DISSERTATION

KINETIC, KINEMATIC AND ELECTROMYOGRAPHIC ANALYSIS OF CANINE CRUCIATE LIGAMENT RUPTURE USING A MONOPOLAR RADIOFREQUENCY ENERGY MODEL

Submitted by

Caroline P. Adrian

Department of Clinical Sciences

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Doctoral Committee:

Advisor: Kevin K. Haussler
Co-Advisor: Christopher Kawcak

C. Wayne McIlwraith
Ross H. Palmer
Raoul F. Reiser, II
Cheryl Riegger-Krugh
Robert A. Taylor

ABSTRACT

KINETIC, KINEMATIC AND ELECTROMYOGRAPHIC ANALYSIS OF CANINE CRUCIATE LIGAMENT RUPTURE USING A MONOPOLAR RADIOFREQUENCY ENERGY MODEL

Canine cranial cruciate ligament rupture (CCLR) is a common cause of pain and lameness in dogs that leads to abnormal pelvic limb biomechanics and ultimately to the development of stifle osteoarthritis (OA). Traditional research into the causes of CCLR has focused on instability secondary to failure of the passive structures within the joint. The purpose of this project was to recognize the role of dynamic components as possible contributors to CCL disease, such as neuromuscular dysfunction of muscles (dynamic stabilizers) surrounding the stifle joint. The present studies were performed to characterize alterations in muscle activity in the limb with CCLR and intact contralateral pelvic limbs, as well as measure biomechanical, clinical and physiologic parameters in all four limbs in dogs with subacute, acute and chronic CCLR (within the same dog during the study). Monopolar radiofrequency energy (MRFE) provided a unique model of CCL injury in which to assess subclinical timeframes.

Electromyographic (EMG) parameters, collected simultaneously with ground reaction forces and kinematics, were assessed bilaterally within the vastus lateralis, biceps femoris and gastrocnemius muscles at 6 timepoints post MRFE-induced CCL

injury and subsequent rupture. The treated limb denotes the pelvic limb that received MRFE surgery and subsequently ruptured the CCL. The untreated limb refers to the contralateral, non-surgical pelvic limb.

Kinematic compensations showed an increase in stifle flexion in the untreated limb compared to the treated limb at all time points post CCLR. Kinetic variables were altered in the treated pelvic limb compared to the untreated limbs post CCLR. No compensatory changes in kinetic or kinematic variables were found in the thoracic limbs at any point post CCL injury or rupture.

This study provided a qualitative description of muscle activity post CCL injury and subsequent rupture. No significant differences were found in muscle onset, activation duration or percentage of peak amplitude normalized to baseline between the treated and untreated pelvic limbs at all time points.

Clinical and physiologic outcome parameters were collected concurrently throughout the duration of the study to evaluate their association with CCL injury and rupture. Joint effusion was the only outcome parameter associated with subclinical CCL injury. However, the majority of these parameters, such as pain, lameness, range of motion, cranial drawer test and radiography, were associated with subsequent rupture of the CCL.

In conclusion, kinematic variables, specifically femorotibial flexion angles, were decreased in the contralateral pelvic limb post CCLR, with minimal changes in subclinical time points at 2 and 4 weeks post MRFE-induced CCL injury. Future studies with larger samples sizes are needed to confirm EMG activity in stabilizing muscles of the stifle to further investigate the role of neuromuscular control in stifle stability.

Several outcome parameters such as thigh circumference, pain, lameness, range of motion, cranial drawer test and radiography, and have been shown to be useful in identifying the presence of CCLR, but not subclinical CCL disease.

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CHAPTER 1

KINETIC, KINEMATIC AND ELECTROMYOGRAPHIC ANALYSIS OF CANINE CRUCIATE LIGAMENT RUPTURE USING A MONOPOLAR RADIOFREQUENCY ENERGY MODEL

