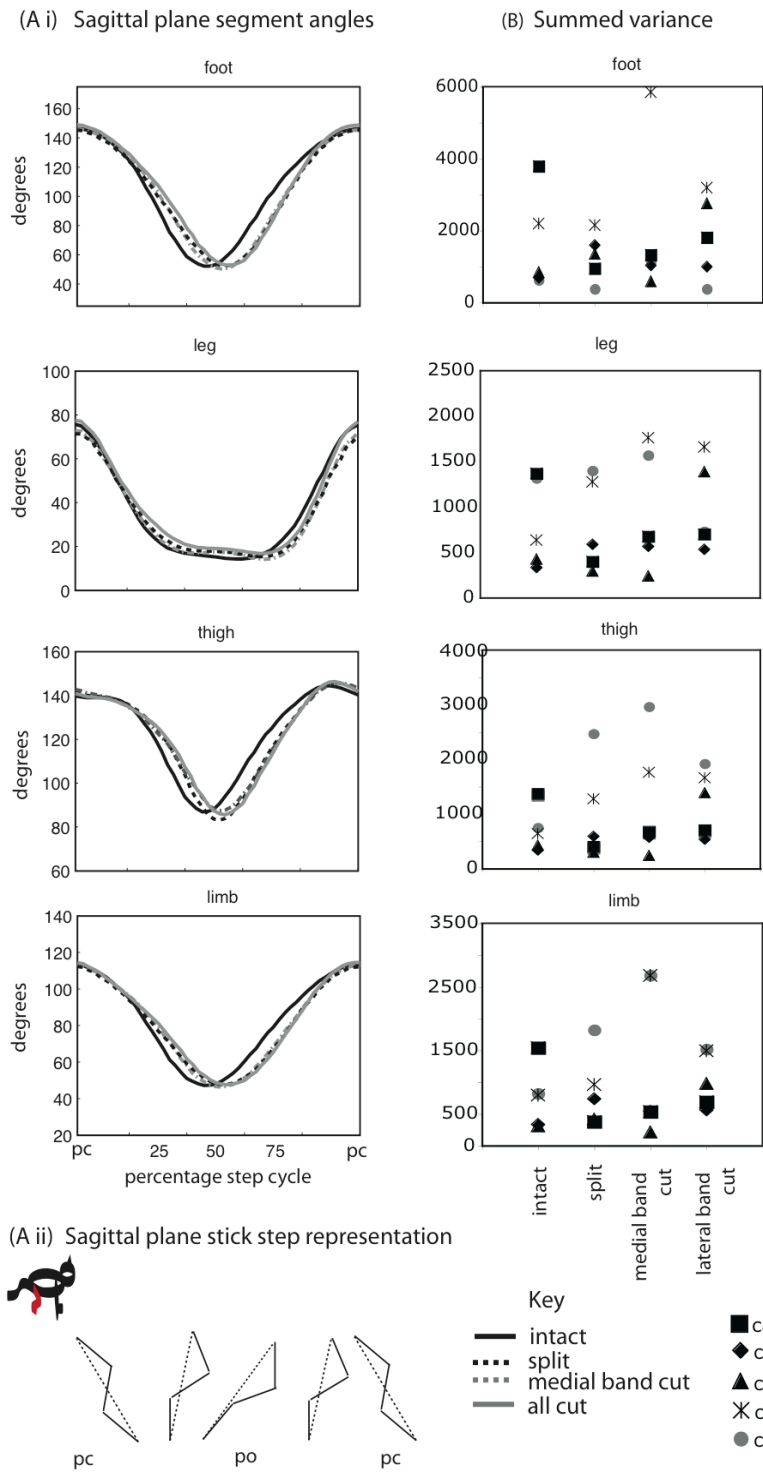
Figure 2.5. (Ai) The mean sagittal plane segment angles under the different fascia disruption conditions are shown. There is little difference between the mean traces. (ii) shows the stick figure representation of the angles of the segments and limb through the step cycle from paw contact to paw contact. (B) The variance for each of the segment angles for each condition for each cat is displayed. The foot shows the greatest variance of all the segments. A general trend to increase the variance post fascia disruption is observed.

percentage change in variance by just splitting the crural fascia. This remains high with the medial band cut but decreases with the lateral band cut; therefore the thigh segment may be more influenced by the lateral band and its ability to transfer force through the sheath.

2.3.5 Frontal segment angles

The frontal plane mean segment and limb angles show an increase in abduction with the consecutive fascia disruptions (Figure 2.6). The angles of the segments and the limb are oriented with the distal section at the origin in Cartesian coordinates as though viewing the angles from the front of the cat (Figure 2.6Ai). These profiles displayed some variation in specific details across cats but the general pattern was consistent across cats. For clarity, the segment angles are represented in a stick figure below the angle trajectories, with the total limb represented with a dashed line (Figure 2.6Aii). In the intact right hindlimb the overall limb orientation in the frontal plane begins in an abducted position at paw contact and then adducts through stance to paw off and then abducts through swing back to paw contact. In the intact right hindlimb, at paw contact the limb is initially abducted with the paw lateral to the body and the thigh and foot segment angles abducted while the leg is adducted. As the limb progresses to paw off the limb and segment angles begin to adduct and the paw is moved more medial to the body; while the foot approaches a vertical configuration. At the start of swing the limb, leg and foot segments abduct while he thigh does not abduct until mid swing resulting in circumduction of the paw.

After splitting the crural fascia the foot and leg segment and the limb angle all shift upward implying that they increase in adduction. The change in angle implies that the limb and foot become more vertical while the leg becomes more horizontal. The largest effect is observed around paw off with the increase in adduction of the leg potentially contributing to the increase in the initial medial swing observed during paw circumduction. Therefore the before noted increase in circumduction can be contributed to more adduction at paw off as well as an increase in abduction during swing. After sequentially disrupting the medial band the angles shift down or abduct to return to closer to the intact trajectories although some cats did not show this decrease in angle (data not shown). But upon the subsequent cutting of the lateral band resulting in the complete fasciotomy the foot and leg increase in adduction, particularly at paw off while paw contact is more conserved. The leg segment angle adducts post fasciotomy resulting in the leg becoming more horizontal throughout the step cycle especially at paw off. These segment changes imply (and are observed in the stick figure representation in Figure 2.6Aii) that the knee become more abducted and the ankle more adducted post complete fasciotomy with a large effect observed around paw off.

Although the frontal plane segment and limb angles show a change in trajectory upon the sequential disruption of the crural fascia, an even more pronounced increase is observed in the summed variance of the angles (Figure 2.6B). The foot segment has the highest variance followed by the leg, limb and thigh in descending order. The overall trend for

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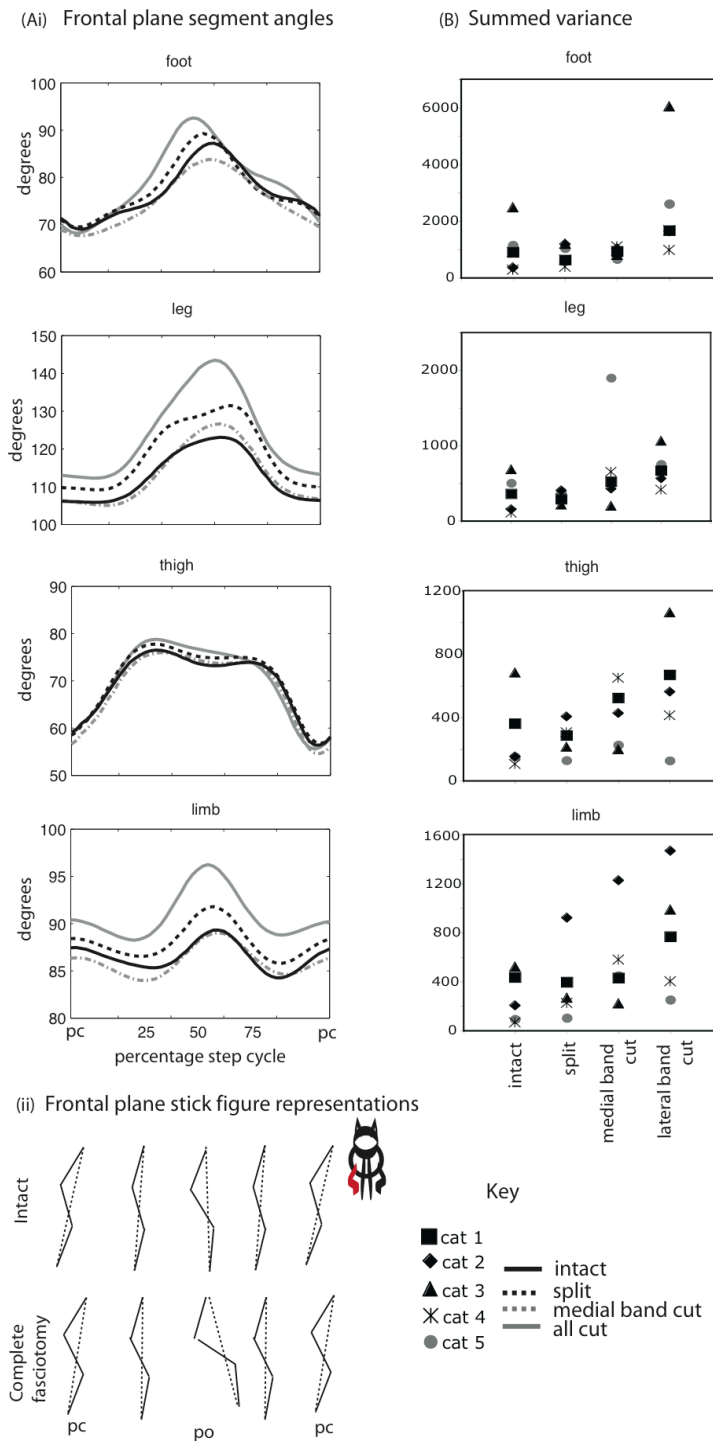
Figure 2.6. (Ai) The mean, normalized frontal plane segment and limb angles show a shift up in angle post fasciotomy (or an increase in abduction). (ii) A stick figure representation of the segment angles for intact and post-fasciotomy show a significant change in limb configuration around paw off. (B). The mean summed variance for each cat under each condition for the foot, leg, thigh and limb are plotted. The foot has the highest variance of all the segments and there is an increasing trend across segment variance post fascia disruption.

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each segment appears to increase upon the sequential fascia disruption steps however when the percentage change from intact is calculated and averaged across animals (Figure 2.7). The post-medial band cut condition illustrates the highest change in variance from intact for the leg, thigh and limb. The mean percentage change for the foot segment increases the most with the fascia split and then slightly decreases with the remaining steps. Intriguingly, the limb summed variance shows that largest change in variance in response to fascia disruption steps of the frontal plane angles.

(A) Percentage change in sagittal plane   **(B) Percentage change in frontal plane**
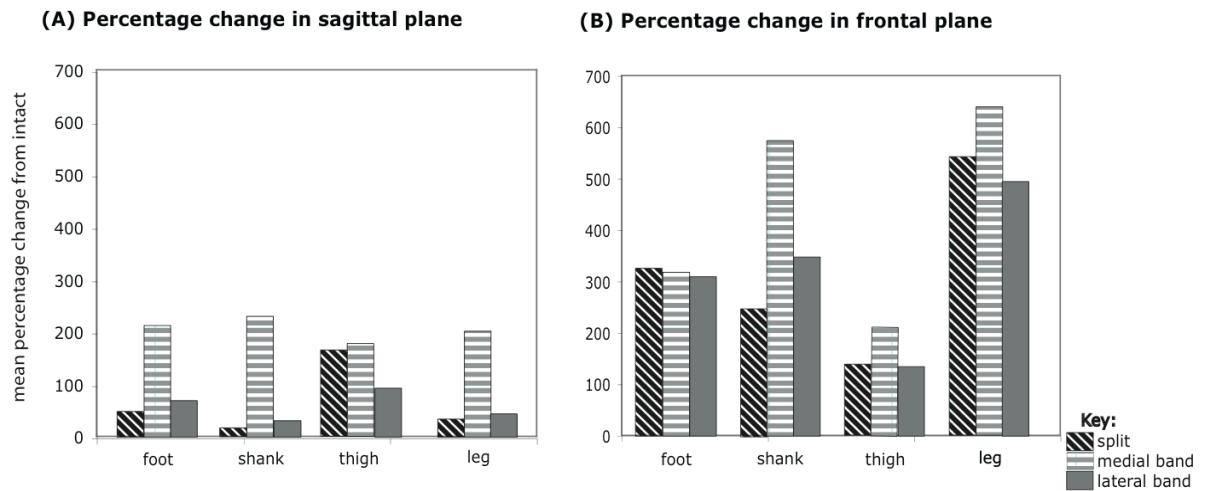
Figure 2.7. The graphs show the percentage change in variance from intact for each segment averaged across all cats for the (A) sagittal and (B) frontal plane segments. The medial band cut condition shows the greatest percentage change for most angles in both the sagittal and frontal planes. Intriguingly, the leg segment shows the greatest change in the frontal plane.

2.3.6 Included angles and acceleration

Included angles (Figure 2.8) in the sagittal plane were also measured to test whether fasciotomy results in reduced acceleration (see Methods). Although the included angles in the sagittal plane changed only modestly across the stages of fasciotomy, the velocities and acceleration showed significant changes at the hip, knee and ankle joints. Each joint will be described first in regard to stance and then in regard to swing. The hip joint is flexed at paw contact and extends with increasing velocity and constant acceleration to about three-quarters of the way through stance. After paw off the hip flexes through swing at a increasing velocity and increasing flexion acceleration to mid swing when the velocity and acceleration begin to increase in the extension direction. The acceleration peaks just prior to paw contact and begins to decrease in preparation for the reversal of the joints direction. Post fascia cutting four out of five cats had a statistically significant loss of extension acceleration at paw off and two out of five at paw contact. The knee is slightly extended at paw contact and flexes through mid stance as the center of mass passes over the leg. It then extends to paw off to provide propulsion. The velocity and acceleration increase in the extension direction through the majority of stance peaking just prior to paw off when max propulsive force is applied. The acceleration then increases in the flexion direction of the joint and flex the joint through mid-swing before extending to paw contact. The velocity and acceleration increase through the majority of swing peaking just prior to paw contact. Post fascia cutting four out of five cats showed a statistically significant decrease in extension acceleration at paw off. The ankle joint is slightly extended at paw contact and flexes softly as the center of mass passes over the joint and then extends to paw off. The velocity and acceleration is constant for the first

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part of stance and then increases in the dorsiflexion direction to just prior to paw contact. The velocity decreases for the first half of swing and then increases in the second half while the acceleration increases in the dorsiflexion direction for the majority of swing. Both velocity and acceleration reverse prior to paw contact. Post fascia cutting three out of five cats showed a statistically significant decrease in plantarflexion acceleration post fascia cutting and three out of five cats at max dorsiflexion acceleration during stance.
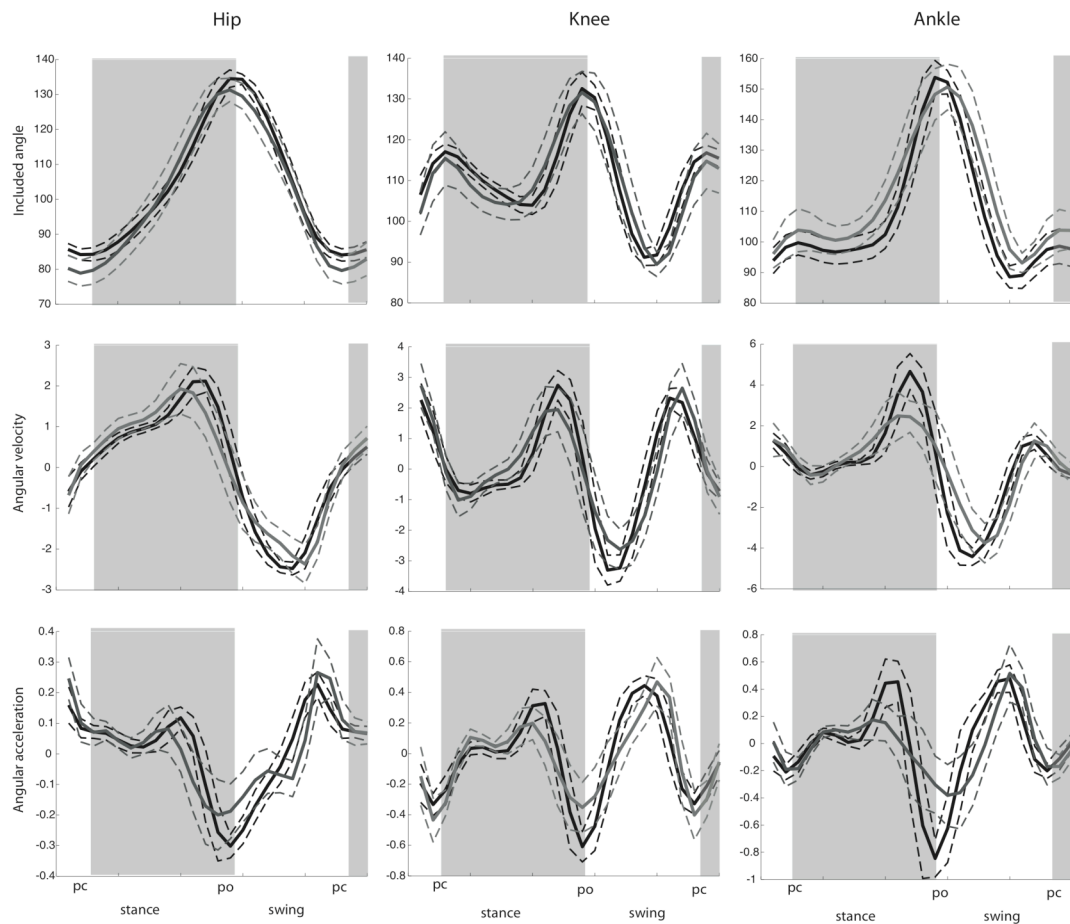
Figure 2.8. The included angle of the hip, knee and ankle joint (columns) in the sagittal plane and the corresponding angular velocity and acceleration (rows) are plotted The hip, knee and ankle included angle doe not show a significant difference between intact and all cut however the deceleration at paw off is very reduced. The knee and ankle also show a loss of acceleration during late stance. The black lines represent the intact crural fascia and the grey after complete fasciotomy. The solid line represents the mean and the dashed the standard deviation.

2.3.7 Electromyography

The electromyography recordings from select muscles in the spontaneously locomoting, decerebrate cat do not change in activation pattern upon the sequential disruption of the crural fascia (Figure 2.9). Although changes in amplitude were observed, it was noted that all muscles for a cat increased or decreased in amplitude in an experiment however the amplitude change was not correlated to kinematic changes and therefore not considered in this paper.

The activity of the propulsive muscles was observed during the stance phase of the walking cycles. These muscles, MG (ankle plantarflexor and knee extensor), aBF and SM (hip extensor and knee flexor) generally began activity just prior to paw contact and continued through most of stance. The timing of these muscles was relatively invariant from animal to animal. The recordings of TA (an ankle dorsiflexor) are characterized with the onset of activity just prior to paw off and continued during early swing.

The recordings from knee flexor muscles showed variable recordings across cats: pBF, ST and G. The recorded EMG activity of pBF in Figure 2.9 illustrates activity at paw contact continuing into early stance. An additional recording (not shown) depicted activity around paw off only. The ST recordings shown illustrate a main burst of activity around paw contact and a small burst at paw off (also observed in a second cat). However we also observed a second pattern of activity in one of our cats where only a burst of activity at paw off was recorded (data not shown). G had distinct phases of activity at paw contact and paw off although the paw off activity was considerably

smaller. A second recording (different cat) only showed a burst around paw contact. Intriguingly, none of the activity profiles changed upon the sequential disruption of the crural fascia.
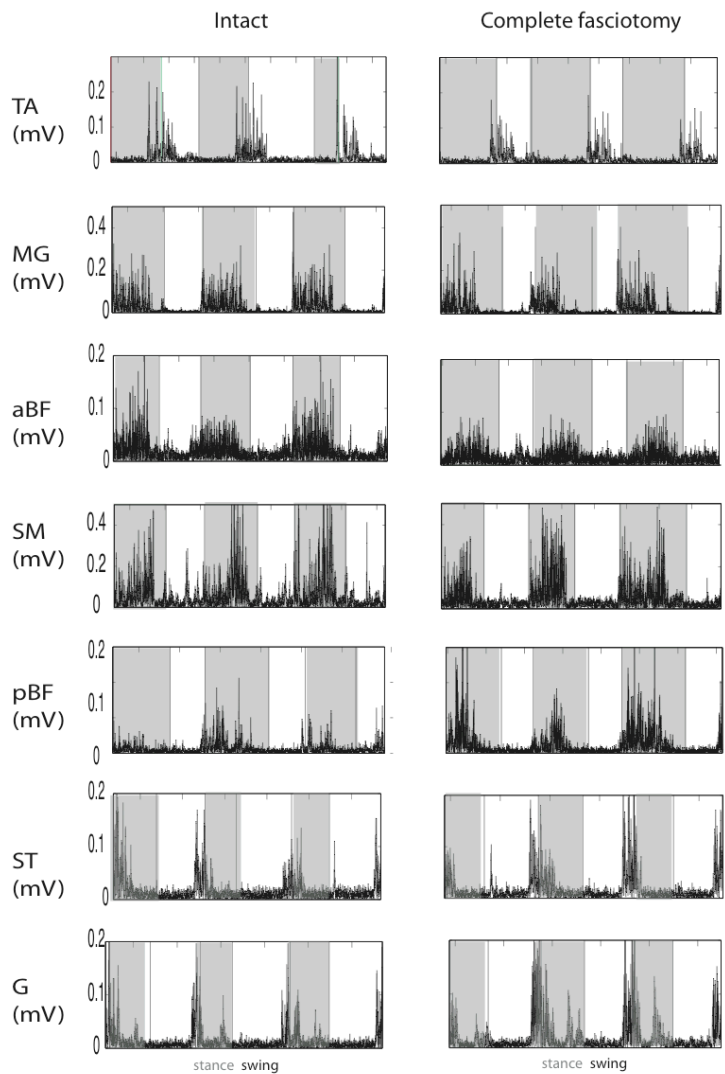
Figure 2.9. Example EMG traces of three steps from different cats under the intact and complete fasciotomy conditions. The grey shaded areas represent stance and the white swing. Although some of the muscles show a slight change in amplitude, they do not show a change in firing pattern.

2.4 DISCUSSION

2.4.1 Summary

We mechanically disrupted the crural fascia in order to investigate the role of this structure in the coordination of the right hindlimb of the spontaneously locomoting decerebrate cat during level walking. In order to fully describe the control of the hindlimb we examined horizontal, frontal and sagittal plane kinematics in conjunction with EMG recordings. In doing so we addressed two main questions. First, does the crural fascia act to transmit propulsive force to the ankle joint from the hamstring muscles? Second, does the crural fascia act to stabilize the distal limb by restricting movement in the medial-lateral direction during swing? We observed no change in duty factor or contact length, and seemingly modest changes in mean segment angles in the sagittal plane following crural fasciotomy. The significant decreases in angular acceleration did, however, support our first hypothesis that the crural fascia contributes to propulsion during level walking. The increased variance of the excursion of the foot during swing and the frontal plane segment angles supports our second hypothesis that the crural fascia acts as a mechanical stabilizer. As we sequentially split the fascia and then transected the medial band, the variance progressively increased. Cutting the lateral band resulted in a decrease in variance, but the variance remained larger than in the control case. Under the conditions of our experiments, the stabilizing effects were apparent primarily in the frontal plane. The lack of change in EMG activity in the observed muscles indicated that the effects we observed resulted mainly from the mechanical actions of the crural fascia rather than from the disruption of any sensory information transmitted by or from the

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fascia. By completing the studies in our decerebrate preparation we were able to investigate the effects of fasciotomy immediately following the procedure, therefore avoiding any possible adaptations that might occur during surgical recovery in a chronically treated and otherwise intact animal. Our reduced preparation also precluded visual and vestibular inputs due to the level of transection and fixation of the head in the stereotaxic frame.

2.4.2 Comparison to Intact Studies: Fascia intact

The kinematics we observed during locomotion were similar to those observed for intact animals walking both overground (Carlson-Kuhta et al. 1998) and on a treadmill (Bouyer and Rossignol 2003; Chang et al. 2009), and for decerebrate animals walking on a treadmill (Akay et al. 2006; Delivet-Mongrain et al. 2008). The similarities include both the phase dependence of joint angles and the ranges of joint angles. We also observed retraction and abduction of the limb in the early swing phase as occurs in the intact animal, followed by protraction and abduction (circumduction) during swing. The only minor difference that we noted between our results and those obtained from intact animals was that, during stance, intact animals exert a small lateral force (Bouyer and Rossignol 2003), while in our case a small medial force was suggested by the foot slipping slightly medially during stance. This frontal plane movement was revealed by the partial weight support.