GENERAL INTRODUCTION

The incidence of cranial cruciate ligament (CCL) rupture in dogs has doubled in the last 30 years[1], with an estimated cost of more than $1.3 billion spent on medical and surgical management in 2003.[2] Multifactorial in origin, this disease process causes abnormal biomechanics of the pelvic limb, ultimately leading to the development of stifle osteoarthritis (OA) and pain, as well as pelvic limb lameness and dysfunction. Traditionally, investigations into the causes of cranial cruciate ligament rupture (CCLR) have focused on instability secondary to failure of the passive structures within the joint.[3,4] Current perspectives on the etiopathogenesis of stifle disease[5] include recognizing the role of dynamic components as possible contributors to CCL disease, such as neuromuscular dysfunction of muscles and tendons (dynamic stabilizers) surrounding the stifle joint.[5-9]

One technique for investigating neuromuscular dysfunction is electromyography (EMG), the study of an electrical signal associated with a muscle activity. In addition to

kinetic (i.e., forces that are able to produce movement) and kinematic (i.e., description of movement) analysis, EMG provides insight into muscle activity and the biomechanical alterations associated with instability of the stifle.[10]

One contributing factor to CCL disease is purported to be due to a dynamic imbalance of muscles surrounding the stifle, where failure occurs in one or more muscles associated with maintaining stability and normal stifle kinematics.[5,11] Identifying abnormal biomechanical characteristics that may predispose dogs to CCL disease could provide insight into its pathogenesis and help to maximize the effectiveness of surgical and nonsurgical treatments for CCLR.

This chapter will provide the reader with an overview of biomechanics related to anterior cruciate ligament (ACL) disease of the knee in humans and a comparison to biomechanics of the stifle joint and its relation to CCL disease in dogs. Models of canine cruciate ligament disease will be discussed, as well as physiologic outcome parameters related to CCLR. Finally, future directions in CCL research are proposed, incorporating the purpose of this dissertation.

The Human Knee and Anterior Cruciate Ligament (ACL) Disease

The Anterior Cruciate Ligament

The human anterior cruciate ligament is a primary stabilizer of the knee, restraining tibial anterior translation and internal rotation.[12,13] Mechanoreceptors present in the ACL provide sensory information to assist with dynamic joint stability, providing a protective mechanism if joint loads approach forces large enough to injure or rupture the

ACL.[14] ACL injury results in abnormal arthrokinematics, leading to tibial subluxation.[14] Chronic instability causes damage to secondary structural restraints (e.g. collateral ligaments and menisci) and articular surfaces.[14]

Incidence and Pathogenesis of Human ACL Injury

The overall incidence of ACL injury is not known, yet estimates of 80,000 annual injuries have been reported, with 50,000 requiring surgical repair in the United States.[13,15] Seventy percent of ACL injuries are categorized as *noncontact* tears which occur during lower extremity deceleration, with the knee at or near full extension and the quadriceps muscle maximally activated.[16] The remaining 30% of ACL injuries are categorized as *contact* injuries sustained when the lower leg is fixed and torque is applied with enough force to cause injury or rupture of the ACL.[17]

Muscle Activity

Soft tissue restraints, such as menisci, cruciate and collateral ligaments, contribute to stability of the knee when mild to moderate loads are applied.[18,19] However, excessive loading and timing of loads applied during vigorous activities, such as stopping and cutting tasks, may exceed the intrinsic strength of these tissues. Therefore, additional extrinsic forces are required to maintain stability of the knee joint.[18]

Muscles spanning the human knee contribute to stability and mobility of the joint. Weight bearing and loads applied to the knee joint by muscle activity provide joint compressive forces that contribute important additional stabilization.[18] Changes in muscle activity, such as muscle onset and intensity, indirectly result in altered joint

stiffness (change in force per unit change in displacement).[18,20]

The ACL has been suggested to play a role in controlling the stiffness of these stabilizing muscles in the human lower limb via mechanoreceptors present in the ACL.[21] Loading the ACL increases mechanoreceptor firing, thereby increasing activity in the gamma-motor neuron system, or neural circuitry that mediates joint afferent neurons and motor reflexes.[21] Muscle spindle tone in knee-stabilizing muscles is increased via activation of gamma-motor neurons, which, in turn, increases excitability of alpha-motor neurons.[21] This results in increased muscle tension, ultimately increasing stability of the joint.[21,22]