The EMG recordings from the spontaneously locomoting decerebrate cat are similar in timing to those recorded from the intact cat. Our recordings of propulsive muscles: MG, aBF and SM, showed activity from paw contact through the majority of stance. The recordings of MG and aBF compare in timing to the recordings from intact cats ((Rossignol 1996) (MG and aBF), (Misiaszek 2006) (MG) and (Carlson-Kuhta et al. 1998) (aBF)) and the recordings of aBF and SM compare in timing to muscular activity in the decerebrate cat during level walking (Gottschall and Nichols 2007). However, recordings from aSM in the intact cat are more variable, either occurring around paw contact and during mid swing or around paw contact and from paw off through most of swing (Rossignol 1996). The full stance activity of aBF and SM suggest that they provide more propulsive force through strong hip extension actions while pBF and ST are only active at the beginning of stance. These muscles have a greater knee flexion torque and may not contribute to propulsion beyond early stance. Furthermore, the recordings of the knee flexors pBF, ST and G are variable in both intact and decerebrate cats. The variable patterns of activity for pBF in our experiments is paralleled in recordings from intact cats, where there are two different EMG patterns described, one with activity around paw contact and one with activity around paw off (English and Weeks 1987; Rossignol 1996) The variable pattern of ST observed in our study, also bears similarities to the patterns observed in intact animals (Carlson-Kuhta et al. 1998; Misiaszek 2006; Rossignol 1996). Our recordings from TA indicated activity from around paw off through most of swing. This pattern of activity is consistent with recordings from intact cats (Carlson-Kuhta et al. 1998; Misiaszek 2006; Rossignol 1996).

2.4.3 Passive mechanical action of the crural fascia

We expected that the crural fascia might provide sensory feedback from sensory endings within the tissue, or from the transmission of stretch to the muscles connected to the crural fascia and thereby might stimulate receptors within these muscles. The alterations in frontal plane kinematics and the lack of gross changes in muscular activation indicate that the crural fascia has important direct mechanical actions that could explain our findings. We cannot preclude that there were variations in the magnitudes of the EMG recordings that we could not detect given the natural variability in this signal, however. An investigation regarding the reflex feedback pathway between the hamstrings and the gastrocnemius muscles was performed by Hyde et al. (1999) using the crural fascia to stretch the hamstrings while simultaneously stretching the gastrocnemius muscles through the calcaneal tendon. The results showed that the hamstrings have a small inhibitory effect on the gastrocnemius muscles (decreasing the force response by 10%). These effects could be transmitted by the crural fascia in response to external perturbations. It is therefore quite likely that the associated sensory pathways would have some effect during locomotion, but these effects were not apparent in the results in the present study, suggesting that the sensory feedback from the hamstrings muscles was influenced predominantly by the changes in length and force brought about by centrally generated activity and movements of the leg relative to the thigh through the major tendinous attachments of these muscles.

The crural fascia may also be acting as a passive mechanism to improve the energy efficiency at the stance to swing transition. The significant decrease in plantarflexion

acceleration at paw off after the complete fasciotomy implies that the crural fascia acts to

change the limb direction at a faster rate suggesting that the crural fascia may be an

elastic structure that becomes loaded during stance and unloaded at paw off. Even if the

crural fascia is not elastic, its connection from pBF and ST and their activation at paw off

may allow for the crural fascia to transmit some of the muscular force to the endpoint and

begin to change the limb direction. By having the crural fascia increase the acceleration

of the joints at the transition point is a more efficient mechanism than using muscles to

achieve the same goal. The use of actuators would require the co-contraction of

extensors and flexors to stiffen the limb, which is energetically demanding.

2.4.4   Effects of fasciotomy on coordination

2.4.4.1 Propulsion

The musculature of the limb provides the flexion and extension of the limb during

locomotion and the corresponding propulsive force. It has been suggested that passive

structures such as the crural fascia may play a role in redirecting the force output (van

Ingen Schenau 1994). Although a significant change in joint angles in the sagittal plane

was not observed post fasciotomy, there was a significant change to the acceleration

profiles suggesting that the crural fascia can potentially play an important role in the

force transmission through the limb. The reduction in dorsiflexion acceleration about

three-quarters of the way through stance after the complete fasciotomy (coinciding with

the approximate end of EMG activity of the propulsive muscles during stance) suggests

that there is a loss of retraction of the limb during stance as the connection from the hip to the ankle created by the hamstring-crural fascia complex will act to retract the shank segment in particular. The retraction of this segment will increase the dorsiflexion acceleration. Furthermore the loss of plantarflexion acceleration in late stance after the complete fasciotomy suggests that the crural fascia could also be propulsive in late stance. At this timepoint ST and pBF are active and the crural fascia could be pulled by the activation of these muscles resulting in an increase in propulsion prior to paw off.

It can therefore be postulated that the crural fascia could facilitate movements with higher propulsive demands such as running, walking uphill or jumping. These tasks have a greater need for joint angle acceleration and usually have a greater metabolic cost than level walking. We can therefore surmise that the crural fascia could play a role in increasing the joint acceleration and provide additional force transmission through the limb with reduced metabolic demand. Furthermore at higher walking speeds there is a decrease in hip sway or intertrochanteric swing (Donelan et al. 2004) as the forces are suggested to be directed more posterior to increase propulsive force and become more efficient. We have observed an increase in intertrochanteric swing post fasciotomy, suggesting that the crural fascia reduces non-sagittal limb movement and increases propulsive force through the limb. This reduction in intertrochanteric swing could be particularly important at higher locomotion speeds where additional extension force is required and reduction in non-sagittal inertial forces is more important.

2.4.4.2 Stability

We found that the kinematics of the treated hindlimb showed greater variability through the step cycle, particularly in the frontal plane, upon sequential disruptions of the crural fascia. In some cases, with reduced weight support, the trajectory of the treated limb gradually drifted and did not return to its original path. The smaller effect on sagittal plane kinematics could be due to the constraints of the experiment, as noted above and also inertial effects that can passively stabilize the limb. The inertial effects would not have been as great in the medial-lateral direction since the accelerations would be smaller in this direction, so removal of the crural fascia would not be as completely compensated.

In order to investigate the roles of the different sections of the crural fascia we applied three sequential disruptions to the right hindlimb: longitudinally splitting the fascia; transversely cutting the medial band; and transversely cutting the lateral band. With each sequential disruption we observed a change in the limb kinematics. First, the substantial effect on variability from the longitudinal split suggests that the sheath may be acting to balance force transmission from the hamstring muscles. These muscles insert medially or laterally onto the tibia contributing to internal and external rotation of the leg. Simultaneous activation of these muscles would tend to stabilize the leg about this axis, but the transmission of some of the force diagonally to the other side would reinforce the stabilizing effect. Second, transversely cutting the medial band may represent the least stable state for the limb since the force transmitted by the lateral band would no longer be balanced. Third, upon completing the fasciotomy and transversely cutting the lateral

band we then observed a decrease in variance, which suggests that there is a restoration of balanced forces within the limb. The variance, however, remained greater than control, and the abduction (circumduction) during swing increased progressively through the stages of disruption.

2.4.5 Functional considerations

The results of this present study suggest that the crural fascia plays a role in the transmission of force between joints in the hindlimb. The crural fascia may be connecting the bi-articular hamstring muscles to the calcaneus thereby effectively making them tri-articular muscles. Previously it has been supposed that the proximal muscles are the major force producing muscles while the distal musculature remains more compliant in order to correct for postural disturbances during locomotion (Daley et al. 2007). The results presented in this paper suggest that the crural fascia acts as the proximal-distal force transmitter reducing the need for additional or larger muscles in the distal limb. This has been previously suggested by (van Ingen Schenau 1994) where it was noted that strong tendons couple the hamstrings to the calcaneus in the cheetah and that this connection allows for the combination of hip extension, knee flexion and plantar flexion required for strong propulsive movements. Given the anatomical similarity of the crural fascia bands to the cheetah tendons and the loss of acceleration at all three joints post complete fasciotomy, we suggest that bands may have a similar role in the hindlimb of the domestic cat.

The increase in medial-lateral variance following crural fasciotomy suggests that the crural fascia stiffens the limb in non-sagittal directions in a force dependent manner. As the hamstrings contract more strongly, during locomotion, there will be an increase in tension in the crural fascia, which will in turn restrict the movement in the medial-lateral direction leaving the distal musculature to regulate stiffness in the distal limb mainly in the sagittal plane. Conversely when a cat is quietly standing the crural fascia may be more compliant and the distal limb musculature may play a larger role in mediolateral stabilization. This mechanism may reinforce the stabilization of the ankle by co-contraction of the ankle stabilizer muscles and the reciprocal inhibition between them (Bonasera and Nichols 1996). Furthermore, the gastrocnemius muscles have been shown to be strong abductors and plantorflexors (Lawrence et al. 1993) and are known to produce propulsive force (Walmsley et al. 1978). In particular MG is known to produce a strong abduction force due to its lateral insertion into the calcaneal tendon. This could be balanced during locomotion by contraction of the hamstrings muscles due to the medial insertion of the crural fascia bands onto the calcaneus. However, during standing or when the hamstrings are not strongly activated, the MG muscle may produce a larger abduction torque, and contribute to the laterally directed forces in response to horizontal perturbations (Macpherson 1988b). Donelan et al. (2004) noted that higher order nervous system centers have been suggested to play a large role in the control of the limb in the frontal plane. Our results suggest that the crural fascia contributes to the stability of the limb in the frontal plane during locomotion, thereby reducing the computational burden on the central nervous system.

Interestingly, the limb orientation angle had the greater percentage change in variance in the frontal plane than sagittal, allowing us to infer that the crural fascia may be acting to globally control the limb angle in the frontal plane. These findings support findings by Chang et al. (2009) whereby they suggest that the limb orientation angle is a global variable. We theorize that the anatomical linkage of the thigh segment to the foot segment through the crural fascia provides for a connection from the hip to the foot and potentially a way for the limb to control the orientation of the overall limb trajectory. In the sagittal plane the percentage change in variance for the limb orientation angle was equivalent to the other segments implying that the sagittal plane results could have been attributed to the increased non-sagittal movements throughout the limb. The sagittal plane effects may be greater in animals running over ground, where body fixation is absent.

2.4.6 Conclusion

The crural fascia provides propulsion to the limb as upon complete fasciotomy there was a statistically significant decrease in acceleration in the limb included angles. Also, the variance analysis presented in this paper shows that the crural fascia acts to stabilize the limb during level walking in the decerebrate cat. Therefore, the crural fascia acts to provide mechanical force transmission and stabilization to the limb, and reduce the degrees of freedom, thus in turn reducing the need for neural control in the frontal plane.

CHAPTER 2 APPENDIX

MTP TRAJECTORY WITH LOW WEIGHT SUPPORT

As mentioned during the chapter the low weight-bearing cat tended to show a medial

lateral excursion of the limb during stance. This can be seen in the example in Figure

2.10A where the MTP trajectory is no longer linear after the complete fasciotomy. This

suggests that the paw is able to slip medially on the treadmill during stance, which could

be attributed to the animal being low weight-bearing. Also note that the placement of the

paw at paw contact and during stance is more lateral following the complete fasciotomy

suggesting that the limb is in a wider stance, which may be more biomechanically stable

(this was noted in some but not all cats). In this example the paw went so medial at the

beginning of swing that the paw hit the left leg and resulted in an irregular excursion as it

attempted to recover.

The box plots in Figure 2.10.B quantify the average and range of the mtp medial-lateral

excursion during the whole step cycle. The increased medial placement of the paw and

the increase in range of mediolateral movement is evident.
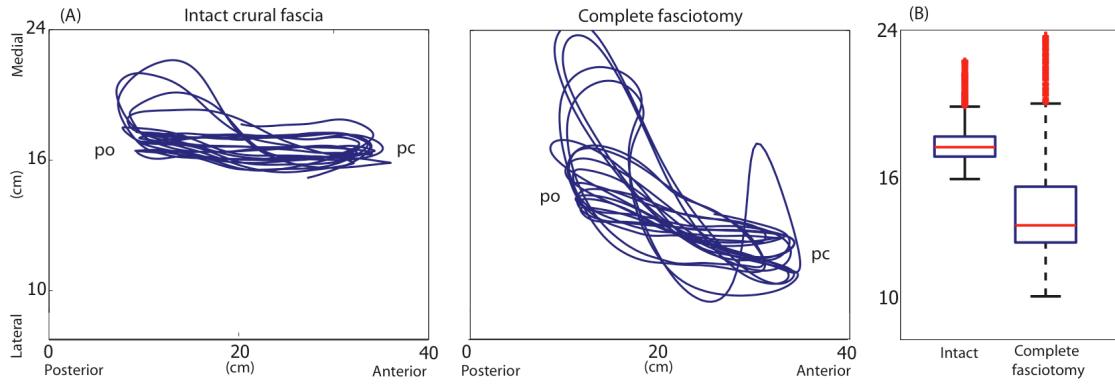
Figure 2.10. (A) The MTP trajectory of the right cat paw during one trial. Note the increased lateral placement of the paw after the complete fasciotomy as well as the increase in variability. The right paw also hits the left paw around paw off resulting in an increased variable trajectory. pc = paw contact; po = paw off (B) The box plots show the mean and range of the medial-lateral excursion of the paw.

CHAPTER 3

ACUTE ASSESSMENT OF THE EFFECTS OF THE DENERVATION OF THE TRICEPS SURAE ON LIMB AND JOINT VARIABLES DURING LEVEL WALKING

3.1 INTRODUCTION

The triceps surae contribute to weight support and propulsion during locomotion and have been investigated in both the human and the cat. Locomotion studies in humans have shown that the triceps surae are antigravity or weight supporting in early stance (Gottschall and Kram 2003; Neptune et al. 2001), however by mid stance the gastrocnemius muscles become propulsive muscles, while the soleus muscle remains an antigravity muscle throughout the stance phase (Neptune et al. 2001). To further evaluate the role of these muscles during locomotion, denervation studies of select triceps surae muscles in the cat have been performed (Pearson et al. 1999) which allowed for the determination of the motor deficit and long-term adaptation during walking. These studies showed a loss of weight support in early stance. Additionally, reinnervation studies in the cat have been performed whereby after an initial period of paralysis of the muscles the motor neurons reform functional connections to the muscles, however proprioceptive connections are not reformed (Cope et al. 1991). During the paralytic stage, an increased yield in the ankle during the weight acceptance of early stance is observed (Abelew et al. 2000; Maas et al. 2007; Pearson et al. 1999), supporting that during weight acceptance the triceps surae plays an antigravity role by plantarflexing the ankle joint.

The hamstrings also contribute to ankle function through the connection to the crural fascia. In the cat the tendon-like connection of the crural fascia from the posterior, proximal muscles to the calcaneus has been suggested to play a role in propulsion (van Ingen Schenau 1994). Furthermore, the whole structure, sheet and bands, has been shown to play a role in stabilizing the limb during locomotion, potentially through reducing the mediolateral torques produced by the gastrocnemius muscles (Chapter 2). Given that the crural fascia is closely associated with the triceps surae it could potentially influence the force of the triceps surae and enhance propulsion in late stance.

Chang noticed preservation of limb level variables, limb length and orientation, during the paralytic stage after the reinervation of parts of the triceps surae (Chang et al. 2009). However, the ankle joint still exhibited an increase in yield during weight acceptance. This conservation of the limb variables may be due in part to the limb biomechanics through an adjustment at the other joints. This is supported by the partial increase in knee extension in response the increased ankle yield during weight acceptance noticed by Abelew et al. (2000). A more likely scenario would be a developing neural adaptation over days and weeks involving a change in the central drive. After a period of adaptation there would likely be an increase in activity in synergistic muscles, flexor hallicus longus (FHL) and plantaris (Plant) to compensate for the increased ankle yield.

The main *objective* is to determine the immediate effect of denervation of the triceps surae or disruption of the crural fascia on preservation of limb variables and the primary

motor deficit of the triceps denervation on kinematics during level walking. This will provide a baseline to evaluate subsequent adaptations in chronic experiments. The secondary *objective* is to evaluate the interaction between the crural fascia and the triceps surae. In particular to investigate if the crural fascia could potentially compensate for the loss of limb length. Furthermore we wish to evaluate propulsion through changes in angular acceleration and to determine the interaction between the triceps and crural fascia and how it is central in propulsion.

To fully understand the distal propulsive mechanisms during level walking we examined the acute affect of deinnervating the triceps surae on limb and joint variables. A number of studies have shown an increase in ankle yield after denervation of some or all of the triceps surae muscles during early stance (Pearson et al. 1999) therefore; we *hypothesized* that the denervation would result in an increase in ankle yield during the weight acceptance during stance. Furthermore to determine if the crural fascia and triceps surae act as synergist structures within the limb we evaluated the change in ankle angular acceleration upon the injury of both systems. We hypothesize that upon the complete fasciotomy there would be a loss of propulsion (angular acceleration) in the distal limb. In order to determine if synergist muscles could compensate for the injury to the triceps surae we also recorded from Plantaris (Plant) and Flexor Hallicus Longus (FHL) to determine if there was a neural compensation within the time corresponding to our experiment (about one hour).

3.2 METHODS

3.2.1 Surgical procedures

The roles of the triceps surae and the crural fascia were investigated in six decerebrate cats walking at approximately 0.7m/s atop a variable speed treadmill. All experimental protocols were approved by the Emory University and Georgia Institute of Technology Institutional Animal Care and Use Committee.

For each experiment, the animal was initially anesthetized using isoflurane gas. While under anesthetic the skin over the gastrocnemius muscle in the right limb was longitudinally split from the popliteal fat pad behind the knee to within one cm of the calcaneus. The skin was very carefully blunt dissected off the crural fascia and resealed using a flat-edged alligator clip. The animal was then supported atop a variable speed treadmill in a natural stance by affixing the head to a stereotaxic frame and clamping the base of the tail. A premamillary decerebration was performed where the brainstem was transected at a 45° angle beginning rostral to the superior colliculi and ending rostral to the mammillary bodies allowing for the preservation of the sub thalamic nucleus. All brain matter rostral and lateral to the transection was removed. The anesthetic was then slowly titrated until eliminated. When the animal exhibited spontaneous stepping the treadmill was turned on and consistent stepping was observed. Occasionally manual stimulation at the base of the tail or locomotor-like movement of the forelimbs was used to encourage a consistent stepping pattern. At the end of the experiment a lethal dose of euthasol was administered to terminate the animal.

3.2.2   Kinematics processing

Three-dimensional kinematics was recorded from the spontaneously stepping,

premammillary cat using six Vicon cameras collecting at 125 Hz. While the cat was still

under anesthetic kinematic markers were placed on the right limb at the iliac crest,

greater trochanter, upper shank (to calculate the virtual knee), lateral malleolus, meta-

tarsal phelangeal joint (mtp) and toe (Figure 3.1). The virtual knee was calculated post-

hoc in Matlab by projecting the vector from the lateral malleolus through the upper shank

marker for the length of the leg. The joint included angles for the hip, knee and ankle

were calculated as shown in Figure 3.1. The limb angle was calculated as the angle from

the MTP to the greater trochanter in the frontal and sagittal planes. The limb length was

determined as the length from the greater trochanter to the MTP. Paw contact and paw

off were demarked post-hoc by video analysis of the animal walking.

Recordings were made under one of two consecutive hindlimb disruption combinations

(a or b pairings described following): 1) intact – no manipulations to the crural fascia or

triceps surae; 2a) denervation of the triceps surae; 2b) complete fasciotomy - a

longitudinal split through the fascia sheet followed by two horizontal cuts through the

medial and lateral bands consecutively; 3a) complete fasciotomy; 3b) denervation of the

triceps surae. After each disruption the skin was resealed with the alligator clip to ensure

comparable conditions for each limb. A minimum of three trials was recorded under

each aforementioned condition, whereby a trial is defined as a consistent, spontaneous

period of walking for at least 20 seconds. Although the aforementioned protocol was our

standard procedure not every cat followed the exact steps. Cat 090213 followed the

procedure of the crural fascia being disrupted first, followed by the triceps denervation

and cats 090611 and 090723 followed the procedure of the triceps being deinnervated

first and then the complete fasciotomy. Cat 081217 had control and triceps deinnervated

trials collected before the tail height was lowered. After the lowering, additional

denervation trials were recorded and then trials post complete fasciotomy were recorded.

During cat 081030 data collection the cat walked uphill during the control and after

complete fasciotomy trials. Cat 080828 was given anesthetic post fasciotomy to allow

for the insertion of EMG's into Plant and FHL and exhibited a new variation in walking

pattern post anesthetic.

3.2.3   Kinematic analysis

The kinematic marker data were analyzed using custom Matlab scripts and each marker

was low pass filtered at 6Hz. The resulting 3D marker traces were then used for all

analysis presented in this chapter. The limb angle and length as well as the included

angles for the sagittal plane limb, hip, knee and ankle joints were calculated. All

variables were time normalized to 25 bins per step and all normalized steps averaged

together across three trials with nine steps per trial. The means of each of the conditions

were compared. In order to further evaluate the changes we investigated the change in

angle during weight acceptance (the minimum of the yield) during stance of the ankle

included angle and the limb length. The mean and standard deviations for each cat before

and after the triceps denervation or complete fasciotomy were plotted against each other. The line of identity was drawn through the graph to represent a lack of change in angle. The placement of the mean angles with respect to the line of identity for all cats depicts the trend in response to the treatment. In order to determine if there is a significant change, a paired t-test was performed across all cats.
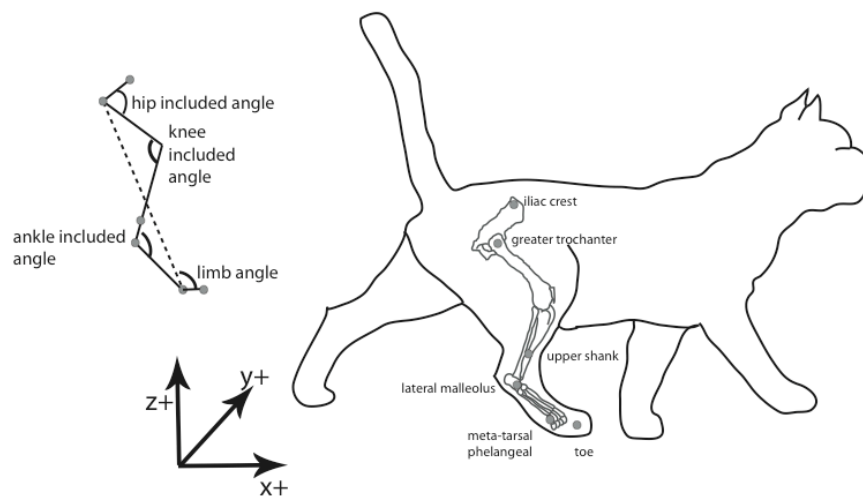
Figure 3.1. The location of the markers are shown on the anatomical drawing of the cat on the right. The axes of the Vicon system are shown at the bottom, where yz is the frontal plane and xz is the sagittal plane. The included angles and limb angle and length are defined at the top left.

The angular accelerations for the included joint angles over the step cycle were determined by differentiating the included angles twice prior to normalization of the data. The maximal angular acceleration of the ankle was averaged across all steps. The mean of the angle before versus after treatment (triceps surae denervation or complete fasciotomy) were plotted against each other to show the trend in change of acceleration across all cats (as described for the angles). Statistical significance was determined using a paired t-test.

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The percentage change in variance for the joint and limb level variables was calculated. The hip, knee and ankle included angles the limb length in the sagittal plane were evaluated as well as the limb orientation in the frontal plane. The summed variance was averaged under each condition and the value before versus after treatment (triceps denervation or complete fasciotomy) were plotted against each other. Statistical significance was determined using a paired t-test.

3.2.4 Electromyography

While the animal was still under anesthetic bipolar electromyography wires were inserted into the muscles of interest. The EMG's were made from insulated wires twisted together, with the last 3 mm of insulating stripped from the end and were tied together using surgical silk with the ends offset from each other. These were then inserted and sutured into gracilis (G), semitendinosis (ST), posterior biceps femoris (pBF), anterior biceps femoris (aBF), tibialis anterior (TA), plantaris (Plant) and flexor hallicus longus (FHL). However, we will only be evaluating the results from FHL and Plant. The other muscles recorded were used as a comparison to determine the strength of the overall EMG signal per cat per trial. The recordings were collected at 1000 Hz and preamplified by a gain of 100.

The EMG recordings for FHL and Plant were time normalized and averaged across each condition using the same criteria as the kinematics (nine steps for three trials per

condition). The normalized mean EMG patterns were plotted and compared to see if there was an increase in duration or magnitude of activity of the muscles.