ACL injury affects joint stability and neuromuscular function, which includes alterations in muscle activity, kinematics and kinetics due to decreased knee stability and OA development.[18,23,24] The human ACL contains sensory and proprioceptive fibers[25], which suggests that neuromuscular control is important and serves a protective role in functional joint stability.[26] For example, when external loads are applied to the ACL-deficient knee, abnormal patterns of muscle activity[27,28] and alterations in muscle timing and magnitude are noted during functional tasks such as walking or jumping.[29] In humans, ACL rupture produces neuromuscular weakness in the knee-stabilizing muscles and a sense of gross instability during rapid turning, acceleration and deceleration movements.[30] Even after static mechanical stability has been restored surgically via ACL reconstruction, human patients may continue to experience functional instability during dynamic activities.[30]

Neuromuscular Control and OA

Neuromuscular control is defined as the "ability to produce controlled movement through coordinated muscle activity".[18] The on/off timing of muscle activity is important in locomotion and in maintaining stability of the joint. ACL injury produces asynchronous muscle firing prior to[31], as well as post rupture, where altered neuromuscular control is characterized by weakness, impaired proprioception, incoordination ('microklutziness') and altered muscle firing patterns, especially within the quadriceps femoris muscle.[32,33] These neuromuscular changes may be the result of disuse atrophy or decrease in voluntary muscle activation, which is speculated to be due to pain, fatigue or joint effusion.[34]

The timing and patterns of muscle activation influence the magnitude and rate of loading on the knee structures. The quadriceps muscle is an important knee stabilizer in both humans[20] and dogs.[20,35] Proper attenuation of impact loads in the normal knee is due to coordinated timing of appropriate muscles during the early stance phase of gait.[36] If stabilizing muscles of the knee are weak, protective muscular activity that normally provides stability to the knee may be reduced, which increases joint loading and the risk of ACL rupture.[37] In humans with knee pain, eccentric muscle loading (a lengthening contraction, as the force on the muscle is greater than the force that the muscle is generating), which is responsible for attenuating impact loads and peak loading rates, is decreased.[38] The subjects for this study loaded their limb rapidly with ineffective shock absorption, which is termed 'repetitive impulse loading' and may produce structural

damage and resultant OA.[38]

ACL rupture causes excessive anterior translation, internal rotation and hyperextension of the tibia relative to the femur. Tibial destabilization causes alterations in tibiofemoral articular cartilage contact points.[39] Destabilization also increases translational forces throughout the entire joint range of motion and increases joint loading in areas of the articular cartilage that typically have little or no contact, which produces cartilage degeneration and the development of OA.[40-42]

In the absence of ligamentous support, increased neuromuscular contributions are required to provide knee stabilization.[23] Increased muscle contraction and co-contraction (i.e., the simultaneous contraction of agonist and antagonist muscles) can increase joint stiffness and reduce tensile forces within the knee ligaments[18,43]; although, increased antagonistic muscle co-contraction over time may contribute to abnormal joint loading by producing increased joint forces that contribute to articular cartilage degeneration.[23] Normal human subjects who simultaneously contract both their quadriceps and hamstring muscle groups produce a 2- to 4-fold increase in knee stiffness and reduce joint laxity by 25-50%.[43] Uncoordinated muscle activity acting on the knee may cause increased ACL strain and ultimately induce ACL rupture.[43]

Humans may compensate for ACL rupture by increasing knee flexion and employing a knee 'stiffening' (stabilization) strategy during weight acceptance.[44] This results in the generation of a greater posterior net force on the tibia relative to the femur in an effort to stabilize the leg and reduce anterior translation of the tibia on the femur.[31]

Reduced knee range of motion and increased biceps femoris and medial gastrocnemius muscle activity (i.e., knee stabilization strategy) may lead to reduced shear forces at the femorotibial joint; however, this compensatory mechanism increases joint loading and joint compressive forces that could lead to degenerative changes.[32,44] Discrepancies exist in the literature related to alterations in quadriceps muscle activity. Reduced quadriceps EMG activity has been reported during weight bearing in humans with ACL rupture[31]; however, most studies report no decrease in quadriceps muscle activity.[45,46] Poor quadriceps muscle control is demonstrated when subjects with ACL deficiency perform static and dynamic tasks. Specifically, the vastus medialis and lateralis portions of the quadriceps muscle fired continuously during a knee flexion task where these muscles are usually inactive in normal subjects. This abnormal activation of the quadriceps muscle leads to anterior tibial shear loads that have been implicated as a cause of knee OA.[32]

Kinetic and Kinematic Measures of Gait Analysis

ACL injuries commonly produce changes in muscle activity, as well as limb kinetics and kinematics, which have been implicated as additional causative factors in the development of OA.[23,44,47] By providing both translational and rotational stability, the ACL plays an important role in the normal kinematics of the knee. Healthy cartilage is maintained in the normal knee when normal patterns of locomotion exist, however alterations in gait are known to occur post ACL rupture, such as anterior tibial translation[48], causing substantial changes in tibiofemoral motion.[24] When the limb is near full extension during weight acceptance and mid-stance, there is an increase in

compressive and shear forces acting on the knee.[23,44,49]

The thickest regions of cartilage lie in the load bearing areas of the tibiofemoral joint. The tibial and femoral cartilage is thicker in anterior weight bearing regions of the medial compartment and thicker in posterior regions of the lateral compartment.[24,47] During stance phase at a walk, when the knee is near full extension, the areas of thickened cartilage are in contact with each another. Kinematic alterations during gait, such as increased knee flexion angles, may shift the normal femorotibial contact areas to regions of thinner cartilage not normally suited to withstand increased loads, resulting in the development of OA.[23,24,44,47]

Increased knee flexion angles are described as a 'quadriceps-avoidance gait' and interpreted as a gait modification to avoid or reduce quadriceps contraction.[50] Quadriceps contraction may lead to anterior translation of the tibia. Therefore, individuals with ACL-deficient knees may reduce quadriceps muscle contraction to avoid instability. However, this theory has not been verified with EMG.[50] A second study evaluating heel strike in individuals with knee pain showed an increase in knee flexion at the beginning of the stance phase.[38] EMG showed a reduced period of activation of the quadriceps muscle during walking in adults with activity-related knee pain, but no pain during testing; however, cadence, velocity, vertical ground reaction force amplitudes and loading profiles were not different compared to age-matched controls.[38] However, pre-osteoarthritic knee pain subjects had a significantly increased loading rate than controls with no knee pain. By loading the limb more rapidly with a shorter period of quadriceps

activation, attenuation of loads acting on the knee was less effective due to reduced eccentric muscle contraction of the quadriceps muscle.[38] Repetitive impulse loading, a pattern of hard heel landing and inadequate shock absorption found in people with activity-related knee pain caused increased vertical ground reaction forces as the heel contacted the ground.[38] This repetitive loading gait consistently produces OA in animal studies.[38,51,52]

The neuromuscular strategies of bipeds and quadrupeds with knee instability post rupture of the ACL remains vague and undefined. A few studies have found decreased quadriceps muscle activity in ACL-deficient knees; however, most studies have not found any alterations in quadriceps activity, suggesting that co-contraction of the quadriceps and hamstring muscle groups may be necessary for knee stabilization.[44-46] Hamstring muscle activity may be effective in reducing anterior tibial thrust during gait in people with ACL deficiency. However, one model simulation assumed kinematics and ground reaction forces were identical to a normal gait and did not take into account alterations in gait, such as an increase in knee flexion, that are known to occur post ACL rupture.[48] Variable results among studies suggest that quadriceps muscle activity may vary in different populations with differing pathology, such as whether an ACL rupture exists, if patients have knee pain prior to OA, or if there is pre-existing OA. Development and progression of OA are influenced by variations in knee kinematics (e.g. increased knee flexion) and altered or increased joint loading.[47]

The Canine Stifle and Cranial Cruciate Ligament (CCL) Disease

The anatomy of the canine stifle (knee) is similar to that of the human knee.[11] Therefore, the canine model is commonly used to study the etiopathogenesis and possible therapeutic approaches to OA in humans.[11] Rupture of the cranial cruciate ligament (CCL), the anatomic equivalent to the human ACL[11], allows excessive cranial translation, internal rotation and hyperextension of the tibia, relative to the femur. Tibial destabilization produces increased translational forces throughout the entire joint range of motion, which often contributes to the development of OA.[40,41] Instability and subsequent OA contribute to significant alterations in pelvic limb biomechanics, such as decreased PVF and increased stifle flexion within the affected limb.[6,53] Compensatory gait patterns are adopted[9,54] in an effort to help protect the injured stifle, but at the risk of contributing to decreases in muscle stiffness, increased joint loading and possible rupture of the contralateral CCL.[8,22,41] CCL rupture (CCLR) of the contralateral limb, which occurs in 37% to 48% of dogs within 16 months after the initial CCLR, remains a substantial secondary clinical problem.[7,55]