3.3   RESULTS

3.3.1   Sagittal plane kinematics

Upon the denervation of the triceps surae, there is an increase in ankle yield and a decrease in limb length during the weight acceptance stage of stance during level walking (Figure 3.2) in the sagittal plane. The weight acceptance stage of stance is the initial period of stance where the center of mass prepares to pass directly over the paw. There is usually a slight yield at the knee and ankle at this time point in the intact limb. In this example cat, the ankle increases in dorsiflexion by 30°, while the leg length decreases by 20 mm.   Although these large changes were observed in these two variables, the hip and knee included angles, and the limb orientation angle remained relatively conserved. It is particularly interesting that the knee included angle did not increase in extension during the same time period.
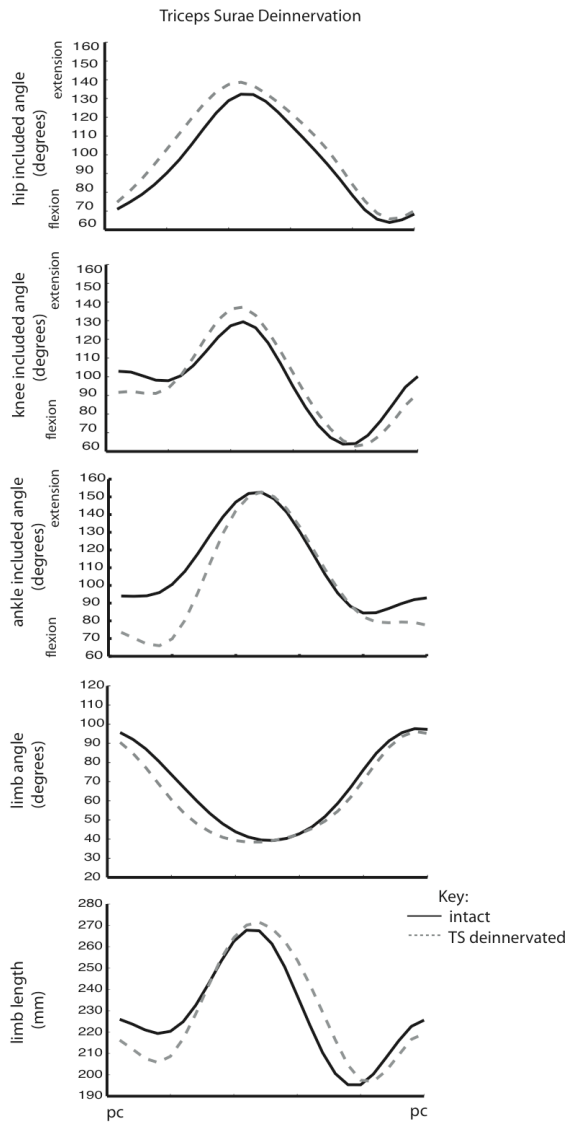
Figure 3.2. The sagittal plane mean joint and limb trajectories are plotted for an example cat over a time normalized step cycle (from paw contact (pc) to paw contact). The black trace represents the trajectories from the intact limb and the grey dashed trajectories from the deinnervated limb. Note the increase in ankle flexion and the decrease in limb length during weight acceptance during stance.

The increase in ankle yield and the decrease in limb length during weight acceptance are

consistently observed across all cats (Figure 3.3). The minimum ankle angle during yield

before the triceps denervation plotted against the angle after the triceps denervation

shows the means for all cats below the line of identity (which represents no change in the

mean angle) implying a decrease in ankle angle for all cats. This increase in ankle

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dorsiflexion during weight acceptance is significant (p=0.02). The limb length also

shows a consistent decrease in length across all cats with all means below the line of

identity again implying a decrease in leg length at that time point. This decrease in leg

length was found to be significant (p = 0.005).
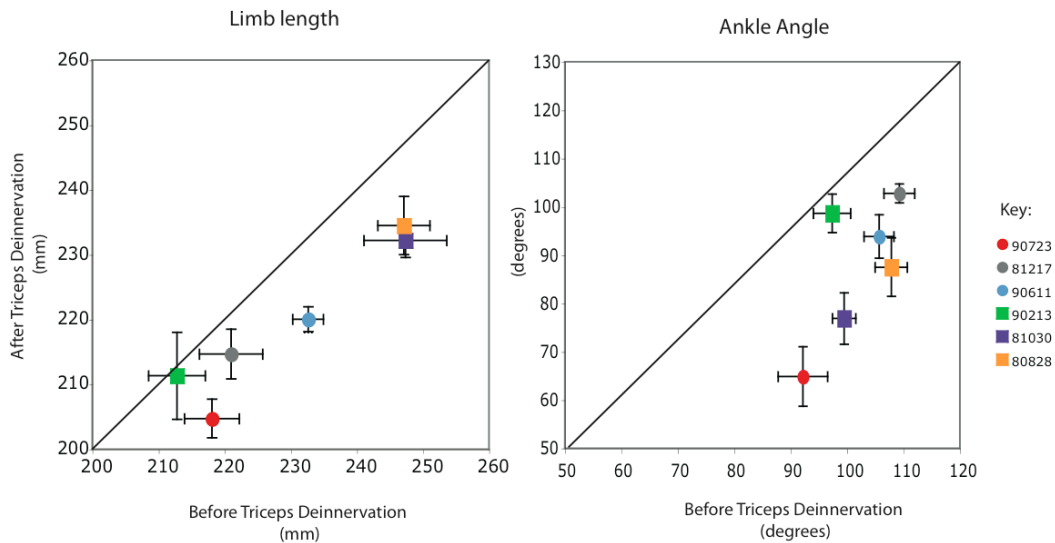
Figure 3.3. The mean minimum limb length and ankle angle during weight acceptance plotted before versus after the denervation of the triceps surae. Note that all cats decrease in limb length and increase in ankle flexion after the denervation.   The 45° line through the graphs represents the line of identity.

Mean limb length and ankle angle were minimally affected by the crural fascia

fasciotomy, as most means lie close to the line of identity (Figure 3.4). The limb length

in particular was highly conserved with almost all points along the line of identity and we

did not find a significant change (p = 0.66). The ankle angle showed a trend to decrease

in angle with four cats means below the line of identity. Overall there was not a

significant change in angle (p = 0.37). However the magnitude of the yield was much

less than the triceps denervation. Interestingly, these trends occurred regardless of triceps

denervation.
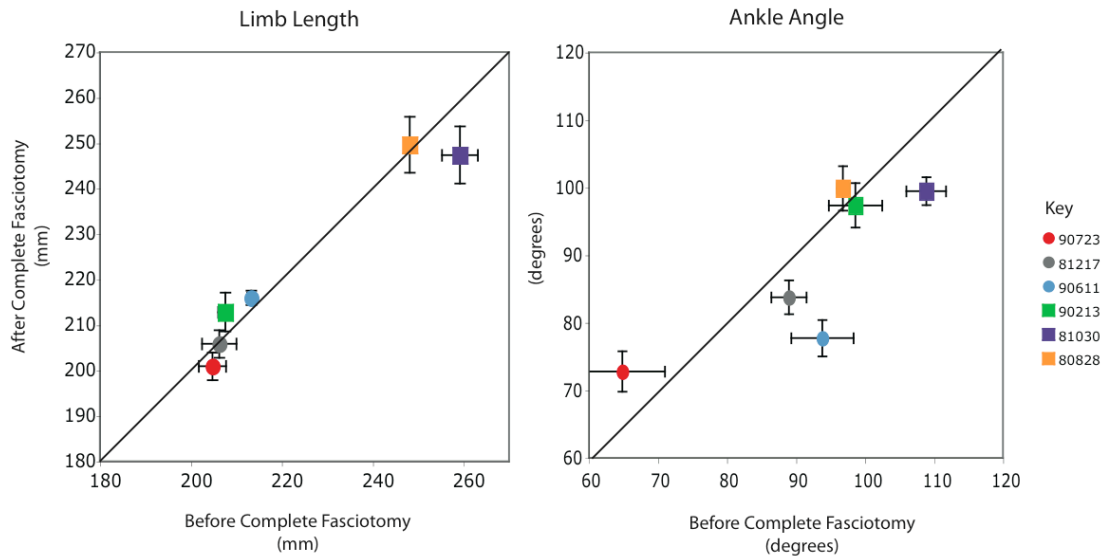
Figure 3.4. The mean limb length and ankle angle during weight acceptance plotted before versus after the complete fasciotomy. The limb length is mostly conserved with most points along the line of identity. The ankle angle shows a trend towards increasing flexion with four cats means below the line of identity.

3.3.2 Ankle Angle Acceleration

The denervation of the triceps surae results in an increase in dorsiflexion acceleration (coinciding with the increased ankle yield) during the weight acceptance stage of stance at the ankle and an increase in plantarflexion acceleration around paw off. The mean trajectories of the ankle angular acceleration for two example cats is shown in Figure 3.5A. These traces line up with the timing of the ankle included angle in Figure 3.2. During the weight acceptance period (grey box) there is an increase in dorsiflexion acceleration upon the denervation of the triceps surae, regardless of the complete fasciotomy likely in response to the increase in dorsiflexion of the ankle joint. This trend is observed across all cats (Figure 3.5B) whereby the mean angular acceleration is above the line of identity (one cat was significantly above the line). During the paw off or maximal extension time period (white box) there is an increase in plantarflexion

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acceleration after the denervation of the triceps surae, regardless of a complete fasciotomy having been performed. Figure 3.5B show the trend of increased plantarflexion acceleration for most cats (three significantly increased) after the triceps denervation around paw off.

While the denervation of the triceps surae results in an increased dorsiflexion acceleration during weight acceptance and an increase in plantarflexion acceleration at paw off, the complete fasciotomy resulted in a decrease in dorsiflexion acceleration during weight acceptance and a decrease in plantarflexion acceleration at paw off. The decrease in dorsiflexion acceleration during weight acceptance after the complete fasciotomy is observed in five cats, however there was not a consistent or significant change in the ankle yield therefore suggesting the loss of propulsion at the ankle. The decrease in plantarflexion acceleration at paw off was observed in all cats (also documented in Chapter 2), although there was no change in ankle angular excursion.
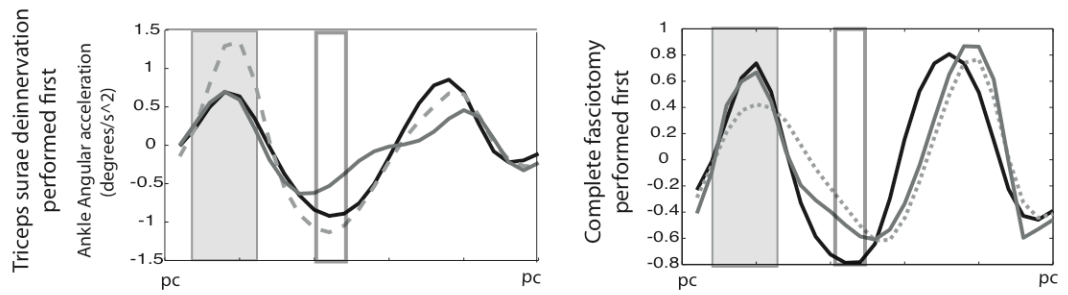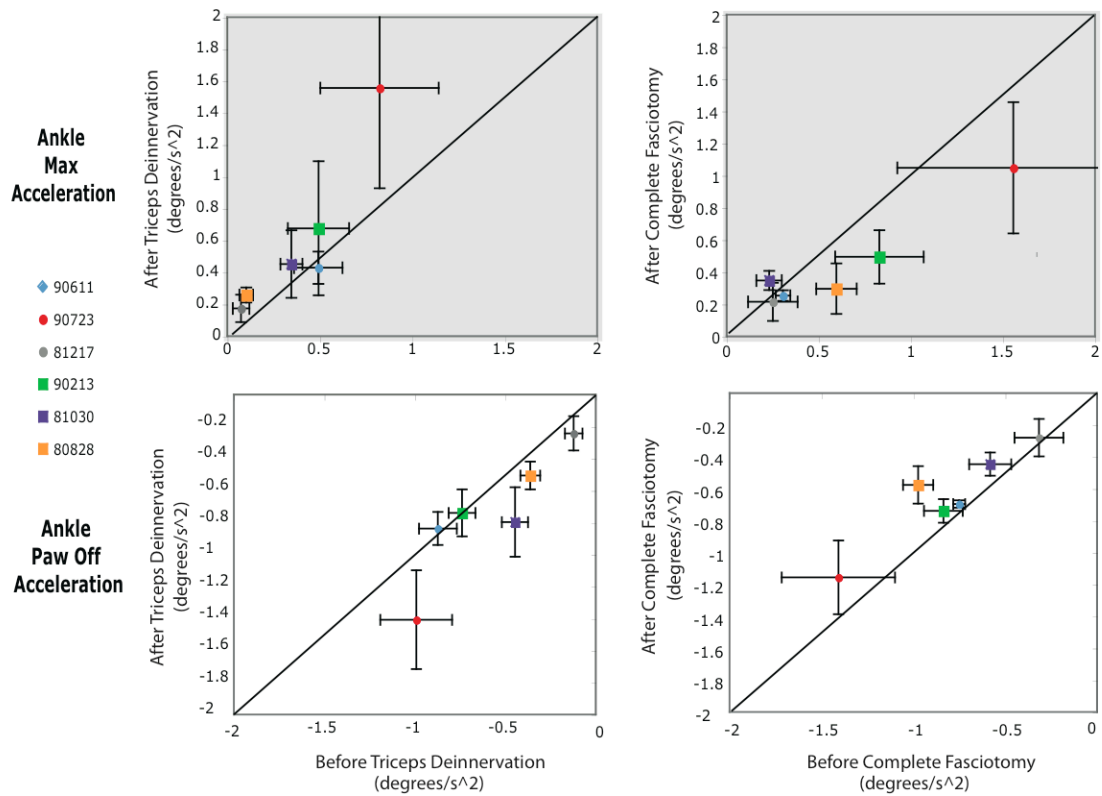
Figure 3.5. (A) Shows the mean ankle angle acceleration through the step cycle for two representative cats. The first figure shows the trajectories of the intact (black), triceps deinnervated (grey dashed) and complete fasciotomy (solid grey) angular accelerations. The second figure shows the trajectories of the intact (black), complete fasciotomy (grey dashed) and triceps denervation (solid grey) angular accelerations. The dark grey box represents the time period of weight acceptance and corresponds to the grey graphs below. The white box represents the time period of paw off and corresponds to the analysis below. (B) shows the mean and standard deviations of the angular accelerations before versus after the treatment is applied. The grey background corresponds to the time period in grey in A and the white background to the time period outlined in A. The denervation of the triceps surae resulted in an increase in acceleration during weight acceptance and an increase in deceleration at paw off, while the complete fasciotomy resulted in a decrease in acceleration during weight acceptance and a decrease in deceleration at paw off.

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3.3.3   Variance

The denervation of the triceps surae results in an increase in summed variance for the joint and sagittal plane limb angles (Figure 3.6). The hip, knee and ankle angles all increase in variance for all cats. While the sagittal limb orientation angle does increase in variance the mean values lie close to the line of identity. The limb length tends to increase in variance with two of the cats means plotting on the line of identity while four cats increase in variance. The frontal limb angle does not show a consistent trend in variance after the triceps surae denervation, with three cats increasing in variance and three cats decreasing in variance, suggesting that the frontal plane may rely more on passive mechanical elements such as fascia for variance control.
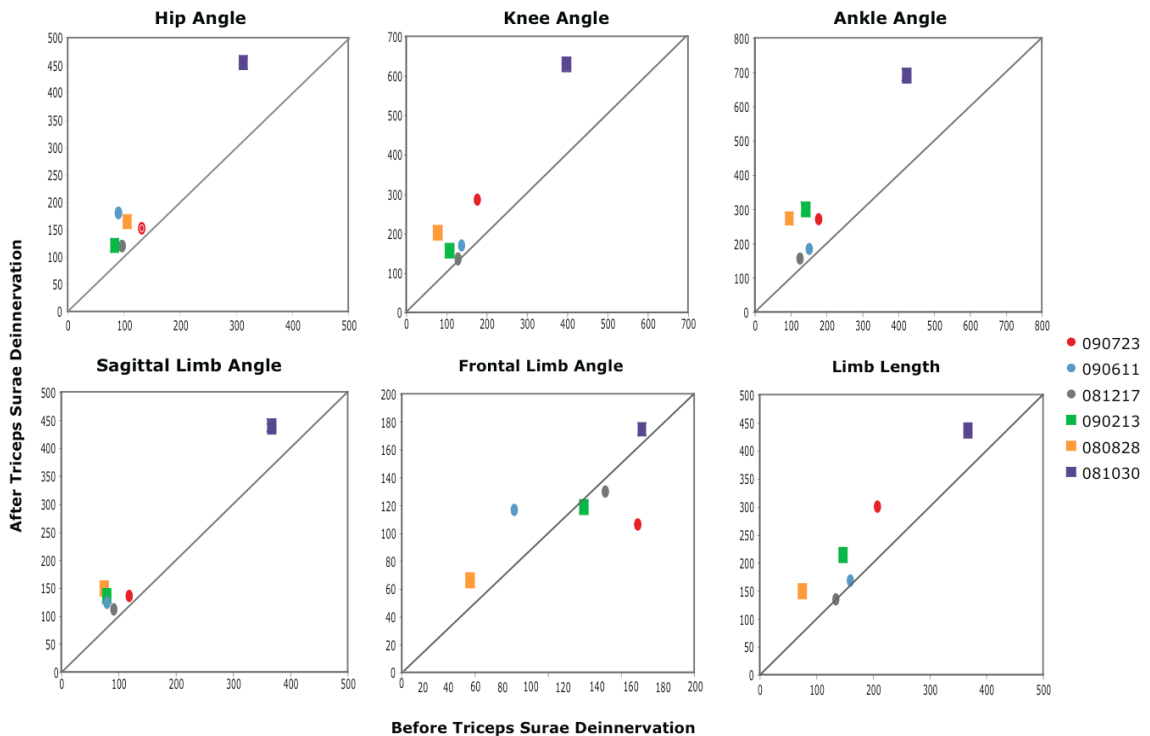
Figure 3.6. The denervation of the triceps surae results in an increase in mean summed variance for the joint and sagittal plane limb angles. The limb length trends towards the increase as two cats are on the line of identity. The frontal plane limb orientation angle has a variable response with three cats increasing in variance and three decreasing in variance.

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3.3.4 EMG recordings

The EMG activity for FHL and Plant did not show a significant change post triceps denervation. The mean EMG traces for three cats are plotted in Figure 3.7 and show that after triceps surae denervation the mean activity remains within the range of the intact mean. Furthermore, most means show a slight decrease in amplitude but this was usually observed in additional muscles in the limb therefore suggested that there was an overall change in the recordings and not a result specific to FHL and Plant. Although the means might vary in amplitude slightly, the duration of the activity remains the same. Furthermore the large standard deviation of the intact EMG means suggest that any variations could be due to subtle variations in step-to-step activity.

FHL
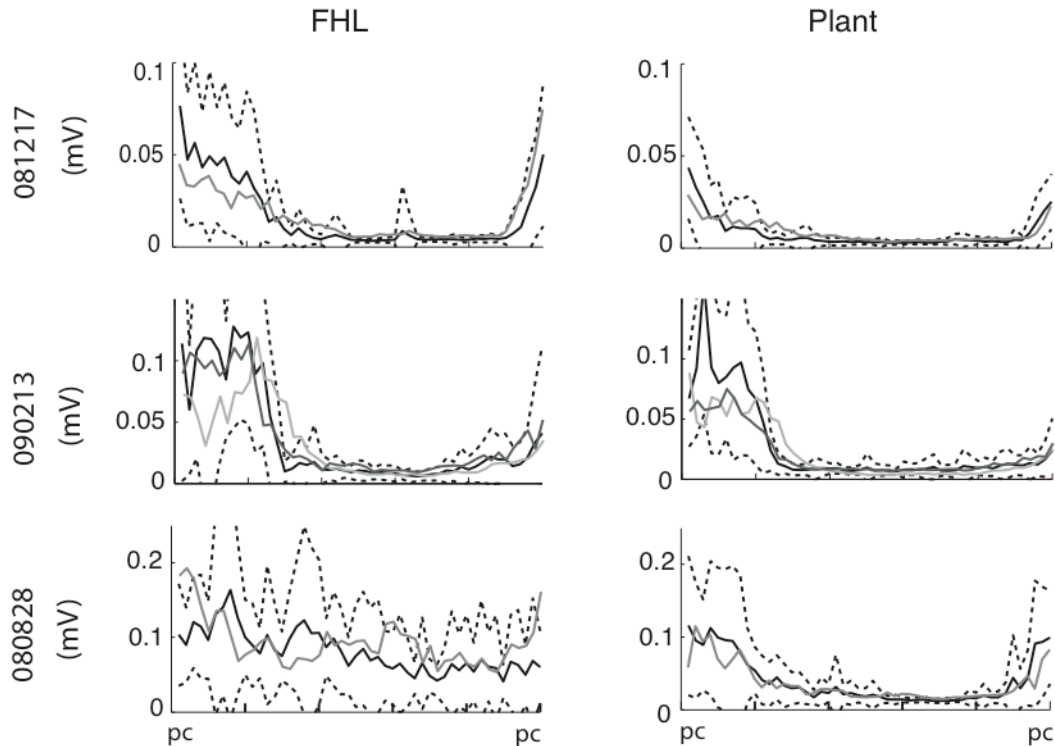
Plant

Figure 3.7. The mean time-normalized traces for FHL and Plant are plotted from paw contact to paw contact (pc). The black line represents the EMG mean in the intact limb condition, the black dashes are the standard deviation for that mean. The dark grey line is the mean EMG trace after the denervation of the triceps surae and the light grey line is the mean after complete fasciotomy (cat 090213 only). Overall there is no change in overall EMG pattern.

3.4 DISCUSSION

3.4.1 Summary

The aforementioned results *support* the hypothesis that the triceps surae are strong antigravity muscles during weight acceptance while the crural fascia assists with propulsion throughout stance until paw off. We deinnervated the triceps surae muscles to investigate the acute effects on locomotion during level walking. In order to describe the effect on the limb we examined the sagittal plane joint and limb angular excursions as well as the limb length, and observed a significant increase in yield at the ankle and a decrease in leg length during the weight acceptance stage in stance after the denervation.

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The angular acceleration of the ankle joint showed an increase in dorsiflexion acceleration upon the triceps surae weight acceptance (increased ankle yield), while at the stance-to-swing transition, paw off, the denervation resulted in an increase in plantarflexion acceleration. The complete fasciotomy did not show a significant change in ankle yield or leg length during weight acceptance but did show a decrease in dorsiflexion acceleration.

3.4.2   Comparison to non-decerebrate cat kinematics

Both acute and chronic preparations show similar results during the paralytic stage of the triceps surae at the joint variable level but differ at the limb level with the lack of conservation of limb length being only observed in the decerebrate preparation. The sagittal plane included angles observed in the intact and post triceps denervated limb show similar effects and ranges of angles between the non-decerebrate (Chang et al. 2009; Maas et al. 2007) and decerebrate cat (presented in this chapter). The acute results presented show the same increase in ankle yield reported by Pearson (1999) in the one to two weeks after denervation of the MG synergists. The decerebrate cat kinematics reported in this chapter will be compared to the acute injury kinematics reported by Chang et al (2009), although this report more specifically addresses the immediate response of the hindlimb to the triceps denervation. Both reports show an increase in ankle yield during the weight acceptance phase of stance. Intriguingly, while both preparations showed a conservation of the limb angle throughout the step cycle, only the decerebrate cat showed a consistent decrease in limb length during the weight acceptance

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phase of stance suggesting that there was a short-term compensation over the time course of Chang et al (2009) experiments.

3.4.3   Effect of the triceps denervation on joint and limb variables

The acute preparation did not conserve joint and limb variables after the denervation of the triceps surae, in particular there was observed increased ankle dorsiflexion and a decrease in limb length during weight acceptance. In the awake, behaving cat the limb length and orientation are conserved after the reinnervation of the triceps surae muscles, while an increase in ankle dorsiflexion is observed during the paralytic stage (Chang, 2009). The consistency in increased ankle dorsiflexion across the two preparations suggests that joint variables are not conserved. While the decrease in limb length in the acute preparation suggests that limb variables require additional time to be conserved. The need for additional time is further supported by the observed recovery of ankle yield in the chronic requiring at least a week (Pearson et al. 1999).

The increased variance observed in the joint variables over the limb variables supports the idea that the limb variables are more conserved than the joint variables in the acute and chronic preparations. Previous studies have suggested that the joint variables show increased variability compared to the limb variables after a treatment was applied to the limb (Chang et al. 2009), again suggesting that the limb variables were more conserved than the joint variables. In the decerebrate cat, the joint variables also showed a greater increase in variability, especially at the ankle after the denervation of the triceps surae

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muscles. Although the sagittal limb angle and the leg length also increased in variability the magnitude was much smaller than the joint angles. These results suggest that the limb variables may have some spinally mediated input to reduce the variability even though a change in kinematic trajectories may be observed. Interestingly, the frontal limb angle had an inconsistent trend with both increases and decreases in variance suggesting that there may be additional influences on the mediolateral stability of the limb.