Muscle Activity

The function of the CCL is important in providing stifle stability during locomotion.[40] Post nontraumatic rupture (disruption of ligament fibers) of the CCL, or CCL disease, stifle flexor muscle groups and the caudal horn of the medial meniscus limit cranial tibial translation by counteracting the forces that occur during weightbearing. This mechanism, called 'cranial tibial thrust', compresses the femoral

condyles against the tibial plateau.[56,57] Varying magnitudes of cranial tibial thrust, the dog's size, activity level, and conformational abnormalities, such as an increased slope of the tibial plateau, are considered risk factors for CCL degeneration.[56] A partial CCL tear is caused by macro- and microfailure of the ligament, and may be initially detected by joint effusion, early radiographic signs of OA, and clinical signs of lameness, although minimal instability may be present.[56] Progressive degeneration of the CCL and articular cartilage matrix occurs due to increased local inflammatory cytokines and abnormal joint loading, until eventual complete CCL rupture ensues.[56]

In normal stifle biomechanics, pelvic limb muscles play an important role in providing joint stability.[58,59] In humans, the ACL is the sole structure limiting anterior motion of the tibia when the knee is near full extension.[40] Due to the increased resting stifle flexion angle of 150° (or 30° of flexion from full extension) in dogs, the CCL is not expected to be the sole stabilizer of the canine stifle in limiting cranial translation of the tibia on the femur.[40] The quadriceps muscle group and patellar tendon provide support to the cranial aspect of the stifle joint[60]; whereas, the gastrocnemius, and hamstring muscle group stabilize the caudal aspect of the distal femur and proximal tibia.[60] Therefore, it is hypothesized that muscular contributions are critically important in providing stabilization to the intact and cruciate-deficient stifle in dogs.

In normal stifles, it is known that a synergistic relationship exists between the CCL, quadriceps, gastrocnemius and biceps femoris muscles to provide stability.[61,62] Cranial thigh muscles (stifle extensors) and the gastrocnemius (stifle flexor and tarsal

extensor) must be active to prevent stifle flexion and pelvic limb collapse during weightbearing. Whereas, the caudal thigh muscles (stifle flexors) act to stabilize the stifle by pulling the proximal tibia caudally (i.e., preventing cranial tibial translation) and extending the hip to create forward propulsion.[61] However, the affect of CCLR on this coordinated relationship is unknown.

Cranial thigh muscles, specifically the vastus medialis and vastus lateralis, originate from the medial and lateral aspect of the proximal portion of the femur, respectively, and insert together on the tibial tuberosity by way of the patellar ligament.[63] Both of these uniarticular muscles act to extend the stifle and are active at the end of swing phase and beginning of stance phase and function to stabilize the stifle.[62,64] The vastus lateralis is a readily accessible muscle for surface EMG and is large enough in mass to reduce crosstalk (i.e., the electrical interference of nearby muscle activity).[65] At a trot, the vastus lateralis is active prior to paw strike which acts to stabilize the knee and remains active for 70% of the stance phase (32% of the stride).[62,65] At the end of the swing phase, the vastus lateralis functions as a stifle extensor in preparation for paw strike.[65]

The gastrocnemius, a triarticular muscle (i.e., crossing 3 joints), consists of a medial and lateral head which originate from the medial and lateral supracondylar tuberosities of the femur, respectively, and insert on the calcaneal tuberosity by way of the common calcaneal tendon.[63] Its function is to produce flexion of the stifle and extension of the tarsal joint.[62] At a trot, the gastrocnemius becomes active after paw

strike and remains active for 73% of the stance phase (33% of the stride).[62] During stance phase, the combined action, or co-contraction, of the quadriceps group (stifle extensor) and gastrocnemius (stifle flexor) provide controlled stability to the stifle joint.[62,66]

The biceps femoris is a biarticular muscle that consists of a cranial and caudal head. The cranial head of the biceps femoris originates on the ventral surface of the ischial tuberosity and inserts partly on the patella and tibial tuberosity.[62,63] The function of the cranial head is to produce hip and stifle extension while the limb is weightbearing.[62,63] When the limb is unweighted, the cranial portion of the biceps femoris is capable of flexing the stifle.[62] The cranial portion becomes active prior to paw strike (25% of swing phase) which acts to decelerate the limb's forward movement and remains active for 46% of stance phase (34% of stride) at a trot to help stabilize the stifle during extension in the stance phase.[62] The caudal portion of the biceps femoris originates on the ventral ischial tuberosity and inserts on the medial aspect of the calcaneal tuberosity.[63] At a trot, the caudal portion of the biceps femoris acts to retract the pelvic limb after stifle stability has occurred[66] and flexes the stifle during the swing phase.[67]

Neuromuscular Control and OA

Mechanoreceptors and free nerve endings are found within the cruciate ligaments and are typically located near the bony attachments, with few receptors located in the mid-region.[20,21] These receptors are responsible for detecting changes in tension,

pressure, amplitude, velocity of movements and nociception.[20,21] Tension in knee structures varies with different positions and with varying degrees of joint flexion. These afferent (sensory) joint receptors influence the gamma-motor neuron system by mediating motor reflexes and muscle activity that contributes to joint stability and prevents potential excessive compressive forces.[21] Mechanoreceptors influence muscle spindle (sensory receptors in skeletal muscles responsible for detecting length changes) activity by regulating gamma-motor neuron activation (Figure 1).[21]

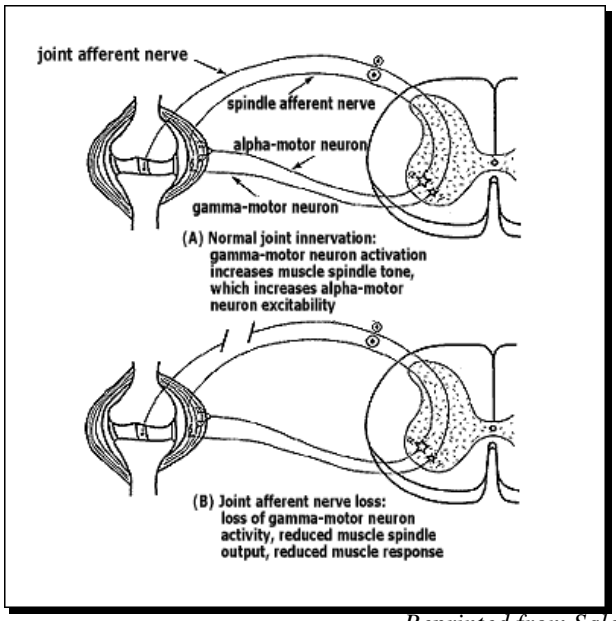

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FIGURE 1: Diagram of the knee with associated spinal cord segment illustrating the gamma-motor neuron and muscle spindle receptor feedback loop. (A) Normally, mechanoreceptors increase firing during ligamentous stretch, which feeds back to the alpha-motor neuron and causes increased muscle tension and increased joint stability. (B) Abnormal condition where CCL injury causes a loss of joint afferent input which causes decreased gamma-motor neuron outflow and secondary decreased muscle force and decreased joint stability.[21]

Joint afferent receptor stimulation in the CCL causes an increased outflow of the gamma-motor neuron system and increased sensitivity of the muscle spindles and muscle tension within the agonist and antagonist muscles acting on the human knee.[21] In response to applied loads, an increase in limb stiffness occurs due to the increased contraction of periarticular muscles.[20,21] By balancing muscle activity acting across the joint, stability is increased, which protects the joint surfaces from mechanical damage related to shear force, but not compressive forces.[21]

Flexor and extensor muscle groups contribute to the production of forces acting across the stifle. During movement, co-contraction compresses the femoral and tibial articular surfaces, which increases knee joint stiffness.[20] When co-contracted, the agonist-antagonist pair produce a balancing torque over the articular surface, distributing the applied load across the joint which helps to properly attenuate impact and compressive loads.[20]