3.4.4 The role of the triceps surae and crural fascia during stance

3.4.4.1 During the weight support stage of stance

During weight support the increase in ankle dorsiflexion angle and acceleration after the denervation of the triceps surae muscles suggests a loss of antigravity action within the limb in the cat. This supports results from previous work in humans that showed that during early stance, the triceps surae muscles provide vertical limb support thereby acting as antigravity muscles during walking (Neptune et al 2001). Although the gastrocnemius muscles are bi-articular there was only a trend for a slight increase in knee extension (data not shown) suggesting that during weight acceptance the gastrocnemius muscles act more to extend the ankle and less to flex the knee (Lan and Crago 1992). However, an increase in knee extension corresponding to the increased ankle dorsiflexion was observed for down ramp walking in the chronic cat 9-12 months after reinnevation of the triceps surae (Abelew et al. 2000). This increased knee extension was presumed to

compensate for the increased ankle yield observed in downhill walking and suggests that the preservation of limb length can be accomplished in different ways depending on the constraints or motor tasks. The decrease in limb length and lack of knee extension in the acute preparation suggests that there was a lack of compensation to the denervation. Also the change in limb length precludes that the mechanical clamping of the tail may restrict the kinematic change, particularly the limb length, after the denervation. The increase in dorsiflexion acceleration of the ankle joint can be attributed to the increase in angular excursion of the limb after the denervation coupled with the conservation of the duty cycles recorded prior to denervation (data not shown).

The crural fascia appears to play a role in limb retraction or propulsion during weight acceptance. The complete disruption of the crural fascia does not significantly affect the ankle yield or the limb length suggesting that it does not play an antigravity role in the limb during weight acceptance, however, a decrease in dorsiflexion acceleration, however, was observed. The decrease in dorsiflexion acceleration can be attributed to a loss of limb retraction after the complete fasciotomy as the leg segment has a change in torque applied along its length. The loss of the connection of the limb retractors or hamstring muscles to the ankle joint results in a loss of retraction force applied to the calcaneus and an increase in force applied more distal on the tibia. This results in the potential for greater knee flexion and less backward pulling of the ankle through weight acceptance and therefore a loss in propulsion. Therefore although the hamstring and crural fascia complex have a similar termination point as the triceps surae, the hamstrings

and crural fascia play a larger role in limb retraction while the triceps surae act as antigravity muscles.

3.4.4.2 During propulsive stage of stance

During the propulsive stage of stance the plantarflexion acceleration increased after the denervation of the triceps surae muscles, which can be attributed to the increase in angular excursion of the ankle joint through stance. Previous studies (Gottschall and Kram 2003; Neptune et al. 2001) have shown that throughout stance phase the soleus muscle provides weight support to the animal while the gastrocnemius muscles are initially weight supporting, but by mid stance become primarily propulsive. Pearson et al. (1999) suggested that the MG muscle in particular contributes to propulsion by ankle extension in late stance, however we did not observe a significant loss of ankle extension after the denervation as reported in the Pearson study, possibly due in part to the extensor bias of the decerebrate preparation. Therefore the increase in plantarflexion acceleration at paw off can be attributed to a "catch up" mechanism in response to the increased ankle yield. The increase in angular acceleration reflects a different purpose beyond the torques produced at the joint and describes the change in excursion of the ankle angle.

When examining the effect of the complete fasciotomy during weight acceptance we observe a decrease in plantarflexion acceleration, again supporting that the crural fascia may be transmitting propulsive force to the ankle joint and thereby acting as a mechanism to transmit the propulsive force of the proximal musculature to the limb endpoint

throughout stance. During most of stance the hip extensor muscles, for example anterior biceps femoris, are retracting the limb backwards thereby propelling the body forward. Given that the crural fascia connects the hip extensors to the ankle joint, it may transmit some of the propulsive force to the distal endpoint.

3.4.4.3 Effect at the stance to swing transition

The propulsive force from the gastrocnemius muscles prior to swing may result in a greater energy demand on the limb to decelerate and change direction. During late stance the gastrocnemius muscles are transmitting almost all of their power to propulsion resulting in an increase in plantarflexion acceleration (Neptune et al. 2001). This increase in acceleration, provides propulsive force, and applies a plantarflexing force (or acceleration) to the ankle joint. However, as the limb prepares to initiate swing it has to decelerate in order to change direction therefore requiring a counter measure to the plantarflexion acceleration of the gastrocnemius muscles. The decrease in plantarflexion acceleration observed at the stance-to-swing transition (paw off) after the triceps denervation suggests that there is a loss of the plantarflexion acceleration from the gastrocnemius muscles resulting in a greater net deceleration of the ankle joint.

The acceleration of the distal limb is affected by the complete fasciotomy in the opposite direction to the denervation of the triceps surae suggesting that even though the crural fascia and the triceps surae are synergistically located they have different functional roles within the limb at the stance-to-swing transition. We have previously shown (Chapter 2)

that the crural fascia contributes to the increased plantarflexion of the limb at paw off, as after the complete fasciotomy there was a decrease in plantarflexion acceleration at the joints. This was supported by the results presented in this chapter where the complete fasciotomy again resulted in a decrease in plantarflexion acceleration at the stance-to-swing transition.

3.4.5 Potential neurological inputs

3.4.5.1 Neurological control of the limb variables

The lack of conservation of the limb length suggests that if there is a neurological compensation that the motor cortex may be involved in the preservation of limb level variables. Joint kinematics are sensed through limb proprioception, either by stretch of muscle spindles or by joint receptors (Boyd and Roberts 1953). An increase in muscle stretch will result in a stretch response and increased activity in the muscle to counter the stretch. The increased ankle dorsiflexion suggests that the FHL and Plant muscles would be stretched given the insertion points of the muscles therefore it is surprising that the EMG did not change. Given that in the decerebrate cat we do not observe a change in EMG, in the muscles surveyed, suggests that there is a lack of immediate spinally mediated neurological compensation to the denervation of the triceps surae.

Recordings from neurons in the dorsal spinal column tract (DSCT) have shown to relay limb length and orientation information to the cerebellum (Bosco et al. 2000). The cerebellum is known to influence posture and locomotion. Although the cerebellum

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conserved in the premammillary decerebration, it may not immediately conserve the limb length variable in the preparation. This compensation may require a few weeks in order for the cerebellum to respond to the change in variable. It is known that the vestibular ocular reflex takes at least five days to change the gain in response to the unilateral removal of the vestibular nerve (Mailoli and Precht 1985). By recruiting muscles that would have biomechanically compensated for the increased ankle yield the limb length could have been conserved. This compensation at the joint level could have been accomplished by either the increased activation of knee extensor muscles, such as the vasti, or ankle extensor muscles, such as FHL or Plant. However, the lack of changes in EMG recordings from FHL and Plant support the suggestion that there is a lack of neural compensation within the distal limb. Furthermore, we did not observe an increase in knee extension suggesting a lack of compensatory activation of knee extensor muscles as observed in Abelew et al. (2000).

Our results depict the immediate response of the limb to the injury and therefore by design did not allow for a period of adaptation that may have taken days or weeks. Generally the data was collected within approximately 30 minutes of the denervation of the triceps surae while the chronic acute time point or paralytic stage was four weeks post injury (Chang et al. 2009). Any additional data collection presented the inability of determining the acute response to the injury versus the possible deterioration of the animal. During the time of data collection the animal was continually subjectively assessed to ensure that deterioration was not observed.

3.4.5.2 Neurological control of stance-to-swing transition

There are two sensory feedback theories as to how the limb knows to terminate stance and initiate swing: the first being that the Golgi tendon organs signal the unloading of gastrocnemius muscles (Pearson 2008); and the second that the spindles sense the stretch of the hip flexors (Grillner and Rossignol 1978). Given that the sagittal plane hip included angle and the limb orientation angle were conserved under the different interventions to the distal limb we can conclude that there are redundant sensory systems within the limb that allow for their conservation. By having denervated the triceps surae we have removed the ability of these muscles to send sensory information to the spinal cord. However the hip flexors are mostly unaffected by both the denervation and the disruption of the crural fascia therefore still providing sensory information to initiate swing phase. Although we did note a slight increase in hip extension post triceps denervation it was not sufficient to alter the timing of the stance to swing transition.

CHAPTER 4

ASSESSMENT OF THE MECHANICAL ACTION OF THE CRURAL FASCIA IN THE CAT HINDLIMB: IMPLICATIONS FOR LOCOMOTION

4.1   INTRODUCTION

The actions of muscles have been described based on both the analysis of the muscle origin and insertion as well as the ground reaction forces produced when the muscle is activated (Honeycutt and Nichols; Murinas et al. 2002). However, the functional action of these muscles, being for example antigravity or propulsive, can change during a task such as stepping. An example of this is the gastrocnemius muscle, which during stance is initially antigravity but becomes propulsive in function by mid stance (Neptune et al. 2001). Previous studies have shown that the action of the muscles may depend on the angles of the joints that they cross (Lawrence et al. 1993; Young et al. 1993). This suggests that the direction of the endpoint force exerted by the muscle changes with the limb configuration. Furthermore, Lawrence et al. (1993) showed that muscles can have strong non-sagittal torques when activated. Intramuscular stimulation (IM Stim) has been previously used to investigate directional output of muscles through the limb (Honeycutt 2009; Murinas et al. 2002; Pratt 1995). In these experiments, intramuscular stimulation electrodes were implanted into the muscles of interest and a twitch stimulation was invoked with the ground reaction forces recorded. Honeycutt (2009) showed that the endpoint force output changes in direction with a change of limb position.

Muscles are also attached to and enveloped by fascia, which may add complexity to the muscle action potentially increasing the articulation of the muscles or redirecting the force output. Studies of the myofascia between and around muscles have shown that the disruption of the myofascia results in a decrease in force output between synergistic muscles (Garfin et al. 1981; Huijing et al. 2003; Maas and Huijing 2005; Maas et al. 2005a; Maas et al. 2005b). These aforementioned studies have all investigated the muscle force output in a linear manner, often with the muscular point of insertion dissected onto a force transducer. This linear experimental design has resulted in an understanding of the amount of force produced by the muscle but not the action of the muscle on the endpoint of the limb.

The crural fascia may affect both the muscles at its origin as well as the muscles it surrounds. The crural fascia originates form the distal end of the posterior thigh muscles, in particular: pBF, ST, SM and G. It then surrounds the gastrocnemius muscles (MG and LG) and terminates on the calcaneus. Within the fascia are two bands: one lateral, initiating on the underside of PBF; and one medial, initiating near the distal tendons of ST and G; both bands terminate on the medial calcaneus. This attachment suggests that the crural fascia could effectively make these muscles tri-articular and affect the direction of the force output. In Chapter 2 we showed that the crural fascia acts to assist in stabilizing the distal limb, promoting propulsion, and suggested that it may reduce the mediolateral directionality of the gastrocnemius when the fascia was pre-stressed. We *hypothesize* that the direct attachment of the crural fascia to muscles, pBF, ST and LG, will have a greater effect on the resulting force direction output, particularly in the frontal

plane, than through an indirect attachment to additional muscles, aBF and Glut. In particular, we *expect* that the complete disruption of the crural fascia will result in an increase in force direction output in the frontal plane for pBF and ST due to the more distal terminal insertion of these muscles onto the tibia.

4.2 METHODS

4.2.1 Experimental Setup

The effects of crural fascia disruption on muscle end point force were investigated in five decerebrate cats with each paw upon a triaxial force plate. All experimental protocols were approved by the Emory University and Georgia Institute of Technology Institutional Animal Care and Use Committee.

For each experiment, the animal was initially anesthetized using isoflurane gas. While under anesthetic, a tracheometry was performed in order to control the anesthetic levels; and an IV line was inserted into the external jugular for fluid and drug delivery. In order to gain access to the crural fascia the skin over the gastrocnemius muscle in the right leg was longitudinally split from the fat pad behind the knee to within 1cm of the calcaneus. The skin was very carefully blunt dissected off the crural fascia and resealed using a flat-edged alligator clip. Intramuscular stimulation electrodes made of Teflon coated, braided, stainless steel wire were sutured into the Semitendinosis (ST), Gluteus Medius (Glut), Lateral Gastrocnemius (LG), posterior Biceps Femoris (PBF) and anterior Biceps Femoris muscles (aBF). Care was taken with the LG insertion to insure minimal

disruption of the crural fascia and only two small holes were made through the fascia by the insertion needle. All insertions were verified at the end of the experiment.

The animal was then supported atop a platform in natural stance by affixing the head in a stereotaxic frame, placing a sling under the chest and clamping the base of the tail. These supports provided to keep the back of the animal relatively straight and aligned. A conservative precollicular decerebration similar to the decerebration described by Honeycutt (2009) was performed. The final transection was rostral to the colliculi but caudal to the mammillary bodies. All brain matter rostral and lateral to the transection was removed. This decerebration was selected as it resulted in a preparation with anti-gravity support but without the preclusion to step as observed in the premammillary decerebrate animals, or the extreme extensor bias noted in collicular decerebrate animals. The toes of each paw were affixed to a triaxial force transducer with double-sided carpet tape and super glue in order to inhibit removal of the foot from the force plate during the data collection. Although all four paws were affixed to individual force transducers, but only the right leg results will be examined. The anesthetic was then slowly titrated until eliminated.

4.2.2   Intramuscular Stimulation protocol

When the animal exhibited steady background tone a twitch threshold of stimulation was determined for each muscle. In order to ensure an adequate stimulation intensity and corresponding force production, the twitch intensity was multiplied three times and set as

the stimulation amplitude for the muscle. Each impulse comprised of a four-pulse chain

stimulation at 200Hz over 20ms was used to produce a twitch contraction in each muscle.

This protocol has been previously described in intact (Pratt 1995) and decerebrate

animals (Honeycutt 2009; Murinas et al. 2002) to create a significant force response

without invoking a full tetanic contraction, which would alter the limb kinematics. This

stimulation protocol was repeated five times per muscle under the different positions and

fascia disruption conditions. The three positions evaluated were: 1) natural, where the

leg was in a weight supporting stance configuration equivalent to mid-stance; 2)

approximately paw contact, which corresponding to approximately 12 cm forward for our

larger cats; and 3) approximately paw off, which corresponded to approximately 12cm

backward for our larger cats. Initial experiments had the limb included joint angles

measured to approximate the angles at those three positions during the step cycle. Each

position was repeated under the four sequential fascia disruption conditions: 1) intact –

no manipulations to the crural fascia; 2) split – longitudinally split over the

gastrocnemius muscles; 3) medial band cut – a horizontal cut through the fascia to medial

side of the medial band; 4) lateral band cut – a horizontal cut through the fascia to the

lateral side of the lateral band.

4.2.3 Data Analysis for Intramuscular Stimulation

The triaxial force transducers recorded the resulting forces in the x, y and z directions

where positive x is a lateral producing force, positive y is an anterior producing force and

positive z is a dorsal directed force (Figure 4.1). In order to examine the resulting force

attributed only to the muscle in question we quantified the direction of the force vectors

in sagittal (yz) and frontal (xz) planes at 35 ms. The selection of this time point allowed

for the occlusion of any force contributions due to the influence of proprioceptive

feedback from the muscle response. An example trace of the recorded force in each of

the three directions is depicted in Figure 1 with the 35 ms time point demarked.

Additionally the force trace had to reach a total minimum of 0.1N for us to include it in

the analysis.   This ensured we had a stable twitch force response. To include a muscle in

our analysis, a minimum of three experiments of data had to be collected at the standards

described.
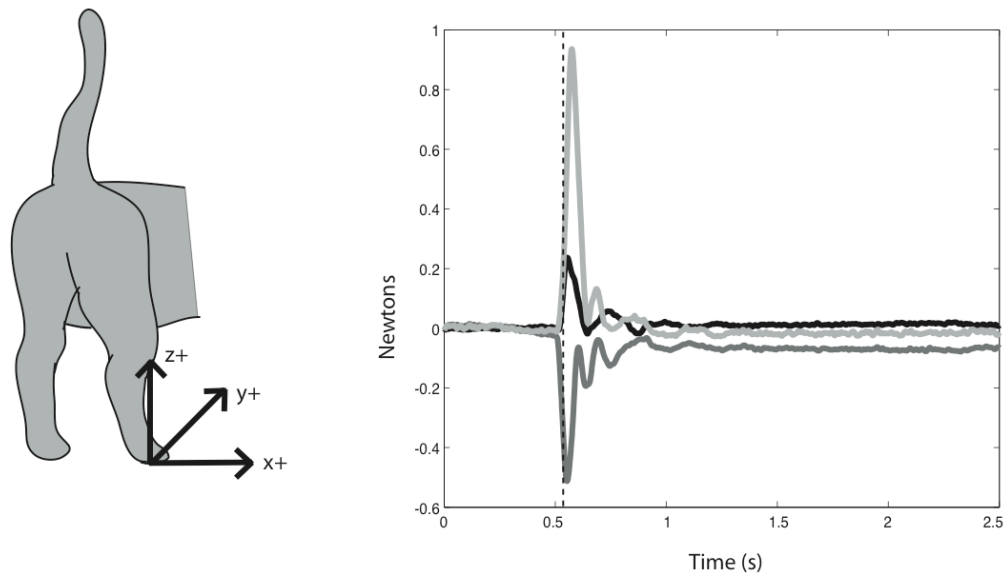
Figure 4.1. This figure depicts the hindlimbs of a cat with the positive force axis's of the force transducer below the paw. On the right we have an example force trance of the x (black), y (dark grey) and z (light grey) force responses to a stimulation. The stimulation onset is at 0.5s and we examine the force response at 0.535 ms (35 ms post onset of the stimulation).

The angular direction of the resulting force vector in each plane: sagittal (yz) and frontal

(xz), as shown in Figure 4.2, was calculated for each muscle, direction and fascia

combination. Due to the low variability between trials (see results), the mean value for

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each muscle, position and cat combination was computed. Furthermore, this allowed for

a single value for each cat that could be analyzed across position or condition. The mean

direction before versus after the complete fasciotomy was plotted against each other to

determine the change in direction. A best fit line and the corresponding 95% confidence

interval was drawn through all of the positions to show the trend in direction change. A

slope of one would imply no change in direction.

Sagittal (YZ) 90 z+   upward   90 z+   Frontal (XZ)

180 ———|——— y+ 0   180 ———|——— x+ 0

posterior | anterior   medial | lateral

                        downward

270   270

Figure 4.2. The coordinate system and corresponding directions for the sagittal and frontal plane.

To quantify the change in force in the up-down (z) direction for pBF, a subset of cats was

selected (see results for selection criteria). The mean z force for each cat was averaged

together for each of the limb configurations.

In order to compare the change in direction due to fasciotomy with the change in

direction of the force output due to position, the absolute change in direction was

calculated and plotted. For the change in direction, the maximal change in force

direction output was calculated between all conditions and averaged across all cats. For

the change within each position, under the different fascia conditions, the absolute

difference between the force direction under the fascia condition and fascia intact

position was calculated. The force direction output from a subset of the pBF cats was used (same selection criteria as previously mentioned).

4.2.4 Kinematics

To calculate the included angles of the limb in the frontal and sagittal plane, reflective kinematic markers were placed on the iliac crest, greater trochanter, upper shank, lateral malleolus, metatarsophelangeal joint (MTP) and toe. A marker was not placed on the knee joint due to the movement of skin therefore the location of the knee was calculated post hoc by projecting a vector from the lateral malleolus through the upper shank marker for the measured length of the shank segment (see methods figure in previous chapters for marker locations). Recordings of the limb position were made each time the limb changed position or a fasciotomy step was conducted using two Redlake cameras place at least 60 degrees apart. Post processing of marker location was done using Motus Peak software version 8.1. The marker positions were exported and used to compute the included angles of the hip, knee, ankle and leg (from greater trochanter to toe) in the frontal and sagittal plane in custom written Matlab code.

The included angles for all the joints in the frontal and sagittal plane were averaged together to determine any kinematic change under the different conditions or different fascia conditions. Validation to determine if any change in kinematic angles was due to a potential change in foot placement was performed by ensuring that the toe marker was

repositioned each time to be within 1cm of the previous conditions placement. Therefore we conserved the location of the endpoint.

A complete fasciotomy was then performed and the recording of the limb being placed in each position was repeated twice. The results of the mean kinematic configuration in the intact and complete fasciotomy case were compared to determine if there is a change in static limb configuration after the complete fasciotomy. Furthermore a control experiment was performed wherein the kinematics were recorded for the intact limb for each position. The limb was then repositioned in each configuration and the kinematics were re-recorded to determine if there was any change in the limb kinematics when the limb was returned to a position.

4.3   RESULTS

4.3.1   Consistency of force direction output of individual muscles

4.3.1.1   Consistency of force direction output

In order to determine the reproducibility of the force directions within a condition (fascia level of disruption) the variability of the force output from the stimulation of each muscle was analyzed. The mean force direction for each muscle had a low standard deviation of the force direction output between the five trials recorded in a position. For example, the average standard deviation for all muscles in the intact position in the sagittal plane is

3.53°. During each trial the limb moved in response to the twitch of the muscle

suggesting the same dynamic movement of the limb during stimulation and that this

movement does not affect the force direction of the limb. Additionally, control studies of

the end point force directions reported by Honeycutt (2009) did not show a significant

change in force direction output when a limb was returned to a position and the muscles

stimulated.

4.3.1.2   Static kinematics analysis

When comparing the limb configuration (joint angles) between intact and complete

fasciotomy, no significant change in configuration was observed across the five cats

analyzed. Due to each cat having a single data point as only one trial was recorded for

each position and condition combination, statistics could not be performed on the

individual cat level. In order to evaluate if returning a limb to a position in the same

fasciotomy state resulted in a change in limb configuration, we performed a control

experiment whereby the limb placed in each position twice in the intact and complete

fasciotomy state in one cat. We found that the returning of a limb to a position did not

result in a large change in joint angles in the intact or complete fasciotomy conditions

(Table 4.1). In addition, the mean magnitude between the angles recorded with intact and

complete fasciotomy conditions did not show a difference beyond the range of the

repositioning change except for the knee angle. Although the knee angle difference after

the complete fasciotomy was greater, the small magnitude of change (5.87°) suggests that

it was not an important change.

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Table 4.1. Shows the mean difference between each of the angles in the sagittal plane. The first row is the mean difference between the two placements of the limb with intact fascia, the second row is the mean difference between the two placements of the limb with complete fasciotomy performed. The third row is the mean difference between the average angle in the intact and complete fasciotomy. Note that the knee has the greatest mean change between the intact and complete fasciotomy.

Difference between:	Limb	Hip	Knee	Ankle
Intact	1.57°	0.89°	1.54°	3.57°
Complete fasciotomy	1.14°	1.42°	1.85°	4.96°
Intact and complete	1.56°	3.91°	5.87°	4.37°

4.3.1.3 Background force of the limb

The background force of the limb was examined (data now shown) to determine if there was any tonic background activity or change in state of the animal over the course of the experiment. We found that the background force was so small that it was negligible and suggests that there was very little background force in the limb. Furthermore, these forces were at least an order of magnitude smaller than the minimum force output produced by the stimulation of the muscle.

4.3.2 Primary muscle direction

The primary direction in the sagittal and frontal planes for each muscle produced by IM Stim is depicted in Figure 4.3 (a reference to the angles and directions is available in Figure 4.2). The directions for the back, natural and forward limb positions are shown. pBF and ST lift the limb upward and act as limb retractors while aBF, Glut and LG

extend the limb down and backwards. In the frontal plane aBF, pBF and Glut are limb abductors.
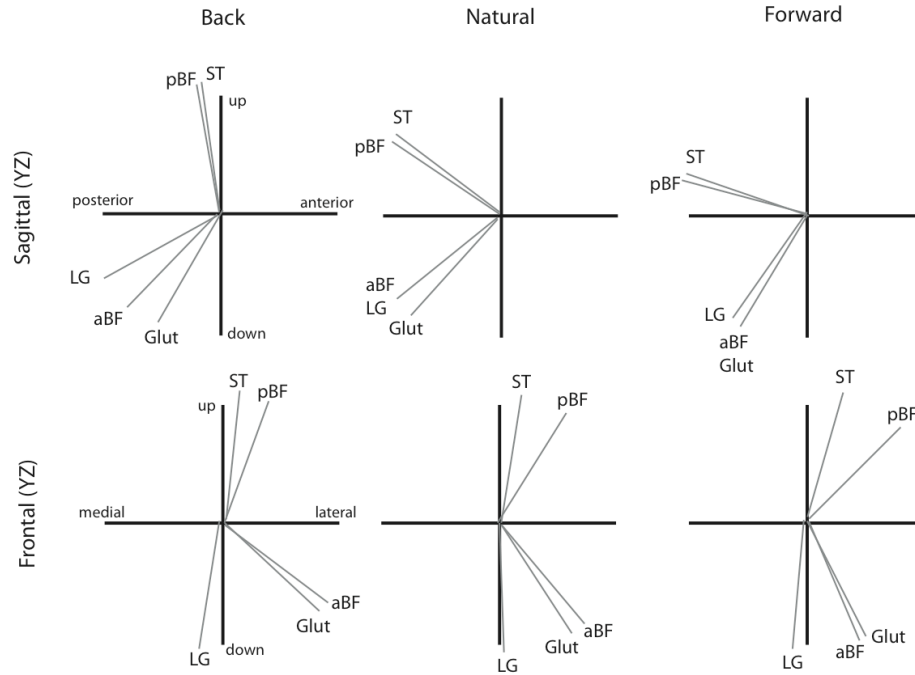
Figure 4.3. The mean direction for each muscle is displayed with a normalized magnitude in the sagittal and frontal planes for each of the three positions assessed. In the sagittal plane aBF, Glut and LG act to extend the limb while pBF and ST flex the limb. In the frontal plane aBF, Glut, pBF and ST act in a lateral direction with LG acting more vertically down or slightly medially.

4.3.2.1 Limb retractors: pBF and ST

In the sagittal plane, pBF and ST force vectors gradually become more upwardly (100.21°+-9.5 and 98.65°+- 4.2, respectively) directed upon the moving of the limb into the back position. Overall these muscles act as limb lifters pulling the endpoint upward when contracted, however at paw contact they have a posterior directed vector to which

would retract the limb (165°+-11 and 161°+-13, respectively) corresponding to function in step cycle. In the frontal plane both muscles abduct the limb (producing a lateral force) upon stimulation. pBF which is a lateral muscle produces a stronger abduction force (58°+- 27) than the ST muscle (81°+-25) which terminates on the medial side of the limb.

4.3.2.2 Limb extensors: aBF, Glut, LG

In the sagittal plane, aBF, Glut and LG act to extend the limb. In the forward position they provide a greater vertical component (239°+-2, 239°+-7 and 235°+-26, respectively) to the force that becomes more posterior (226°+-18, 240°+-13 and 209°+-3, respectively) as the limb is moved to a back position potentially depicting the ability to be propulsive muscles. This is most notably observed for LG and least for Glut. In the frontal plane, aBF (310°+-19) and Glut (303°+-6) are strong limb abductors that do not vary with the limb position while LG (272°+-16) is more purely a limb extensor showing slight variations around the vertical axis upon changing limb position.

4.3.3 Effect of complete fasciotomy on muscle direction output

Although there is limited change in direction upon complete fasciotomy for each muscle, the largest change is observed in the frontal (XZ) plane, particularly for ST, PBF and LG (Figure 4.4), which are directly attached to the crural fascia. Each muscle's intact direction is plotted for all directions and all cats against the direction after complete

fasciotomy. A slope of 1 (line of identity – shown in grey) through the points would imply that there is no change in the direction. The details of the changes for each muscle in each plane and limb configuration are described following.

4.3.3.1 aBF and Glut

aBF and Glut have relatively narrow confidence intervals, particularly in the sagittal plane, and show a similar range in pre and post fasciotomy directions with most of the points clustering around the linear regression fit. The different positions (limb configurations) represented by different colors in the plots do not show distinct directions but tend to overlap within the output directions. Although Glut in the Frontal (XZ) plane has a larger confidence interval it could be attributed to the one point (blue circle) that appears to be an outlier for the data.
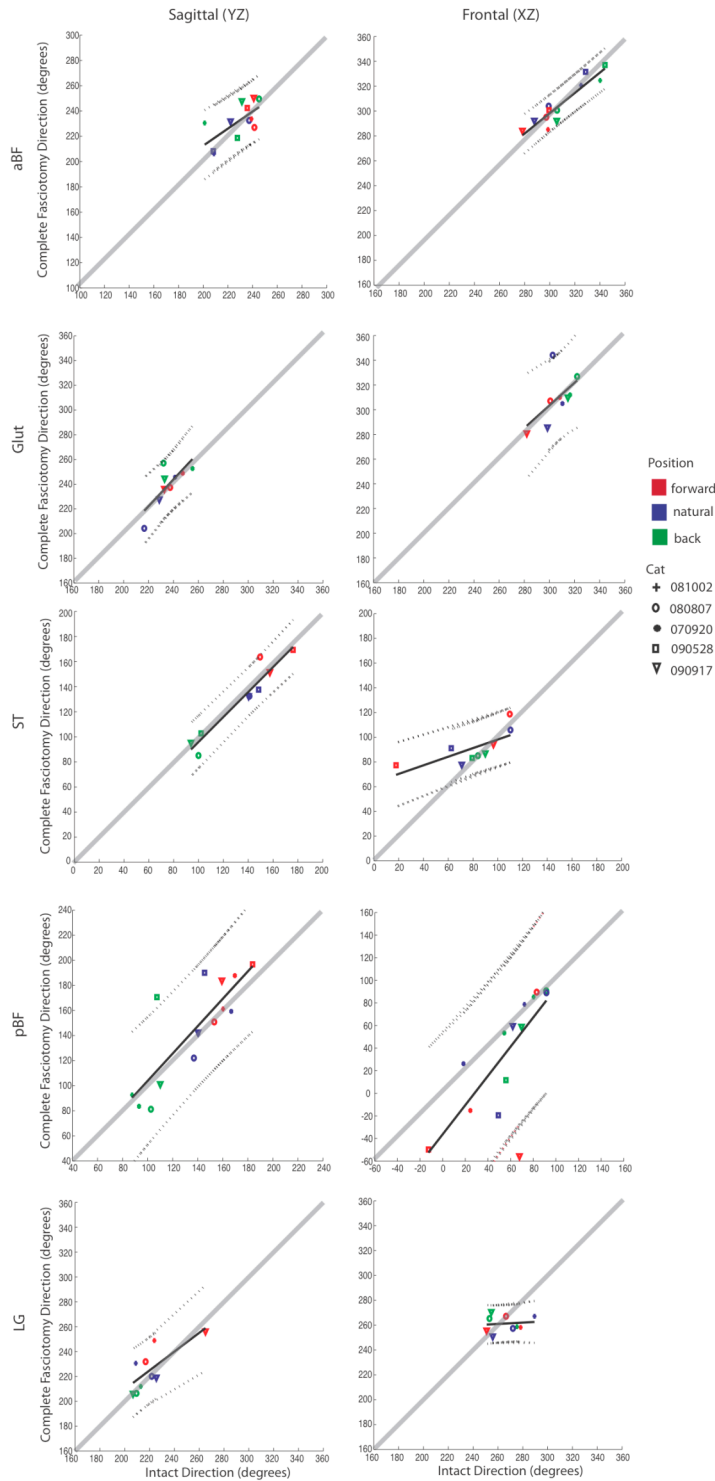
Figure 4.4. The direction of each muscle in the complete fasciotomy (post) is plotted against the direction in the intact limb (pre). The sagittal plane is represented in the left column, and the frontal plane is represented in the right column. A regression line is fit for all data points and the 95% confidence interval is plotted in dashed lines. The line of identity is shown in light grey. Each limb configuration is represented by a different color and each cat by a different symbol. The colors and symbols are kept consistent across all muscles. pBF has the largest confidence interval range. ST and LG's regression fit is almost horizontal in the frontal plane.

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4.3.3.2 ST and LG

ST and LG had a surprising and interesting effect after the complete fasciotomy, with all

endpoint force directions for all positions in the frontal plane converging towards a more

vertical direction (92°+- 14 and 261°+-6.5, respectively) and reducing the variability of

endpoint force directionality across cats. Figure 4.5 shows all LG plots of force direction

before and after complete fasciotomy (this is the same data represented in Figure 4).

Although the initial directions are more variable the final positions in the frontal plane

tend to be closer in direction than the directions for the intact case.   The same is

observed in ST in the frontal plane (data not shown).

In the sagittal plane, the force directions tend to have narrower confidence interval

ranges. Interestingly, ST in the sagittal plane shows distinct grouping of force direction

based upon the limb configuration, which is maintained upon the fasciotomy. LG does

not show as great of a distinction in force direction based upon the limb configuration

however it does show an increase in directional range after the complete fasciotomy.
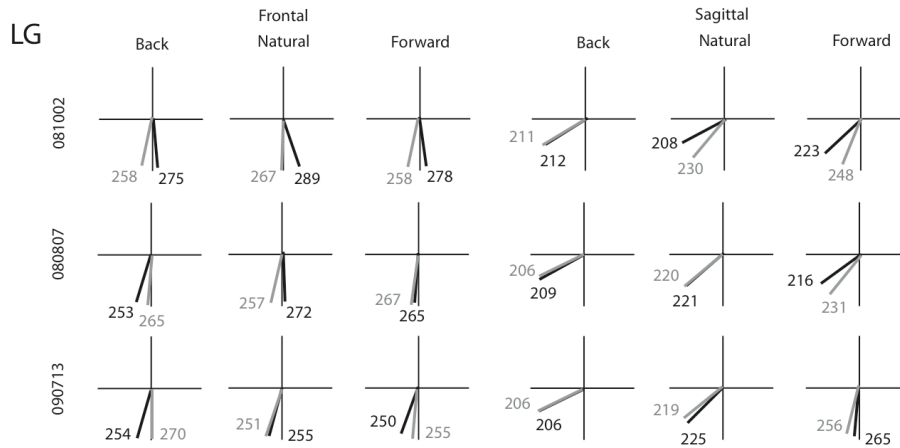
Figure 4.5. The intact (black) and complete fasciotomy (grey) force directions are plotted for all cats in all positions and in both planes for LG. LG in the frontal plane shows a convergence of the force output direction after the fasciotomy across all cats, regardless of the original direction.

4.3.3.3 pBF

pBF has a highly variable endpoint force direction after the complete fasciotomy for all limb positions in both planes. The large confidence intervals in the sagittal plane are due to the large changes in direction after the complete fasciotomy as well as the variability in direction of change. In the frontal plane there is a greater spread of force directions after the complete fasciotomy, particularly in the forward configuration (red marks on the graph). One red mark is actually denoted as an outlier to the 95% confidence intervals but upon closer inspection, two subgroups of force output direction are observed in the forward configuration.
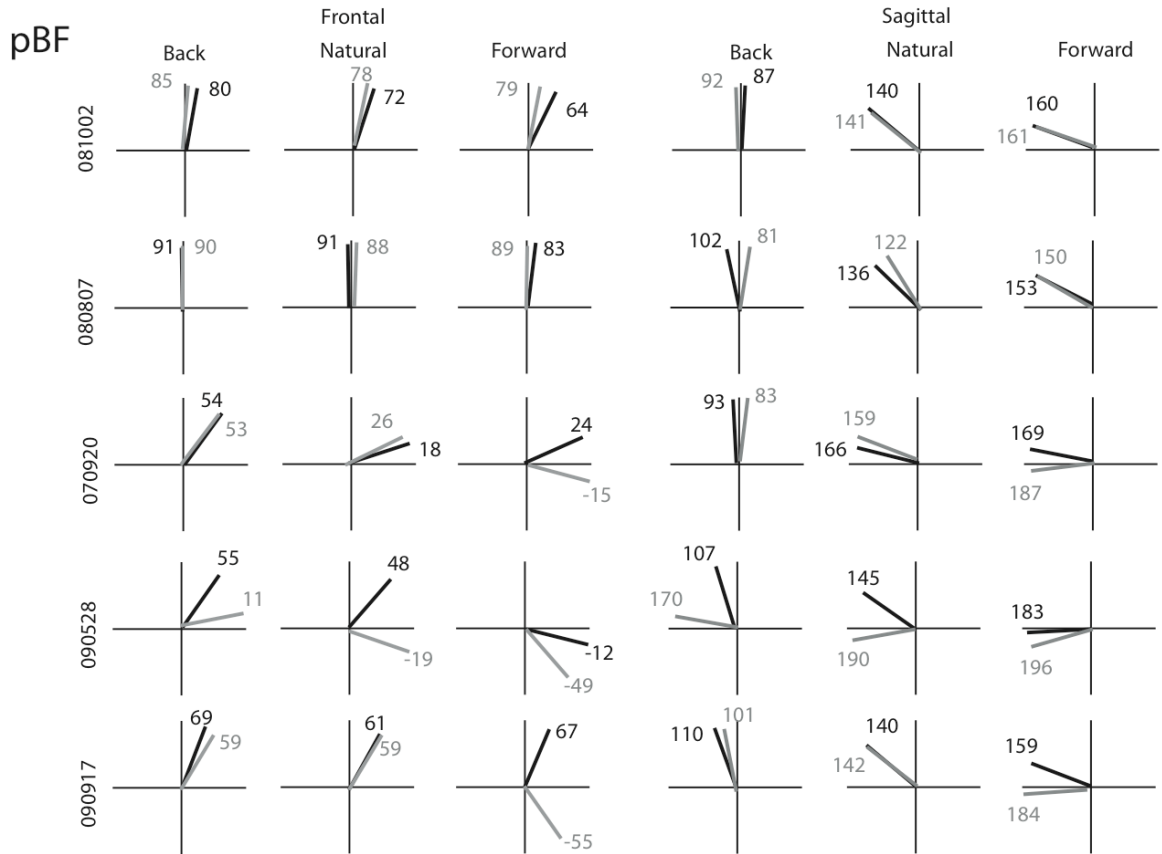
Figure 4.6. The intact (black) and complete fasciotomy (grey) force directions are plotted for all cats in all positions and in both planes for pBF. pBF has a variable initial direction and response to the fasciotomy. The two trends of force output directions for pBF upon the complete fasciotomy in the forward positions are shown.

The force output direction in the frontal plane upon the complete fasciotomy in the forward position can be separated into two subgroups. The first subgroup (cats 081002 and 080807) does not show a marked change in force direction while the second subgroup (cats 070920, 090528 and 090917) has a large change in force direction after the complete fasciotomy. This second subgroup is composed of three cats and shows a large change in force direction with the output crossing the 0 horizontal axis. The crossing of the axis implies that the force has changed from an upward to a downward directed force. This is further shown in Figure 4.7 where the normalized magnitude in

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the z direction for the three cats is plotted for the limb configurations pre and post

complete fasciotomy. The forward position shows a change from an upward force to a

downward directed force after the complete fasciotomy. The natural position is not

associated with a significant change in force magnitude. The back limb configuration has

a large standard deviation after the fasciotomy.   This is due to a high variability in the

results with one cat having almost no change in magnitude, one producing a downward

force and one cat having an almost 0 force output after the complete fasciotomy.
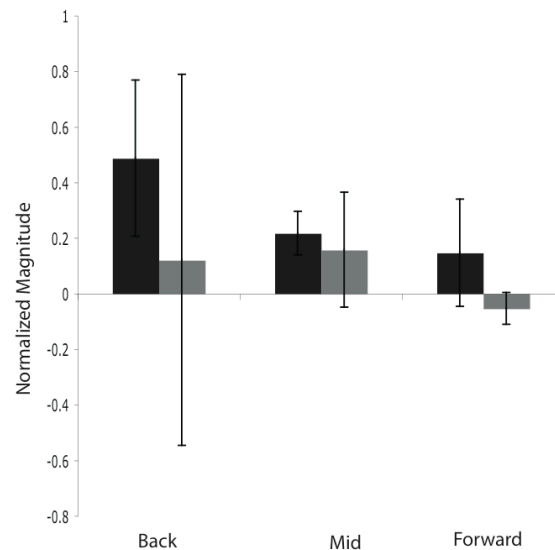
Figure 4.7. The normalized magnitude of z force produced by pBF upon IM stimulation. The forward position shows a change in the force direction from upward to downward after the complete fasciotomy. The black bars represent the intact limb magnitude and the grey bars the complete fasciotomy magnitude.

The frontal plane has a greater variability in response to complete fasciotomy regardless

of the direction of the initial force direction. The variability in force direction of pBF,

even with the intact limb, across all cats is shown in Figure 4.6 (this is the same data

represented in Figure 4.4).   In all positions and all planes there is a variable intact force

direction as well as a variable change in direction of the force output. In the sagittal

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plane forces, in the back and natural position, forces tend to become more anterior after complete fasciotomy if the initial force direction was posterior (with the exception of cat 090528 which always became more downward directed). In the forward position forces either did not change or became more downward directed (as described in the paragraph preceding).
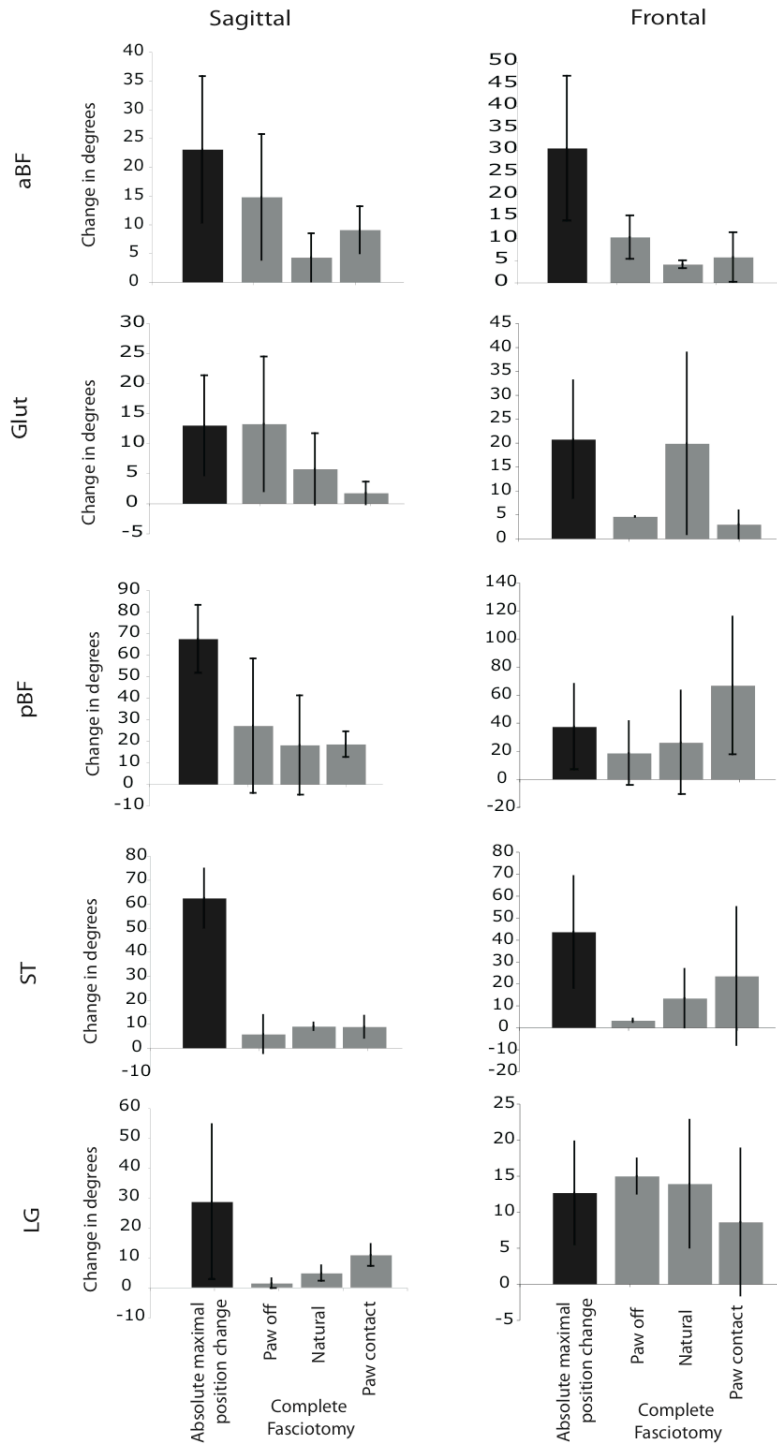
Figure 4.8. Comparison of the change in direction due to changing the limb configuration (black) versus the change in direction due to complete fasciotomy (grey). Most muscles show a greater change in direction with change in limb configuration in the sagittal plane. The frontal plane shows a trend for a greater or equal change in force direction output for Glut, pBF and LG.

4.3.4 Comparison of absolute change in muscle direction: positional vs. fasciotomy

By comparing the change in direction due to the complete fasciotomy versus the change in direction with a positional change we can determine the functional importance of the change in endpoint direction. pBF showed a bigger change in direction upon complete fasciotomy in the frontal plane in the forward position (which is when the muscle changes to pulling in the opposite direction). LG showed a comparable absolute change in direction upon fasciotomy to the change in position. All muscles were affected more by the positional change than the fasciotomy in the sagittal plane. Glut had a comparable change between fasciotomy and change in position in the back position. In the frontal plane Glut also showed a comparable change in direction in the natural stance and the positional change but this could be attributed to the outlier point observed in Figure 4.4. Overall, the change in force direction output changed more with the limb position change in the sagittal than in the frontal planes (comparing black bars in Figure 4.8).

4.4 DISCUSSION

4.4.1 Summary

Overall, our hypothesis that the muscles are directly attached to the crural fascia would have the greatest directional effect was supported. Additionally, we did observe the greatest effects in the frontal plane as expected.

4.4.2 Limitations of the study

The kinematic limb configuration results did not show a significant change upon sequential fasciotomy suggesting the changes in force direction are due to internal redirecting of force as oppose to a change in limb kinematics. Furthermore, control studies performed by Honeycutt (2009) whereby the limb was replaced in a position after relocation did not show a significant change in force direction output. The control experiment did not show a significant change in the joint angles allowing us to ignore the subtle kinematic changes when evaluating the effect on the ground reaction forces. Future experiments should be designed so that the limb configuration is preserved between fascia disruption steps to prevent any debate over the effect of the complete fasciotomy on the limb.

Although IM stimulation does not result in physiological recruitment of a muscle, it does allow for minimal disruption to the anatomy of the limb. Although muscles produce contractions through the asynchronous recruitment of muscle fascicles, our stimulation protocol only recruited braches of axons in the local area of the electrode insertion resulting in partial activation of the muscle. Furthermore, we were not investigating the effect of a full tetanic contraction, which would have produced significant more force but would have resulted in an unpredictable limb configuration and would have invoked reflex actions in additional muscles. Tetanic studies typically involve the dissection of a muscle out of a limb and the linear force recordings of the muscle, which prevent the recording of the action of the muscle on the endpoint force. Additionally, the tetanic

stimulation often involves the dissecting out of the muscle nerve, which is an invasive procedure. Therefore by using our intramuscular stimulation protocol we were able to get a more physiological and intact description of muscular action on the endpoint of the limb.

4.4.3 Effect of limb position

Honeycutt (2009) previously showed that muscles change endpoint force direction output with static changes in limb position. Our results of the primary limb direction in the intact limb support these findings. Furthermore, we show the primary direction in the frontal plane and suggest that the muscles do not display a positional-dependence in this plane. However, we cannot exclude this result as potentially being a side effect of the experimental paradigm as we did not change the limb position in the frontal plane and only the sagittal plane. Future studies, moving the limb in the frontal plane should be conducted. However, the goal of our experimental procedure was to compare the changes in force output due to different limb positions that correspond to stance in the step cycle and limited frontal plane movement is observed during stance. The results did support that while the direction of force output for some muscles, such as pBF and ST, changed in the sagittal plane across stance, the force direction remained consistent in the frontal plane. This supports the function of the muscles during stance providing propulsion and limiting non-sagittal torques on the limb therefore resulting in a more stable limb and preventing changes in the frontal plane functionality during locomotion.

4.4.3 Internal limb changes on force direction

The complete fasciotomy of the crural fascia appears to have a limited overall change in the force direction output of all muscles in the sagittal plane as most of the linear regressions lie close to the line of identity. Although pBF and LG did show larger confidence intervals compared to the other muscles suggesting that the resulting force directions did have an increase in variability.

The frontal plane showed a greater change in force direction output after the complete fasciotomy with pBF having a variable response, while ST and LG show a convergence onto a single direction.   These three muscles are closely associated with the crural fascia, with pBF and ST connecting to the origin of the muscle and LG being enveloped by the muscle. aBF and Glut had relatively no change in endpoint force direction after the complete fasciotomy and do not have a direct connection to the fascia.

The high variability of the force output of pBF is particularly intriguing as this muscle has been noted to have variable EMG patterns between cats during locomotion (discussed in Chapter 2), as well as having been suggested to be an unstable muscle towards the end of swing (Harischandra and Ekeberg 2008). Late swing is just prior to paw contact, which corresponds to our forward limb position. The forward limb position had the greatest variability in effect and actually had a large change in endpoint force direction with the muscle exhibiting greater limb extension than flexion force after the complete fasciotomy.   Harishandra and Ekeberg (2008) had suggested that the pBF muscle may require additional neural feedback during late swing to stabilize the muscle but our

results suggest that the crural fascia may act as a stabilizer to the muscle essentially becoming a distally located controller. By having the crural fascia distally stabilize the variable activity and force output of pBF during locomotion the limb maintains a consistent trajectory from step-to-step without the need for additional compensatory mechanisms.

Although it was initially hypothesized that the crural fascia would limit the mediolateral force direction outputs of the muscles, it appears that when the limb is not pre-stressed or does not have a strong background tone that the crural fascia does not restrict the range of muscle movement. This suggests that under quiescent conditions the limb is able to readily respond to perturbations with greater flexibility. This is supported by the results depicting the convergence of force output direction of LG and ST in the frontal plane after the complete fasciotomy. Although LG and ST attached to the crural differently: ST is attached at the distal end of the muscle and mostly through its tendon; while LG is surrounded by the crural fascia with the muscle fibers in series with the fascia and limited attachment at the tendon; both the crural fascia and LG tendon terminate on the medial side of the limb. This medial side insertion can be considered to be an inline termination point with the crural fascia as compared to muscles such as pBF, which terminate on the opposite (lateral) side of the limb. Future studies evaluating the endpoint force direction in ST and LG with the crural fascia pre-stressed, potentially through the activation of the hamstring muscles, may result in the loss of directionality convergence observed after the complete fasciotomy.

4.4.5 Implications for locomotion

Although the changes in force output direction for all muscles were relatively small compared to the positional-dependence, the changes can have a large effect during one step when the cat is walking. Studies by Lawrence et al (1993) showed that small differences in muscular torque could have a large affect on the trajectory of the limb during locomotion. In order to calculate how the small changes in force direction of the muscles could affect the trajectory of the limb post fasciotomy we performed a basic calculation. An average contact length of approximately 25cm for the toe from paw contact to paw off has been observed during our locomotion experiments, with a medial-lateral excursion of the toe generally being around 5cm through stance and swing. Upon complete fasciotomy a resultant direction change in the limb endpoint direction is approximately 5° over the step length given the changes in output direction for individual muscles with IM stim. This change in magnitude in the frontal plane was selected as it represents the mean smallest change in direction for a muscle in the frontal plane after the complete fasciotomy. The resultant medial-lateral excursion of the limb would be approximately 2.19cm, which is almost 50% of the entire mediolateral excursion range of the limb.

By having the crural fascia limit the amount of non-sagittal force directing of the muscle force, the forces produced by these muscles will remain in the sagittal direction and will provide more propulsive force to the endpoint of the limb. The small changes in force direction post fasciotomy in all muscles can also affect the total force direction of the limb, particularly at paw contact and paw off. In particular, we observed a large effect of

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cutting the crural fascia in the frontal plane with pBF, particularly at the forward or paw contact position. Given that paw contact is the time point that sets up the position of the limb for the weight acceptance portion of stance, it is important that the limb be in a stable and controlled position. By having the crural fascia limit the variability in muscular force, the limb will be less variable in its configuration and will therefore be more conserved in its configuration from step to step. Furthermore, aBF, Glut and LG show the largest change in force direction output upon sequential fasciotomy at the back or paw off position. Although these muscles are not typically active at paw off during level walking the forces produced by the muscles are still acting in the limb.

The pulling direction of a muscle could be transmitted to the ankle through the crural fascia given the strong anatomical connection between these muscles and the fascia. During stance this could act to decelerate the limb and changing its direction. For example, in mid-stance both the gastrocnemius muscles and the crural fascia are working in parallel to provide propulsion but again become juxtaposed at the stance-to-swing transition. At the stance-to-swing transition the gastrocnemius muscles are still transmitting residual propulsive force while the crural fascia, probably due to its connections to pBF and ST, appears to assist in the limb deceleration and change in direction. At the transition the residual propulsive force from the gastrocnemius muscles is inefficient as it is working against the direction of limb movement. The intact limb intramuscular stimulation results show that in the back limb position the pBF and ST muscles act more as limb retractors, which benefit the initiation of swing.

4.4.6   Comparisons to Hindlimb Modeling

Modeling of the cat hindlimb has shown that interjoint coupling may be an important biomechanical consideration in endpoint control (Bunderson et al. 2007; van Antwerp et al. 2007). In particular, coupling the hip and ankle joint has shown to greatly affect the force direction output of proximal, pBF, and distal, gastrocnemius, muscles (van Antwerp et al. 2007). These models do not include any soft tissue structures, such as fascia, in their anatomical design and therefore use mechanical locks to represent the coupling. It could be suggested that the crural fascia could act to provide this interjoint coupling, particularly between the hip and the ankle through its anatomical initiation from the hip extensors to the termination on the calcaneus and subsequently reduce the movement at both of the joints. Therefore, we predict a physiological range of force output would be observed from muscles in a modeled hindlimb with the addition of the crural fascia. A model of the finger tendon-network by Valero-Cuevas et al (2007) showed that an anatomical structure can switch the tensions of the feasible force set thereby acting as a local computational unit modifying the input signals from the nervous system. In a similar manner, a structure such as the crural fascia, that could localize stiffness control of the distal limb in a situation such as the need for a rapid response to a perturbation. This is particularly important as distal musculature has been suggested to play a significant role in perturbation correction, while the proximal musculature is suggested to play a stronger role in propulsion (Daley et al. 2007).

CHAPTER 5

DISCUSSION

5.1 SUMMARY

In this thesis I sought to lay the groundwork for understanding how force is transmitted in the limb by muscles and their associated connective tissues. The crural fascia, in the distal hindlimb of the cat, originates from the posterior thigh muscles, surrounds the gastrocnemius muscles and terminates on the calcaneus. The linking of the posterior thigh muscles to the calcaneus suggested that the crural fascia increases the articulation of the muscles as well as plays a role in transmitting force to the endpoint. Furthermore, the sheet-like structure of the crural fascia was investigated as potentially playing a role in reducing the medial-lateral movement of the limb. The synergist location of the crural fascia and the triceps surae muscles suggests that they may play complementary roles in the limb and the interaction between the two structures, especially during stance was investigated. Furthermore, consideration was given to the manner in which these structures may affect task or limb level variables.

To investigate the aforementioned topics I used three experimental paradigms to gain insight into how force is directed between tendon and fascia in the distal hindlimb of the decerebrate cat. First, I mechanically disrupted the crural fascia in the right hindlimb of the spontaneously locomoting decerebrate cat during level walking to investigate the role of this structure in providing propulsion and stabilizing the distal limb (Chapter 2). Second, I deinnervated the triceps surae muscles in the spontaneously locomoting

decerebrate cat to determine the acute effect of this treatment and determine if there is a short-term compensatory mechanism mediated by the spinal cord and brainstem being elicited during level walking (Chapter 3). Third, I examined the change in direction of the ground reaction forces produced by the intramuscular stimulation of select hindlimb muscles in different static limb configurations before and after the complete fasciotomy of the crural fascia (Chapter 4). By completing the studies in the decerebrate preparation I was able to investigate the effects of these interventions immediately following the procedure, therefore avoiding any possible adaptations that might occur during surgical recovery in a chronically treated and otherwise intact animal. The reduced preparation also precluded visual and vestibular inputs due to the level of transection and fixation of the head in the stereotaxic frame.

By conducting these studies I was able to address two main questions: first, to what extent do muscles and fascia in the distal limb transmit propulsive force; and second, do these structures also act to stabilize the distal limb? Our findings suggest that the crural fascia and triceps surae both act as propulsive mechanisms in the limb, as observed by the loss of ankle angular acceleration during late stance after the complete fasciotomy, and the combined increase in ankle yield in E2 and increased deceleration of the limb at the stance-to-swing transition after the triceps denervation. These structures also act to stabilize the limb as injury to the crural fascia or triceps surae results in an increase in variance of the limb segments and joint angles during the step cycle. Furthermore, the complete fasciotomy results in a change in force output direction when individual muscles are stimulated. These results suggest that the crural fascia acts to redirect force

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within the limb to reduce non-sagittal movement during level walking and reducing variance results in a more stable limb. The triceps surae provides antigravity force to counter the weight support increase demand upon weight acceptance in early stance and propulsion during late stance. The implications of these results will be discussed following.

5.2 COMPARISON OF DECEREBRATE CAT TO INTACT CAT STUDIES

The decerebrate cat was selected for this study as it is has been well established as preparation for evaluating neurological feedback systems (Baev et al. 1991; Donelan and Pearson 2004; Gottschall and Nichols 2007; Mori 1987). Furthermore, the preparation allows us to evaluate the acute response to a treatment in the limb. In order to validate that this preparation was comparable to the kinematics and EMG recordings in the intact cat I discussed a detailed comparison between the locomoting decerebrate cat and the awake, behaving cat in Chapters 2 and 3. This section will provide an overview of the comparisons.

The sagittal plane included angles and phase relations of the untreated, right hindlimb of the decerebrate cat (Chapter 2 and Chapter 3) during level (and uphill – see Appendix A) walking are within the ranges of the awake, behaving cat (Carlson-Kuhta et al. 1998). Although the decerebrate cat does show a slight extensor bias with less joint flexion than the awake, behaving cat. Overall these results presented in this thesis suggest that the

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spontaneously, locomoting decerebrate cat is a viable preparation to investigate acute, invasive treatments and lays the groundwork for understanding adaptations subsequent to injury.

Overall, the EMG recordings from the intact hindlimb in the decerebrate cat represent the timings of recordings reported for the intact cat and support the decerebrate cat as a viable preparation for understanding the muscle activation patterns during walking. Most muscles, MG, TA, aBF, ST, SM showed consistent recording patterns. However the recordings of pBF and ST have variable recordings in both the awake, behaving animals (Carlson-Kuhta et al. 1998; Misiaszek 2006; Rossignol 1996) and the decerebrate cat. Harischandra (2008) suggested that these muscles (pBF and ST) are unstable muscles during late swing, which may account for some of the inter-animal variability between recordings and suggested that these muscles require neurological feedback to stabilize. Possibly these differences in feedback between animals accounts for the variability of the recordings.

5.3 COORDINATION

5.3.1 Propulsion

The musculature of the limb provides the flexion and extension of the limb during locomotion and the corresponding propulsive force. It has been suggested that passive structures such as the crural fascia may play a role in redirecting the force output (van Ingen Schenau 1994). Although a significant change in joint angles in the sagittal plane

was not observed post fasciotomy, there was a significant change to the acceleration profiles suggesting that the crural fascia can potentially play an important role in the force transmission through the limb. Therefore the crural fascia acts to increase the torque at the joints by providing a greater moment arm at the joints given its connection from the hamstrings to the calcaneus.

It has been suggested that the proximal muscles are the main propulsive muscles in the hindlimb, while the distal muscles more readily respond to the environment or task required (Daley et al. 2007). The results presented in Chapter 3 (triceps denervation) suggest that the triceps surae muscles change in functional task during the step cycle – initially being antigravity muscles and by mid stance acting as propulsive muscles. Although the triceps surae contributed more to propulsion than the crural fascia, the crural fascia may still have a propulsive role throughout stance. The juxtaposed actions of the crural fascia and triceps surae, although synergistically located, suggests that the distal muscles act more to maintain posture, while the crural fascia acts as an extension of the proximal muscles to transmit the propulsion to the distal limb during early stance. The crural fascia acts as a mechanical link to transfer propulsive power from proximal muscles to distal joints. Although both the proximal and distal musculature is able to provide propulsive force, the proximal muscles tend to be bigger in size and weight and are able to produce larger sagittal forces. The lighter distal muscles allow for greater efficiency when moving the leg during locomotion as there less mass results in less torque required to move the distal segments. Therefore, the distribution of the heavier proximal muscles and lighter distal muscles sets up an efficient distribution of mass

within the limb. Furthermore, distal muscles have been suggested to be more compliant and able to respond faster to perturbations (Daley et al. 2009). By having the crural fascia connect from the hamstrings to the calcaneus the propulsive force can be transmitted to the endpoint efficiently with a negligible increase in mass and thereby conserving the efficient distribution of mass within the limb while increasing the propulsive output.

The connection of the crural fascia from the thigh to the shank segment allows for the direct mechanical coupling of joints and the regulation of interaction torques. The limb is comprised of multiple segments and the torques of the joint come not only from the muscles that act at those joints but also from the interaction torques from the attached segments. A limb segment can affect an adjoining segment without the activated muscle being attached to the adjoining segment. Instead the adjoining segment can be moved through inertial coupling as described in Zajac et al. (1993) whereby the activation of soleus to move the leg segment resulted in a corresponding movement in the thigh segment due to the two segments being mechanically coupled at the knee. Previous studies investigating muscle torques have dissected the muscles free from any surrounding connective tissue in order to describe the corresponding output (Young et al. 1993).   By evaluating muscle torque with connective tissue such as fascia attachments maybe more complex to evaluate but may simplify the overall control of the limb. Given that inertial torque is proportional to the joint acceleration, the decrease in angular acceleration after the complete fasciotomy suggests that the interaction torques between

the segments may be decreased. Therefore supporting the idea that connective tissues act to mechanically link segments and help control the interaction torques.

By performing a complete fasciotomy I expect that the cat would not be able to jump as high. Having initiated the examination of the kinematic effects of the complete fasciotomy in the decerebrate cat under higher propulsive demand i.e. uphill walking (Appendix A), I found that after the complete fasciotomy there was a decrease in the paw contact time with the treadmill during late stance. This suggested a loss of the limbs' ability to remain in contact with the ground and therefore transmit propulsive force. Further studies evaluating greater propulsive demands should be conducted: such as jumping. This has already been suggested as an action that may utilize the crural fascia (van Ingen Schenau 1994). Furthermore, Zajac (1985) reported on the role of the thigh muscles in the cat during jumping from a crouched position. These results showed that the double joint posterior thigh muscles (pBF, ST and G) are active during the initial propulsive phase until the heel leaves the ground.   They are then inactivated for the second propulsive phase, which lasts until the toe leaves the ground. Zajac (1985) postulated that this was due to the potential negative work created by the knee flexion action of these muscles. Zajac also claimed that the cat thigh muscles do not utilize previous storage of elastic energy. However, G, pBF and ST are all connected to the crural fascia and during active contraction in the crouched position could potentially load the crural fascia through stretch (ankle dorsiflexed and muscles shortened). Furthermore aBF and SM are active after heel off, peaking about 30ms before toe off therefore potentially keeping the crural fascia under tension and aiding in propulsion. This

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discussion suggests further studies to understand the potential propulsive role of the crural fascia during jumping.

5.3.2 Stability or restrictions on variability in limb movement

Stability can be considered to be describing a trajectory as free from change or variability. Due to the increased variability of the trajectories of the limb segments after the complete (or sequential) fasciotomy (Chapter 2) and the potential for impaired balance, I conclude that the crural fascia aids in stabilizing the distal limb.

Furthermore, I showed that the different structural components of the crural fascia play a role in stabilizing the limb. The sheet appears to balance the force transmission from the posterior thigh muscles as upon splitting the sheet there is a substantial increase in segmental and endpoint variability. Upon inspection of the tissue with a dissecting microscope, there appears to be striations in the tissue that run perpendicular to each other and with a pinnate angle. This cross-structure may be the mechanism for the cross transmission of force. Further studies quantifying the mechanical properties of this tissue and the angles of the striations would provide insight into the mechanism.

The two bands on the medial and lateral sides appear the most like tendons in the crural fascia and could be the main propulsive force transmitters in the limb. They appear to act to balance the forces at the calcaneus as after the disruption of one of the bands (and after splitting the fascia) the greatest variance is observed in the limb trajectories. This

variance decreased upon the disruption of the second band eliminating the asymmetry in the limb. Therefore together the two bands could be acting to reduce the mediolateral movement of the ankle joint. Although the crural fascia may be mostly limiting the ankle joint, the increase in mediolateral variability in all limb segments can be ascribed to the interaction effects of the different segments.

The greatest increases in variability upon disrupting the crural fascia were observed in the frontal plane suggesting that the crural fascia acts to reduce movement in the this plane. In comparison the triceps surae showed a greater (and more consistent) increase in variability in the sagittal plane after denervation. The intramuscular stimulation directions for LG showed little action in the mediolateral direction and more in the upward and posterior directions. Therefore it is not surprising that the denervation of the triceps surae did not affect the frontal plane as much as the sagittal plane and can be explained by the activation of the triceps surae muscles stiffening the distal limb and therefore reducing the variance in the trajectories.

However, it has been argued that mediolateral control requires cortical integration during walking (Dean et al. 2007). The decerebrate cat supports this suggestion, as the tail has to be clamped in order to assist with stabilization and weight support of the hindquarters during locomotion. But within the limb there are additional structures to reduce the variability of the segments in the frontal plane. Structures such as the crural fascia may help to reduce the need for proprioceptive feedback from the limb and thereby reduce the neural computation demand on the nervous system. Furthermore, by using a structure

such as the crural fascia to stabilize the limb, the need for muscular co-contraction can be reduced which is both neurally and energetically more demanding on the system.

5.3.3. Functional implication of Coordination Control

I have shown that the crural fascia (Chapter 2) and triceps surae (Chapter 3) contribute to propulsion and stability within the hindlimb. The ability of the crural fascia to act as a propulsive mechanism within the limb implies that it makes the posterior thigh muscles tri-articular. Furthermore I have shown that the denervation of the triceps surae muscles results in a lack of conservation of the limb or task variables, particularly limb length, during weight acceptance. These findings provide insight into the field of motor control, which will be discussed in this section.

It is often easier to consider the coordination of the limb in terms of a goal, collapsing the degrees of freedom; otherwise the consideration of all the degrees becomes an ambitious task. A central question in the field of motor control is how the central nervous system generates the appropriate muscle activation in order to achieve a particular movement. This is particularly pertinent given that multiple muscles cross more than one joint and have actions in more than one plane (Lawrence et al. 1993; van Ingen Schenau 1994). By the crural fascia linking segments together and limiting the movement of multiple segments the number of degrees of freedom in the limb that the nervous system has to consider if reduced. The role in reducing the degrees of freedom becomes more apparent when the limb is producing higher forces such as during running. The increased

activation of the muscles results in potentially increasing the non-sagittal torques but simultaneously increases the fascia stiffness thereby limiting the non-sagittal movement of the joints or segments. By limiting these non-sagittal movements the possible degrees of freedom to be considered is reduced.

Although there has been a lot of consideration of how the nervous system may control the limb, the idea that the control of the limb may be embedded in the anatomy is beginning to be developed. Bunderson et al. (2008) and Honeycutt (2009) have both shown that the nervous system is able to resolve the redundancy at the peripheral level through the biomechanics of the limb for the postural task. Although these studies have mostly investigated the role of muscles within the limb, it could be suggested that other mechanical structures such as fascia would be involved in the peripheral control of the limb as well. Structures such as the crural fascia allow for the linking of segments and therefore could potentially reduce the number of degrees of freedom that the nervous system has to consider (this is further explored in the section on tensegrity). The crural fascia in particular links the thigh and foot segments due to its connection originating from the hamstring muscles and terminating on the calcaneus.

Furthermore, the idea that the controller for motor coordination may be embedded in the anatomy and not exclusively originating from the nervous system is beginning to be explored. A study of the tendon network in the finger has shown that the tendons are able to perform logic that enables the correct output torque to be produced. The network is able to self-regulate the torque output based on the pre-stress within the network itself

through the distribution of the input force throughout the network (Valero-Cuevas et al. 2007). This network presents an example of how connective tissues could act to reduce the requirement for nervous system control while still maintaining a high degree of flexibility. Although the structure of the crural fascia is not as complex as the finger tendon network it may still be acting to provide the correct output torque. For example, during quiescent standing the crural fascia may not be pre-stressed and therefore be in a more relaxed state and allowing for greater flexibility, while a vigorous task, such as running, tenses the fascia through the activation of attached muscles resulting in a stiffer structure that can limit the degrees of freedom (supported by force buckle traces in Appendix B). In this way, the potential non-linearity of the recruitment of the structure can be used to change the stiffness of the tissue and its role in a task dependent manner.

5.3.4 Postural regulation

Although the studies presented in this thesis are focused on the role of the crural fascia during locomotion, it could be surmised that the crural fascia may play a role in postural regulation. During quiescent standing the crural fascia may be less stiff due to reduced muscular activation and therefore the distal limb may readily be able to respond to large perturbations. Usually in this state there is not much movement of the center of mass beyond slight postural sway and the two bands of the crural fascia may be acting to reduce the small mediolateral movements. It has been suggested that the retinacula near the malleoli act to limit mediolateral torques thereby limiting the movement of the limb (Young et al. 1993). Similarly, fascia bands could be acting to reduce the mediolateral

movement at the ankle or through the leg. The sheath could be adding to the structural

support by allowing the cross transmission of force to maintain a symmetry of force on

the calcaneus. However the forces applied to counter the movement in the limb by the

crural fascia would be small and have a small limitation the non-sagittal forces produced

by a full activation of the distal muscles.

However, the crural fascia can be tensed by the activation of the muscled enclosed by and

attached to it. In this tensed state the crural fascia could act to limit the non-sagittal

torques produced by the distal muscles. This would limit the flexibility of response of the

distal musculature to respond to the perturbations, particularly in the non-sagittal

directions. However, this would keep the forces produced by the muscles in a propulsive

direction and therefore not allow the perturbation to alter the endpoint direction greatly.

Given that the crural fascia's stiffness may be regulated by muscle activation it could be

suggested that the crural fascia could act as a distal switch to allow or reduce the non-

sagittal torques of the distal muscles and potentially assist in the response to

perturbations.   At low forces or fascia stiffness it is able to allow for the greater dynamic

muscle output range while at higher stiffness it will limit the output range of the muscles.

The crural fascia may facilitate the biomechanical force constraint strategy used by the

cat to maintain posture. Forces produced in response to horizontal plane perturbations

are determined by the biomechanics of the limb and show a bias at each paw to align in

the diagonal direction pointing toward the center of mass of the cat (Honeycutt and

Nichols; Macpherson 1988a). Given that the medial and lateral bands of the crural fascia

insert onto medial malleolus, it could be surmised that the attachment supports this force constraint alignment. Future studies evaluating the force response strategy in the hindlimb of the cat with and without complete fasciotomy should be performed.   I would expect to see a more radial constraint strategy after the complete fasciotomy instead of the ellipse as observed in the intact limb.

5.4 NEUROLOGICAL FEEDBACK

The evaluation of EMG recordings during locomotion allowed us to investigate if there was an immediate brainstem or spinal cord compensation for the treatments applied to the limb. I expected that a perceived change in proprioception within the limb would result in a change in duration or amplitude in EMG recordings.

Although sensory pathways may have had an effect during locomotion in the decerebrate cat, these effects were not apparent in the results presented in this study. Previous studies have established that reflex pathways can modulate muscle activity in the decerebrate cat both in the isolated muscle (Bonasera and Nichols 1996; Hyde et al. 1999; Ross and Nichols 2009) and in the whole limb (Honeycutt et al. 2009; Honeycutt and Nichols). Within our studies, the length and force feedback from muscles (except for the triceps surae after denervation) was still intact, however treatments to the limb, both the complete fasciotomy and triceps surae denervation, did not have a major impact on the excitation of muscles during locomotion. Instead, it appears that the hindlimb muscles were dominated by a centrally generated activity. This is supported by the lack of change in activation of the hamstring muscles upon complete fasciotomy as well as the FHL and

Plant muscles after denervation of the triceps muscles. Both treatments had the potential to influence the force and or length feedback from the muscles and consequently change the activation patterns. However, the lack of change observed in the EMG recordings suggested that our recordings were not sensitive enough to the subtle changes in activation. This issue remains unresolved and future attempts to determine subtle changes in muscle activation, such as recording from transducers attached to tendons, should be considered.

The decerebration might limit the ability of the animal to respond to treatments as the ability for cortical influences or integration has been removed. This may effect the conservation of the limb variables, which are often considered to be task variables. Although it has been suggested that the cerebellum (which is conserved in the decerebration) is involved in the regulation of locomotion and posture, in particular, limb length and orientation (Bosco et al. 2000), I did not observe a conservation of the limb length after the triceps denervation. Furthermore the limb orientation angle showed the greatest percentage increase in variance after the complete fasciotomy. These results suggest that there is not an immediate compensation in the spinal-cerebellar tracts. There may be a cerebellar response but that usually takes more time than the length of our experiments. For example, it has been shown to take at least five days to change the gain of the vestibular ocular reflex after unilateral removal of the vestibular nerve (Mailoli and Precht 1985).

One central question regarding fascia is whether or not it provides sensory information directly from innervation of the tissue. Previous histological studies have shown sensory endings of free nerve endings, Raffini and Pacnini corpuscles in fascia (Stecco et al. 2006; Yahia et al. 1992), these studies did not determine the functional role of these endings. In particular, sensory capsules around joints have been documented (Boyd and Roberts 1953) but their functionality has been mostly ignored even though they could be potentially providing information about the limits of the joint range of movement. Furthermore, direct investigations into the types of endings in the crural fascia have not been conducted. Future experiments to determine the types and locations of sensory endings in fascia should be conducted and would provide insight into the functions of fascia within the limb.

5.5   OTHER POTENTIAL FUNCTIONAL CONSIDERATIONS

5.5.1   Potential limitations of fascia on muscle fiber shortening and corresponding increased force output

The synergistic location of the crural fascia and the gastrocnemius muscles suggests that they may influence each other's force output magnitude and efficacy to the endpoint. The details of this will be explored in this section.

Pinnate muscles are described to have shortening and thickening forces that are produced by the muscle fibers upon the contraction of the muscle. The thickening forces have been considered to be inefficient forces produced by the muscle (Gans and Bock 1965). However, given that a muscle, like the gastrocnemius muscle, is surrounded by fascia it could be suggested that the thickening forces are directed to move the fascia out of the way and therefore transmit work through the fascia to the endpoint. This argument assumes that the fascia is an elastic structure. Furthermore, this design would not restrain the shortening of the muscle.

However, studies of muscles surrounded by compartment fascia have shown a decrease in muscle force upon complete fasciotomy (Baskin and Paolini 1966; Garfin et al. 1981; Huijing et al. 2003; Mozan and Keagy 1969). Baskin and Paolini (1966) had suggested that fascia acts to contain muscle volume expansion and therefore increase the interstitial pressure and transmit forces. Furthermore, Mozan and Keagy (1969) had conducted preliminary studies on the frog gastrocnemius muscle whereby they had shown a decrease in muscle contraction force post fasciotomy and then reversed the experiment by putting the muscle in a glass tube and finding an increase in force output.

Given these studies on muscles in compartment fascia it can therefore be suggested that the crural fascia has limited elasticity and limits the amount of shortening within the muscle resulting in an increase in interstitial pressure. By limiting the amount of shortening of the muscle fibers it correspondingly increases the amount of active contraction force produced by the muscle (Figure 5.1). However, initial studies

investigating the mechanical properties of parallel fiber organized fascia, such as the

fascia lata, have shown that it exhibits the non-linear visco-elastic properties of other

collagenous tissues (Wang et al. 2009). Therefore there is some elasticity of the

structure, which may allow for it to act as a way to absorb the thickening force and

transmit that force to the endpoint. Future studies into the mechanical properties of fascia

would quantify the elasticity of the tissue providing insight into its functionality. This

would be particularly pertinent when considering the non-uniform alignment of the fibers

within the sheet.

Length-Tension Curve
of a Single Muscle Fiber
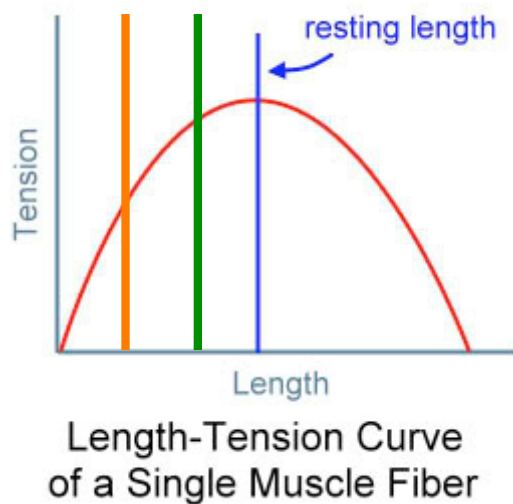
Figure 5.1. The green line represents a force produced by a muscle fiber in fascia (limited shortening). The orange line represents the force produced by a muscle fiber post fasciotomy (additional shortening allowed). The orange line has a corresponding lower tension or force than the green line. In this way the fascia acts to increase force produced by the fibers by limiting shortening.

Another way to investigate if the crural fascia were to limit the shortening or thickening

forces within the gastrocnemius muscle would be to place sonomicrometry crystals in the

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muscle and evaluate the recordings during locomotion before and after the complete

fasciotomy. It could be hypothesized that the crural fascia would restrict the thickening

of the gastronemius muscle and therefore after the complete fasciotomy I would expect to

see a thickening of the muscle body. By placing four crystals in one head of the

gastrocnemius muscle one would be able to show the length and width changes in the

body of the muscle. I expect that there would be an increase in inter-fascial width and an

increase in muscle fascicle shortening after the complete fasciotomy. The placement of

the crystals in the gastrocnemius muscle would require a small incision of about 2 mm

through the crural fascia for the insertion of each crystal. However this small incision,

especially if inline with the direction of fibers in the fascia at that point, will not affect the

overall force or functionality of the crural fascia.

5.5.2 Tensegrity

The results investigating the role of the crural fascia during locomotion (Chapter 2)

suggest that the stiffness of the crural fascia may be regulated through the activation of

the muscles associated with it. Furthermore, this regulation of stiffness or tension may

assist in maintaining the integrity of the limb. The idea of using tension to maintain a

structures' integrity is best explored through the principle of tensegrity. The term

tensegrity is an abbreviation of the terms tensional integrity and the principles dictate that

through a balance of tensional and a few compression structures the structure will

maintain equilibrium. It is important to note that the compression structures are

discontinuous with each other and linked through the continuous tensional structures.

Due to the principles of tensegrity, structures, such as the Georgia Dome, can be built to be both lightweight and strong and provide the benefit of weight to strength ratio. Furthermore these structures are able to mechanically stabilize themselves due to tensile pre-stress. Therefore, when an external perturbation is applied to the structure only a transient change will occur and the structure will return to its original equilibrium state.

It has been suggested that the human body is a tensegrity structure with the bones pulling up against gravity by the muscles, tendons, ligaments and fascia, and that the shape or stiffness of our bodies depends on the activation of our muscles or tensile pre-stress (Ingber 2008). Although the human body may not fit the exact definition of a tensegrity structure as it has compression structures (bones) in direct contact with each other, the principles may be applied on a subsystem level. In fact, most work considering tensegrity has been in regard to cells with the cytoskeleton being a tensegrity structure that is able to maintain the shape and integrity (Ingber 2008).

It has been suggested the cat hindlimb may utilize the design of a tensegrity, which results in the ability of the cat to reach high speeds, such as the cheetah, or jump high distances (Vogel 1998). The elastic structures that provide the pre-stress within the limb also have the potential for high-energy storage and release. A structure such as the crural fascia in the cat hindlimb would provide for the mechanism to pre-stress the limb as well as potentially store elastic energy required for large movements such as high jumps.

The principles of tensegrity are beginning to be applied to robotics (Paul et al. 2006). In these robots the actuators are considered the cables (muscles and elastic components) whose lengths can be changed, also known as cable collation actuation (Paul et al. 2006). Furthermore an actuator at one location in the robot produces movement at multiple locations and across "joints" therefore requiring a smaller number of actuators and the ability to have a more global control signal. The crural fascia could provide for a mechanism to transmit the actuator signal from the hamstring muscles to the distal calcaneus thereby reducing the need for actuator control in the distal limb during strong propulsive movements. Although I did not observe a change in joint angles upon complete fasciotomy I may not have been using the best experimental design as I was examining the role of the crural fascia during level walking. An activity such as maximal jumping may have elicited a stronger propulsive transmission result.

5.6 CLINICAL RELEVANCE

5.6.1 Intra-compartment pressure: syndrome and socks

5.6.1.1 Compartment Syndrome

Compartment syndrome is the compression of muscles, nerves and blood vessels within a fascial compartment and most commonly occurs in the lower leg. Acute compartment syndrome is often the result of a blunt force trauma that causes increased inflammation, edema or hematoma within the compartment. Chronic compartment syndrome occurs

regularly for a short period of time brought about by vigorous activities such as running, which temporarily increases swelling within the compartment (Biedert and Marti 1997). The increased pressure within the compartment can be attributed to the limited elasticity of the fascia comprising the compartment, as it does not expand in relation to the increased pressure. Chronic compartment syndrome is thought to be due to an increase in stiffness of the fascia properties (Hurschler et al. 1994). The increased pressure results in compression of nerves and blood vessels within the compartment, which leads to severe pain and reduced blood flow, which if left untreated will result in permanent nerve damage and tissue necropsy. The syndrome is diagnosed by recording the pressure within the compartment by inserting a needle attached to a pressure meter. If the pressure exceeds 45 mmHg then the syndrome is confirmed. The only intervention is to perform a surgical longitudinal split of the fascia. For compartment syndrome resulting from blunt force trauma the prognosis is very good as long as it is diagnosed early enough as permanent nerve damage can occur within 12 hours of the onset of compression.

After the fasciotomy has healed, the potential of scar tissue formation could affect the functioning of the fascia. The stance to swing timepoint should be examined to determine if there is an increase in muscle co-activation to stiffen the limb or if there is a decrease in the deceleration at the joints affected, both will suggest a loss of efficiency at the transition. Furthermore the loss of elasticity could affect the force output for strong propulsive movements. It could be expected that there would be an increase in muscle activation in order to increase the propulsive force. Although a stiffer tissue, such as scar tissue, may be more effective in transmitting the force it may result in a more abrupt

change in force that in turn could result in an injury. No kinematic studies (at time of writing) have been performed before and after distal limb fasciotomy to relieve compartment syndrome. Subjective evaluations post treatment mostly focus on the relief of symptoms and ability to return to a prior exercise program (van Zoest et al. 2008). Therefore our studies provide the first insight into the acute effects of the fasciotomy, particularly longitudinally splitting the crural fascia. The decrease in limb stability that I reported may influence the post operative care of the patients suggesting an external brace might facilitate patients until the fascia has healed. Alternatively a tissue engineered fascia graft could be used to increase stability and reduce the amount of scar tissue formed in response to the surgery.

5.6.1.2 Compression Socks

Although too much compression can result in tissue damage, too little can result in swelling of the extremities due to reduced vascular return. One intervention for reduced compression is to wear support or compression socks. Compression socks are becoming more popular with recreational runners although few studies have been conducted to determine how they improve the running (Ali et al. 2007; Kemmler et al. 2009). Compression socks are designed to provide a graduated pressure from the distal to the proximal end of the sock. The graduated section of the sock begins at the ankle and slowly decreases in pressure towards the knee. The graduation is thought to help improve circulation by assisting in the return of blood from the feet to the body resulting in less cramping and edema in the extremities and potentially stabilize the leg. Although there are few studies investigating the effect of the socks during running there does

appear to be evidence that they assist with clearance of lactic acid from the muscles (Kemmler et al. 2009). Given the similar location of the compression socks and the crural fascia it could be suggested that the crural fascia could have a similar effect in the leg. The compartment created by the crural fascia surrounding the gastrocnemius muscles could result in an increased pressure that would result in improved venous return. Furthermore I have shown (Chapter 2) that the crural fascia does act to stabilize the leg. One substantial difference between compression socks and the crural fascia is that the crural fascia is proximally connected to the hamstring muscles while compression socks terminate as a band without any connection to the proximal limb. Although I do not know if the crural fascia has a graduated design, it may be that the tensile force of the fascia can be adjusted by modulating the activation of muscles. I have already suggested that the crural fascia may be under less tension during quiescent periods and increased tension during periods of vigorous activity. Furthermore the studies performed by Garfin et al. (1981) showed that there is an increase in compartment pressure when muscles within the compartment are active. Therefore during periods of increased activity it may be contributing to removing lactic acid and improving circulation. In this case, an increase in compartment pressure is actually helping to improve circulation and tissue health and using the potential non-linearity of the tissue properties to an advantage.

5.6.1.3 Importance of regulating compartment pressure

The compartment syndrome and compression socks suggest that crural fascia's stiffness and modulation of the internal compartment pressure can both hinder and help structures within the compartment. Too much internal pressure results in nerve compression and

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constricted venous flow resulting in tissue necropsy while too little pressure will result in

insufficient venous return and edema in the distal limb. Therefore the role of the crural

fascia in venous return should be further investigated in future studies, including

comparative studies of different animals, such as the giraffe, where the venous return

could be difficult to control given the height of the animal. Furthermore, studies

investigating if there is a decrease in leg variability should be performed evaluating

runners wearing compression socks, and before and after the treatment of compartment

syndrome.

Rolfing, a bodywork technique, claims to be able to manipulate or reorganize the

connective tissues or fasica in the body through modified massage. One claim is that it

will allow the body to regain its full functional range of motion. This assumes that the

technique is most effective when there is an injury to the system resulting in an increase

tension or stiffness of the fascia. This increased stiffness would limit the range of

movement or flexibility of the segments associated with it. However, if the technique

were to be performed on a healthy tissue it may result in a decrease in stiffness which

may reduce the stability of those segments and possibly result in an injury.

5.6.2   Force transmission: prosthetics and orthotics and exoskeletons

By understanding the role of the crural fascia during locomotion in the hindlimb I am

able to present new design considerations for exoskeltons, prosthetics and robotics. One

major issue that is common to all three device designs is how to overcome the ability to

transmit propulsive force to the endpoint while reducing the number (and weight) of actuators thereby increasing the efficiency of the actuators. One solution would be to introduce a crural fascia-like structure into the design allowing for the placement of the actuators to be at the proximal end of the limb reducing the weight at the distal end of the limb thereby reducing the power requirement to move the distal limb. Furthermore this connection through a fascia-like structure will allow for the power from an actuator to cross joints (see section on tensegrity robots above). This would follow the suggestion that the proximal muscles in animal hindlimbs have been suggested to be the large propulsive muscles while the distal musculature is utilized for the stabilization of the limb (Daley et al. 2007).   The cat can therefore use the crural fascia to transmit this propulsive force across joints to the ankle.

Not only can the linking of the segments be used to transmit force from the actuators to the endpoint but if the structure is elastic it can also be used to increase efficiency for changing limb directions at transition points such as paw off or paw contact. Currently to change a limb direction in robotics they will use the co-contraction of the flexor and extensor actuators to stiffen the limb but this is inefficient. The ability to load or stretch an elastic component and then release it would result in a faster and more efficient transition from one direction to another. This is supported by the complete fasciotomy of the crural fascia resulting in a decrease in angular acceleration at paw off. This design could also be implemented at the contact time point and use the same principles as paw off.

Although most linking structure designs are often only thought of as a linear connection, the crural fascia has a three dimensional sock-like shape. The whole shape should be considered when translating the structure to applications. I have already shown that just by splitting the crural fascia there is an increase in variance of the limb trajectory and that if only one band is intact the variability is the greatest due to the asymmetry of force transmission. Therefore translating the full structure in to a device will result in a more stable design. Not only will the structure allow for less medial-lateral variability but it may allow for the force transduction from multiple actuators to the endpoint. Although this may be a redundant system it may provide for greater flexibility depending on the task at hand.

5.7   FUTURE STUDIES

This research has laid the groundwork for understanding the mechanical role of the crural fascia during locomotion. Furthermore it has provided insight into the different roles of the sheath and the bands and how they interact to provide stable locomotion.

Ground reaction force recording during stance during locomotion could be used build upon the kinematic studies and determine if there is a decrease in downward or backward directed force and an increase in mediolateral force after the complete fasciotomy during stance. Further studies into the role of the crural fascia during higher propulsive

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movements such as galloping or jumping should be considered, particularly to further explore the questions regarding propulsion and the segmental interaction torques.

In order to understand the potential mechanical role of the crural fascia, mechanical testing of the tissue, both the sheath and the bands should be conducted to determine the viscoelastic properties of the tissue in multiple pulling directions. The mechanical testing would also determine if the crural fascia was elastic and therefore able to store and release energy during the step cycle.

Conducting comparative studies of fascia across different animals, particularly between those who walk plantargrade versus digigrade would elicit if passive mechanical systems play a larger role to stabilize a limb with the ankle in the air, like the cat, instead of on the ground, like the human. Additionally it would provide insight into how different animals may use the structure for stronger propulsive movements, like pouncing in the cat, or venous return, like giraffes with long legs.

Given the limited EMG results further exploration into the question of whether or not the crural fascia is innnevated and the potential type of sensory endings should be conducted. This will determine the potential sensory role of the crural fascia within the limb. This may be particularly important at the joints as it may signal when a joint is nearing the limits of its range of motion to reduce the risk of injury.

APPENDIX A:

**DISRUPTION OF THE CRURAL FASCIA DURING UPHILL WALKING
RESULTS IN LOSS OF PROPULSION**

A.1 INTRODUCTION

Cats and humans encounter a number of different demands when walking, such as going up or downhill. Walking uphill requires additional propulsion. The neuromuscular system achieves this by increasing the activation of all muscles during the step cycle (Carlson-Kuhta et al. 1998), the extensor muscles to carry the animal forward during stance and the flexor muscles to lift the foot higher for placement up the slope.

The best way to quantify propulsion would be to examine the ground reaction forces. A ground reaction force is the external force acting on the body in response to its contact with the ground resulting in an acceleration of the body. As propulsion and therefore acceleration increases, so would the corresponding ground reaction force. However we were not able to record the forces, as we did not have the technical capabilities. Therefore we elected to examine propulsion by collecting kinematics and elucidating propulsion information from the trajectory of the limb. The evaluation of the trajectory of the paw would provide information about the contact of the paw with the treadmill. A decrease in contact with the treadmill would result in less ground reaction force and therefore less force to propel the body forward. By evaluating if there is a change in contact with the treadmill belt after the complete fasciotomy we would be able to suggest if the crural fascia played a role in propulsion in uphill walking.

The anatomy of the cat family has been suggested to be designed for strong propulsive movements, like jumping (Vogel 1998) and fast running (van Ingen Schenau 1994). Although we did not observe a substantial change in the limb kinematics during level walking upon complete fasciotomy we did find a loss of angular acceleration during stance, which suggests that the crural fascia may play a propulsive role. Therefore our objective was to elicit the potential propulsive role of the crural fascia during a greater propulsive demand, such as uphill walking. We hypothesized that the paw has less contact with the treadmill during late stance, therefore less ground reaction force would be applied to the limb to accelerate the animal forward resulting in less propulsion.

A.2   METHODS

A.2.1   Surgical preparation

The effects of the complete fasciotomy of the crural fascia was investigated in three decerebrate cats walking at 0.7m/s atop a variable speed treadmill during level and uphill walking conditions. All experimental protocols were approved by the Emory University and Georgia Institute of Technology Institutional Animal Care and Use Committee.

The animal was initially prepared as described in locomotion chapter. The skin was initially opened and blunt dissected off the crural fascia, kinematic markers placed on the right hindlimb (Figure A.1) and biopolar EMG electrodes were inserted into the muscles of choice (to be described later in the Methods) while under anesthetic. Once the animal

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was affixed atop a level, variable speed treadmill, a premammillary decerebration was performed and the anesthetic was then slowly reduced until eliminated.

A minimum of three trials was recorded under each condition evaluated and each trial consisted of a minimum of 20 seconds of continuous stepping. Stepping was usually spontaneously evoked upon the running of the treadmill, but occasionally stimulation at the base of the tail or through manipulation of the forelimbs in a walking pattern was required to establish a consistent pattern. Once consistent stepping was observed the treadmill was manually lifted to an angle of approximately 22°. The angle of the head was kept level with respect to the treadmill and not adjusted to a downward position as would be observed in an intact cat (the vestibular system was also kept intact). The tail height was adjusted if the animal displayed an inability to keep the paw on the treadmill throughout the step cycle with an intact limb.

A surgical treatment of disrupting the crural fascia was applied to the limb during uphill walking. This disruption was achieved by first longitudinally splitting the crural fascia, then cutting the medial band before finally cutting the lateral band (see Chapter 2 Figure 2.1 for details). The skin was resealed after the treatment with a blunt-edged alligator clip. At the end of the experiment the animal was euthanized with 1ml euthasol administered through the intravenous line.
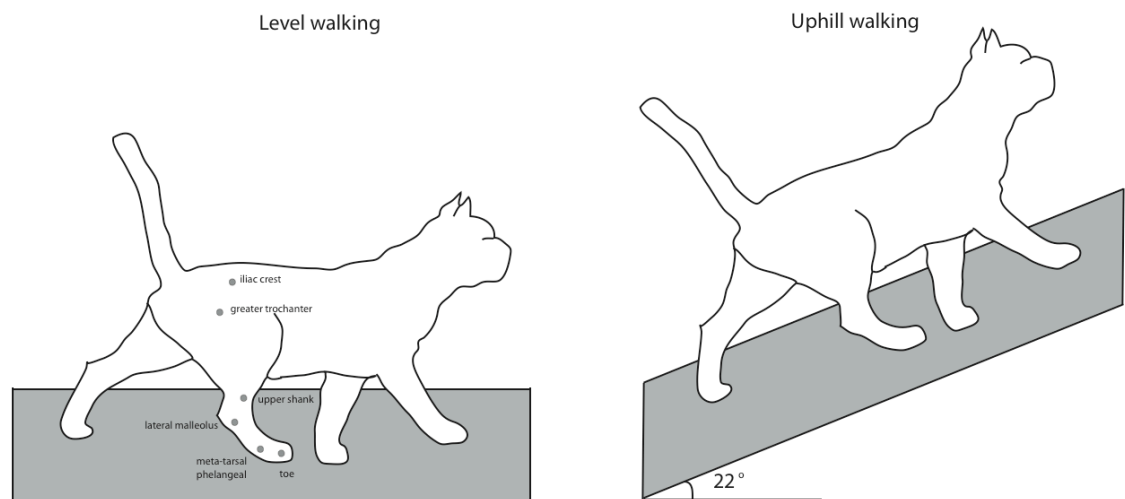
Figure A.1. To simulate uphill walking the treadmill was lifted and supported at the anterior end at an angle of 22°. Kinematic recordings of the markers placed at the iliac crest, the greater trochanter, upper shank, lateral malleolus, meta-tarsal phelangeal (mtp) and toe were collected under both level and uphill walking conditions.

A.2.2 Kinematic recordings

The three-dimensional trajectories of the reflective markers placed on the right hindlimb were used to describe the mechanical changes observed between level and uphill walking and complete fasciotomy in the uphill only condition. The marker trajectories were recorded by Vicon cameras at 125 Hz and paw contact and paw off were demarked during post-hoc video analysis of the animal walking. All data was filtered and processed in custom Matlab scripts.

The included angles of the hip, knee and ankle were calculated in order to compare to data presented on uphill walking in intact cats as well as elicit any changes in propulsion in the limb upon the complete fasciotomy. The calculation of the angles has been described in the previous chapters. The resulting angular trajectories were then normalized to 4% bins in order to average nine steps from three trials under each

condition together. These results were compared between level and uphill walking, and between intact and complete fasciotomy conditions during uphill walking.

In order to fully visualize the net effect of complete fasciotomy of the crural fascia during uphill walking, the trajectory of the MTP marker in the sagittal and horizontal planes was reconstructed for a representative trial under intact and complete fasciotomy conditions.

A.2.3 EMG electrodes

Pairs of bipolar electromyographic wires were inserted into semitendinosis (ST, 2 cats), posterior biceps femoris (pBF, 1 cat), tibialis anterior (TA, 2 cats), Sartorius (aSart, 1 cat), Vasti Lateralis (VL, 1 cat), and medial gastrocnemius (MG, 1 cat), and sutured into place with surgical silk. Not every cat had the same combination of muscles. Mineral oil was applied to the muscles and the skin incision closed with staples. The EMG recordings were high pass fourth order Butterworth filtered and rectified post-hoc for each trial. The first 10 consistent steps of each trial were compared to determine if there was a change in EMG activity profiles. The EMG recordings were time normalized from paw contact to paw contact using 25 bins (each representing the mean for 4% of the step cycle). We were only able to record EMG from two of the cats, as cat 081030 had noise interference that preventing the recording of EMG activity.

A.3 RESULTS

A.3.1 Comparison of level to uphill walking

A.3.1.1 Kinematics: sagittal included angles

Uphill walking resulted in an increase in hip, knee and ankle extension at paw off compared to level walking (Figure A.2). Only two of the three cats are depicted in Figure A.2 due to cat 081030 hitting its toe on the treadmill during swing resulting in a highly variable trajectory. The increase in extension at the hip is observed for all three cats but the increase in knee and ankle extension is only observed in two of the cats (cat 081030 did not show the trend). There is a phase shift forward in the timing of maximal extension (for two cats) for uphill walking resulting in maximal extension (near paw off) to occur earlier in the step cycle than level walking suggesting an increase in acceleration through the stance phase in uphill walking.
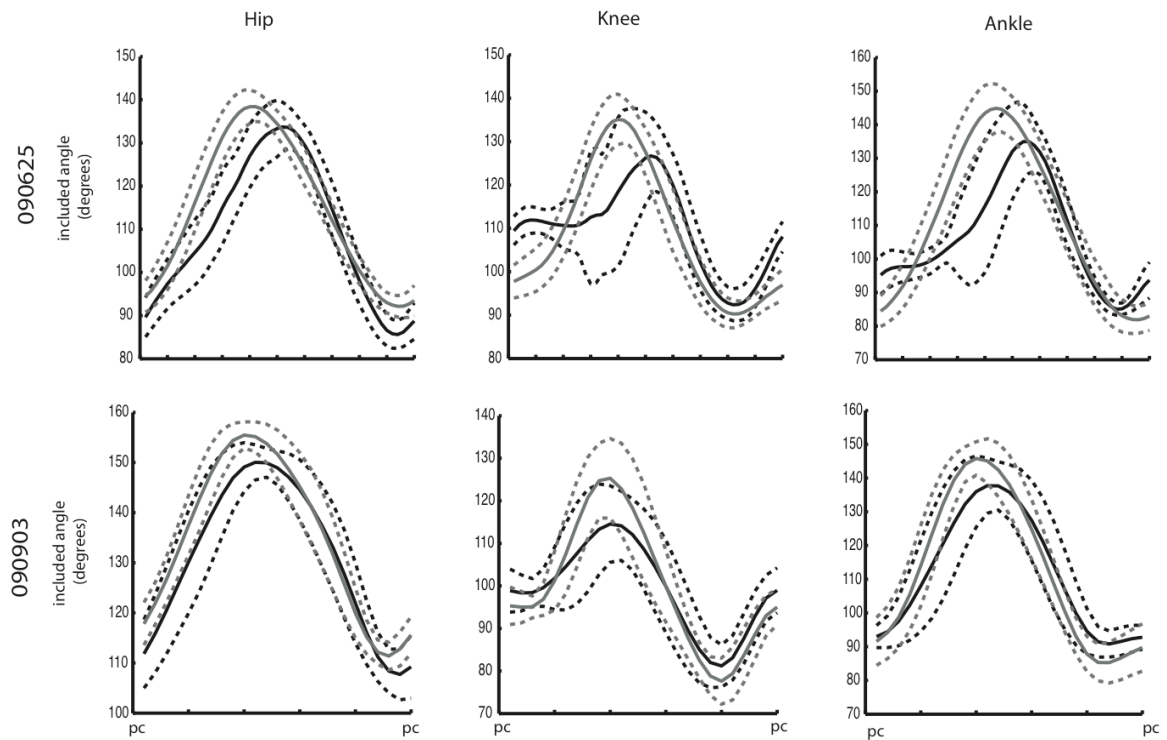
Figure A.2. The normalized sagittal plane included angles for the hip, knee and ankle angles during level (black) and uphill (grey) walking. The mean angles (solid line) across all trials in the condition and the standard deviations (dashed lines) are shown. There is an increase in extension in the hip, knee and ankle included angles during uphill walking compared to level walking at approximately paw off (maximal extension). Also there is a shift forward in timing when the maximal angles are reached at the end of stance.

A.3.1.2 Muscle activation response: EMG recordings

There was a variable response in muscle activity from level to uphill walking (Figure A.3). TA, ST, aSart and VL had an increase in activity and MG and pBF had a decrease in activity. TA consistently increased in amplitude for both cats. ST had an increase in amplitude around paw off for cat 090903 while 090625 had a shift in the timing of the paw off activity of ST resulting in earlier activation. This earlier activation coincides with the shift in the joint angle kinematics that suggested a shorter stance duration. aSart and VL showed an increase in amplitude and an earlier peak activation time when

walking uphill. pBF did not have the same pattern of EMG bursting as observed in ST

but these muscles have been reported to have variable patterns of activity as described in

our locomotion chapter. pBF and MG had a decrease in overall activity upon uphill
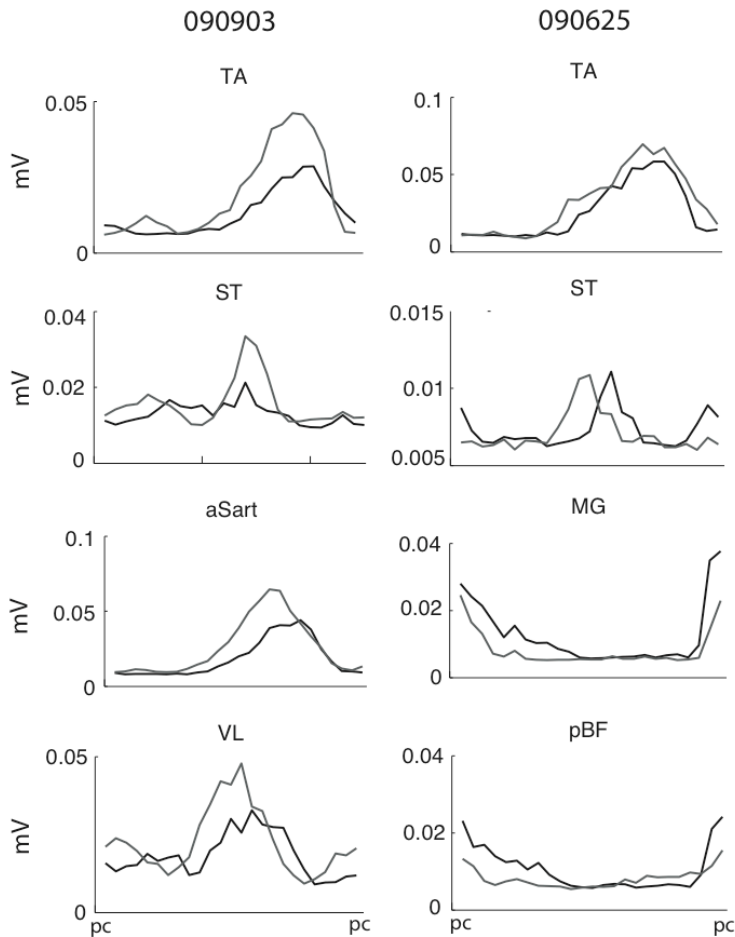
walking.

Figure A.3. The mean, normalized EMG traces for example muscles from two of the cats are presented. The mean is from paw contact (pc) to paw contact. An increase in activity when walking uphill (grey lines) is observed in TA, aSart, VL, ST (in one cat), while MG and pBF show a decrease in activity.

A.3.2   Effect of complete fasciotomy during uphill walking

A.3.2.1   Evaluation of endpoint trajectory

There appears to be two effects on the limb endpoint during uphill walking upon

complete fasciotomy. The first effect is best observed by examining the trajectory of the

mtp in the sagittal plane (Figure A.4A). The step cycle begins at paw contact and moves

down the treadmill during stance and is then lifted off the treadmill and swing back to

paw contact. Upon the complete fasciotomy we observe an increase in vertical lift of the

paw (appearing as a tail on the trajectory) around paw off (cat 081030 and 090903). This

increase in vertical excursion depicts a lifting of the paw off the treadmill before maximal

extension is reached.

The second effect of the complete fasciotomy during uphill walking is best viewed in the

horizontal plane (Figure A.4B). The step cycle begins at paw contact and moves

posterior (left) to paw off and then typically returns as a lateral swing to paw contact,

although cat 090903 showed a variable swing trajectory. The uphill mediolateral

trajectory of the intact limb was observed as a similar trajectory in level walking.

However, cat 090625 shows a medial excursion of the paw during stance that becomes

exaggerated upon the complete fasciotomy. Due to the medial paw off position, the paw

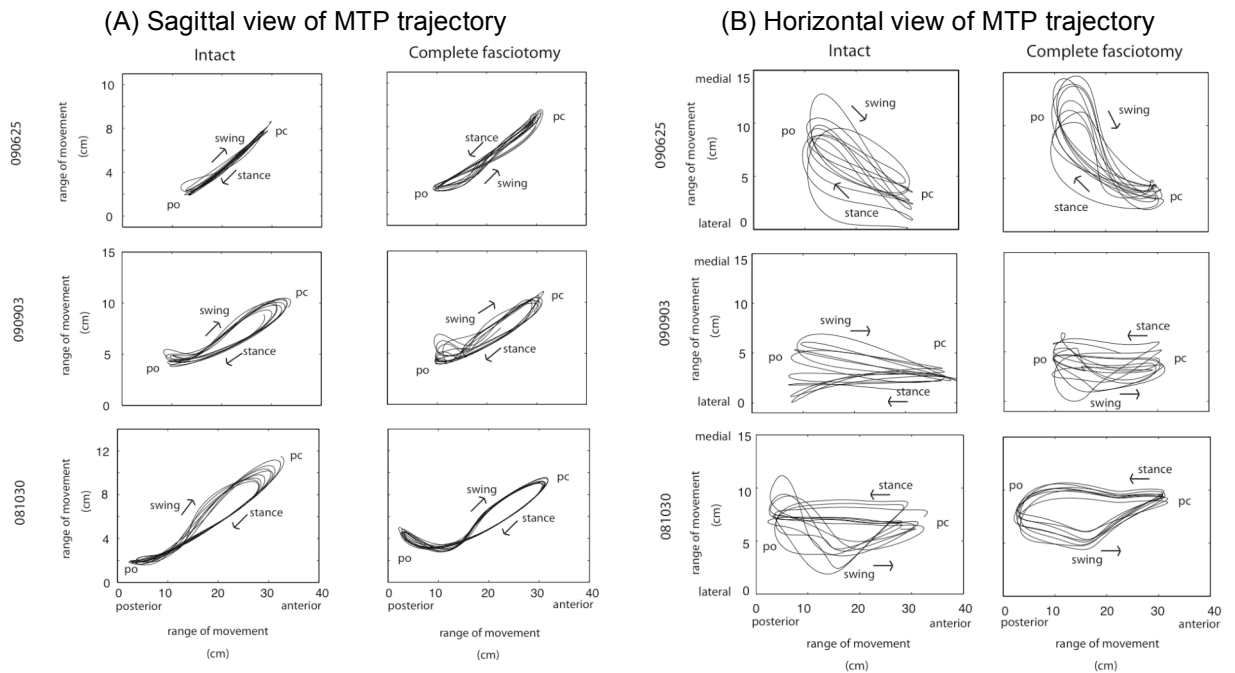returns to paw contact with a medial swing.

Figure A.4. The (a) sagittal and (b) horizontal view of the MTP trajectory during uphill walking for the three cats with intact and complete fasciotomy conditions. Two cats (090903 and 081030) have an increase in vertical projection at the paw off time point after the complete fasciotomy, while cat 090525 does not exhibit the vertical change it does exhibit and increase in medial movement of the limb through stance and at paw off.

A.3.2.2   Kinematics: sagittal included angles and angular acceleration

The angular excursion of the joints did not significantly change during uphill walking

upon complete fasciotomy (Figure A.5). Although two cats (090625 and 090903) exhibit

a phase shift forward in peak extension at all joints after the complete fasciotomy. This

suggests that they have a lower percentage of the step cycle in stance than the intact limb.

Cat 090903 has a second bump during early swing (after maximal extension), which is

due to the toe hitting the treadmill shortly after maximal extension. Cat 081030 has an

increase in flexion at all joints during early weight acceptance suggesting a loss of

antigravity action within the limb.
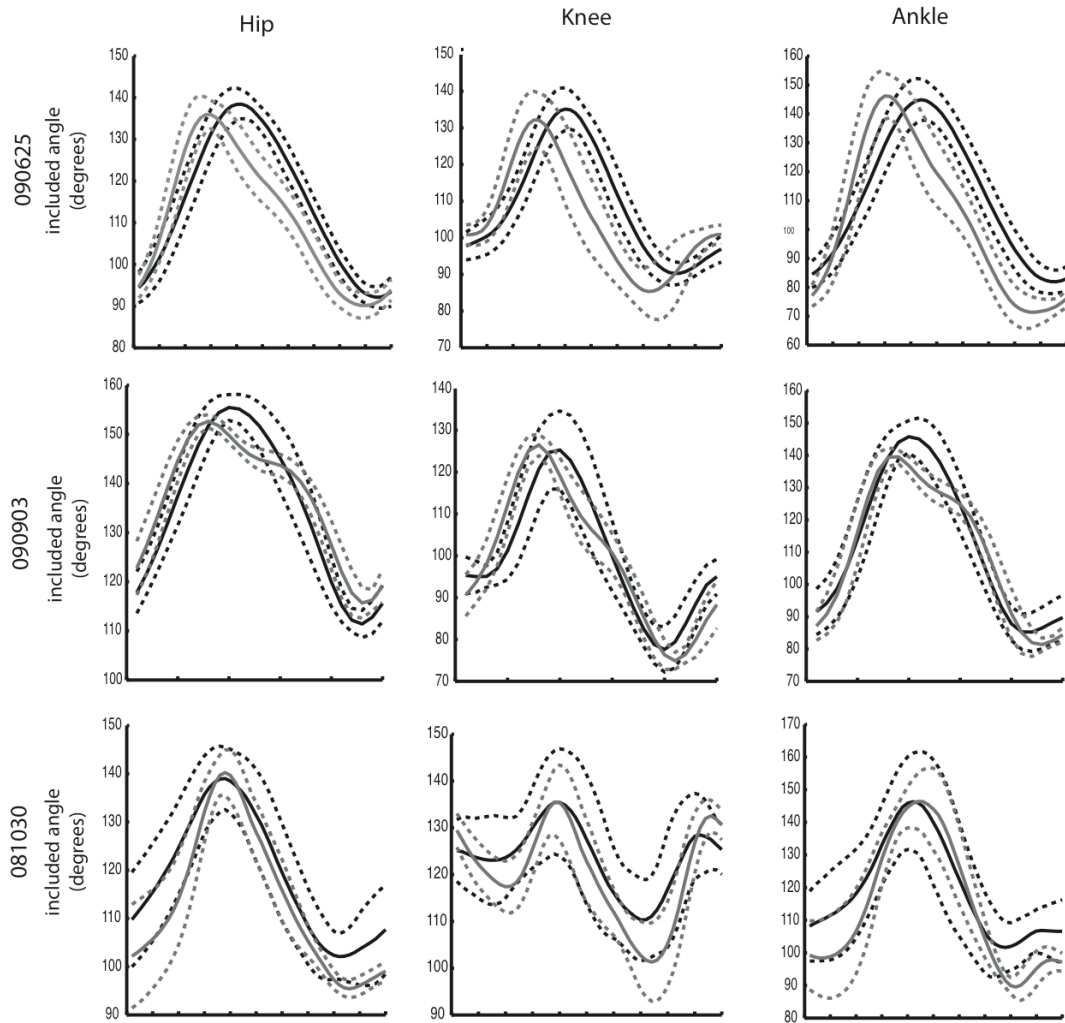
Figure A.5. The joint kinematics of the three cats walking uphill pre and post complete fasciotomy. Two cats show a phase shift forward in timing of maximal extension at all joints. One cat has an increase in joint flexion during weight acceptance.

A.3.2.3   Muscle activation response: EMG recordings

The EMG recordings from the two cats (090903 and 090625) show different trends in

activation amplitude after the complete fasciotomy in uphill walking (Figure A.6).

While cat 090903 (which lifted the foot up at paw off after the fasciotomy) showed a

decrease in amplitude for all muscles, cat 090625 (which pushed the foot medially after

the complete fasciotomy) showed an increase in amplitude for ST and pBF.

Interestingly, both cats exhibit an increase in the duration of EMG activity of ST near

paw off, furthermore cat 090625 showed an increase in activity in pBF beginning in mid

stance and continuing to after paw off suggesting a change in the activation patterns of

muscles that have a connection to the crural fascia.

Figure A.6. The mean and normalized EMG traces for a subset of muscles for two cats. The black lines represent the mean EMG trace for the intact limb and th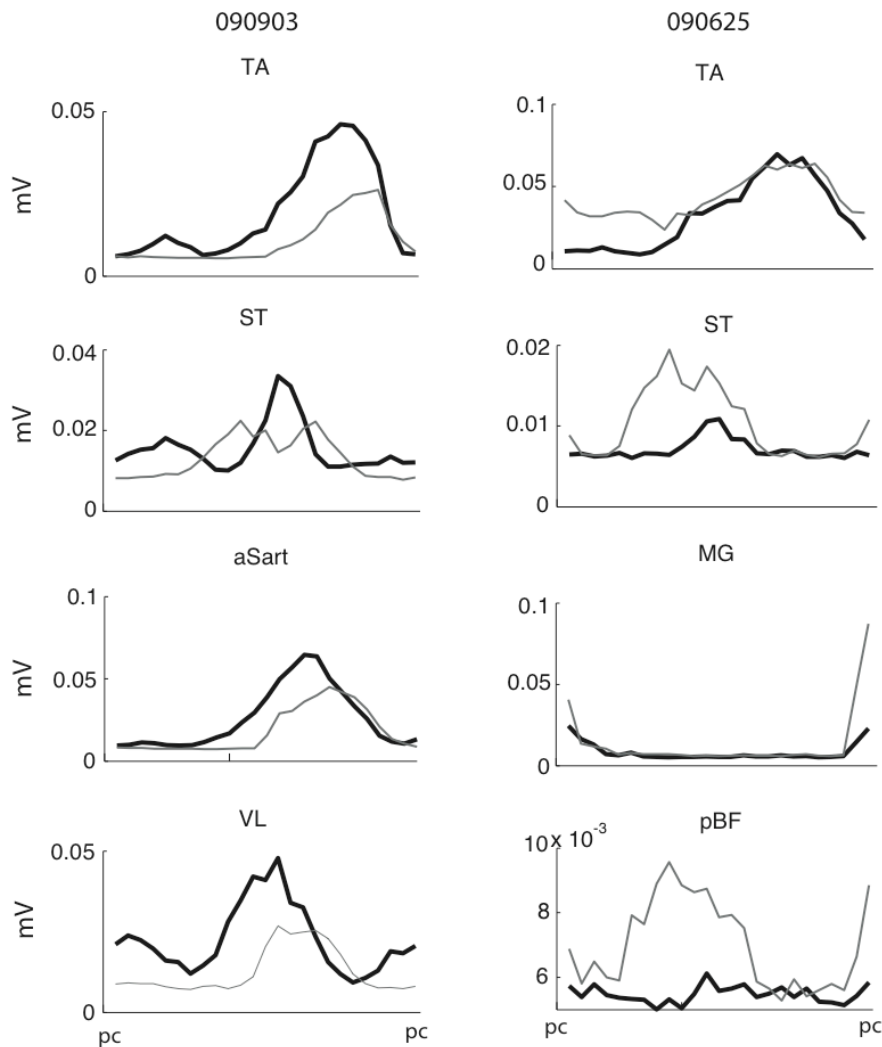e light grey lines represent the mean EMG trace after the complete fasciotomy. Cat 090903 shows a decrease in amplitude for all muscles upon the complete fasciotomy while cat 090625 shows an increase in amplitude for all muscles surveyed upon complete fasciotomy.

A.4 DISCUSSION

A.4.1 Summary

When the decerebrate cat went from level to uphill walking there was an increase in extension for all joint angles at paw off. There was a corresponding increase in EMG activity for TA, aSart, VL and ST muscles but not MG and pBF. When the cat then underwent a complete fasciotomy while walking uphill there were two observed responses: either an increase in vertical lift of the paw at paw off or an increase in medial movement of the limb during stance and at paw off. The mean angular excursions of the joints suggested a shorter stance as there was a trend to reach maximal extension of a joint earlier in the step cycle during uphill walking. Although there were two trends in the movement of the limb endpoint there was a consistent increase in EMG duration around paw off for ST and pBF.

A.4.2 Level to uphill walking: comparison to intact cat

The angular excursions reported for the decerebrate cat showed a slight increase in extension of the joint angles compare to those reported for intact cats walking uphill (Carlson-Kuhta et al. 1998; Maas et al. 2009). This increased extension could be attributed to the extensor bias of the decerebrate cat. The observed shorter stance percentage of the step cycle in the decerebrate cat when walking uphill appears to be consistent with the intact cat as compared to the markings of paw contact and paw off timing in the intact cat as reported by Carlson-Kuhta et al (1998).

The EMG signals from our decerebrate cat showed a variable response in EMG amplitude during uphill walking as not all muscles increased in amplitude. This is different to the results recorded from the intact cat where the extensor muscles had a increase in activity during stance and the flexors had an increase in activity during swing (Carlson-Kuhta et al. 1998; Gottschall and Nichols 2007). Additionally, we do not observe the increase in EMG activity of ST during early stance in both cats (only for cat 090903) as observed in intact cats (Carlson-Kuhta et al. 1998). One important difference between the intact cat and our decerebrate cat was that we kept the head level with the treadmill when we increased the slope of the treadmill. This is different to the observed head position of the intact cat (Carlson-Kuhta et al. 1998), which remained level with respect to gravity (or downward facing with respect to the treadmill). However, the slope of the treadmill should be sufficient to elicit the uphill walking response without the need for the headtilt (Gottschall and Nichols 2007).

A.4.3 Effects of fasciotomy on uphill walking

Although we observed two changes to the limb trajectories during uphill walking after the complete fasciotomy, they can be explained based upon the initial trajectories. The two cats that showed a relatively linear trajectory during stance had an increase in lift of the foot at paw off upon the complete fasciotomy. This could be attributed to the redirection of force from the hamstrings to the tibia after the complete fasciotomy resulting in an increase in knee torque which will lift the foot upward earlier. The earlier

knee flexion may also have a contribution from the earlier onset of the EMG recorded from ST around paw off. By no longer having the paw on the treadmill during late stance there will be a loss of propulsive force transmitted to the endpoint after the complete fasciotomy resulting in a decrease in efficiency when walking uphill. This is due to the loss of force from the limb being transmitted onto the ground and therefore no corresponding reaction force accelerating the body forward. In the case where the paw went more medial during stance to paw off after the complete fasciotomy, the cat was already sliding the foot medially in the intact limb. This suggests that there may not have been sufficient weight support (tail held too high) for the animal during stepping. The increase in mediolateral trajectory after the complete fasciotomy supports our earlier findings during level stepping (Chapter 2) where the treated hindlimb had increased mediolateral variability. The increase in medial projection of the limb during stance and early swing suggests that the crural fascia was helping to keep the limb in a more linear overall trajectory. Having a trajectory that is more sagittally directed would suggest greater forces being directed in the anterior direction resulting in greater forward acceleration of the animal. Increased non-sagittal forces would decrease the force projection in the sagittal plane. The trajectories of the limb endpoint support the hypothesis the crural fascia plays a role in limb propulsion during uphill walking.

These responses may have been exaggerated due to the preparation having partial weight support by the tail clamp. This mechanical fixation might have prevented the hip from lowering in response to the complete fasciotomy allowing for the maintenance of paw contact with the treadmill. However, we have previously shown (Chapter 3) that the limb

length is able to decrease after the denervation of the triceps surae with the tail clamped suggesting that the limb should have been able to change length to maintain contact with the treadmill. Therefore, the effect of lifting the paw off the treadmill during late stance is contributable to the complete fasciotomy.

Although the general trends in EMG amplitude were different for the two cats, there was a consistent increase in ST during around paw off and into the flexion phase of swing. This suggests that there is an increase in muscular contribution to knee and ankle flexion during early swing that may have been previously achieved by the crural fascia. The crural fascia's connection to the calcaneus could pull or lift the posterior foot off the ground during early swing by creating a greater knee flexion moment arm.   After this has been removed the knee flexion musculature is recruited to increase knee flexion. Furthermore, cat 090903 showed a delay in the activation of the limb extensor muscles during swing, which would counter the flexion.   Overall suggesting a changing in the timing of the CPG pattern for proximal flexors and extensors upon the complete fasciotomy.

APPENDIX B

FORCES RECORDED FROM THE TENDINOUS BANDS OF THE CRURAL FASCIA DURING LEVEL WALKING

B.1 INTRODUCTION AND METHODS

Force buckles have been used to record individual muscle forces during locomotion (Biewener et al. 2004; Prilutsky et al. 1996). To capture the muscle force the buckles are placed on the tendons of the muscle of interest. Given that the medial and lateral bands of the crural fascia appear tendinous in structure we placed two force buckles on the bands in our spontaneously locomoting decerebrate cat. Care was taken during the insertion to limit the disruption of the crural fascia to small holes on either side of the bands to allow for the insertion of the buckle. Recordings were collected during the locomoting trials as described in Chapter 2.

B.2 RESULTS AND DISCUSSION

The recordings from one cat (090326) are shown in Figure 1 and depict a peak force at paw contact, which decreases through stance and the first part of swing before rising to return to paw contact. Although we have recordings from additional cats, most had technical issues, however one other cat shows a similar pattern in the buckles through the step cycle.
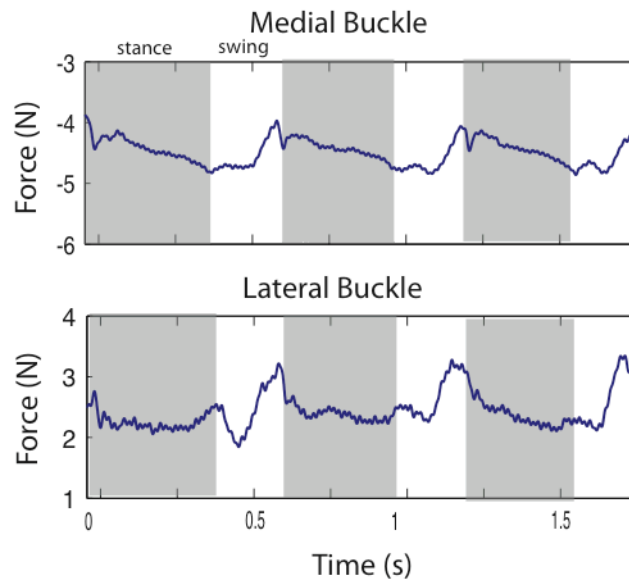
Figure B.1. The force buckle traces from one example cat. The top trace is the medial buckle and the bottom trace is the lateral buckle. The grey shaded areas are stance and the white is swing. Note the increase in force around paw contact, which declines through stance. There is a small increase in force around paw off. The force remains low through swing until nearing paw contact.

These recordings show that the crural fascia force increases during paw contact, which is when most muscles are active during the step cycle (Rossignol 1996). Although there are many muscles active at that time the total change in force magnitude is only about 1N in each band. Given the structure of the crural fascia and the multiple muscles associated with it, it is difficult to determine exactly which muscles are contributing to the increase in force. It should be noted that these traces do not represent the total force potentially being transmitted through the crural fascia, as we do not have recordings from the fascial sheet. The force decreases during stance, with the medial band shows a more gradual decrease than the lateral band. This suggests that the medial band may be transmitting more force to the endpoint than the lateral band throughout stance. This may contribute the increased adduction of the endpoint of the limb during stance observed in the decerebrate cat. The slight increase in force in both buckles around paw off suggests that

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the activity from the limb retractors is transmitted through the crural fascia and therefore used to lift the paw. This is most likely a contribution from pBF and ST. It appears as though the force is greater in the lateral band, which could signify a greater contribution from pBF, which also supports the initiation of the lateral swing of the paw during circumduction.

These results show that the crural fascia has changes in force through the step cycle that appears to coincide with the activation of the muscles associated with it. Therefore supporting the idea that the crural fascia's stiffness is modulated by the activation of the muscles attached to it.

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