Mechanoreceptors in the canine CCL are capable of regulating muscle tone and influencing stiffness within the adjacent stifle musculature, which affects stifle stability due to the activity of the gamma muscle spindle fibers.[22] Stimulation of intrafusal skeletal muscle fibers (muscle spindle) by the application of low forces (5-40 N), indicates their low thresholds to mechanical deformation.[20] Large loads (~130 N) applied to the cruciate ligament evokes a direct effect on extrafusal skeletal muscle fibers, which are innervated by alpha motor neurons, before changes in EMG signals from the quadriceps and hamstring muscles were observed.[20] These mechanoreceptor reflexes are

elicited only at high amplitudes of mechanical force. On the other hand, the regulation of intrafusal skeletal muscle fibers innervated by gamma motor neurons is designed to adjust the coordination of muscle activity surrounding the knee joint at low amplitudes of mechanical force.

In a canine model, electrical activity within the stabilizing muscles of the stifle increase in response to experimentally-applied tensile forces to the CCL. Loading of the CCL from 5 to 60 N elicited a corresponding increase in the EMG amplitude within the canine quadriceps muscle, which illustrates the influence of mechanoreceptors on increasing muscle stiffness. This is a strategy which may be used to compensate for ligamentous instability.[22] Increased amplitudes of co-contracted (coordinated) muscle activity can increase joint stiffness and reduce tensile forces within the knee ligaments.[18,20]

Meniscal Role in CCLR

The menisci are composed of fibrocartilage and are located between the medial and lateral condyles of the femur and tibia. Menisci form congruent surfaces and are primarily responsible for reducing concussive forces.[68] The menisci contribute to stifle joint congruency to seat the femoral condyles and reduce the void between these two bones.[69] Post CCLR, the medial meniscus acts as a secondary stabilizer against cranial tibial translation by compressing the caudal horn between the femur and tibia as the tibia advances cranially, which is believed to cause the high rate of medial meniscal tears that occur post CCLR.[69] Three months post CCL transection, the menisci are grossly normal,

but by 6-12 months post CCLR, meniscal damage is evident.[70]

Kinematic Measures of Gait Analysis

Kinematics are used to describe limb movements independent of the forces that cause the movement. Outcome parameters may include linear and angular displacements, velocities and accelerations that can be measured from a variety of methods and anatomical landmarks (e.g., body segment center of gravity, joint centers of rotation, distal limb segments, anatomical prominences).[10] Kinematic analysis of healthy and CCL-injured dogs has been evaluated at the walk and trot.[59,65,70-72]

CCLR causes changes in neuromuscular function and secondary alterations in stifle kinematics.[9,53,59,70] A common finding post CCL transection includes a decrease in the stifle flexion angle of ~5°-14° in the affected limb during the stance phase at a walk and trot; presumably in an attempt to avoid pain associated with weight bearing and to reduce joint instability during the stance phase.[59] Dogs compensate for CCL loss by carrying the affected limb in greater flexion throughout the gait cycle, but are not able to prevent tibial subluxation during stance. At the swing to stance transition, the CCL-deficient stifle demonstrates marked cranial displacement of the tibia that was sustained throughout stance.[59] At the beginning of the swing phase, the CCL-deficient joint returns to a cranial-caudal alignment seen in the intact stifle joint. It has been suggested that the repetitive, mechanical subluxation of the tibia that occurs with each weight bearing step may be a primary cause of joint degeneration following CCLR.[59]

Patterns of flexion and extension change within the CCL-deficient limb post

rupture.[9,53,73] Reduced stifle extension at the end of stance may reduce limb propulsion and be a similar protective adaptation to preventing pain and joint instability. Coxofemoral and tarsal joint angles are more extended during stance phase, in contrast to the stifle joint. Increased extension in these joints may help to preserve gait by maintaining paw contact and limb propulsion in the presence of a painful, flexed stifle joint. Recognition of specific kinematic patterns of musculoskeletal pathology may be possible by understanding primary versus compensatory gait.

Increased stifle joint flexion may also be due to reflex inhibition (decrease in voluntary activation) of the quadriceps muscle related to joint effusion.[59] Post CCL transection, joint effusion and/or cranial tibial motion causes pressure and deformation changes that are detected by mechanoreceptors in the joint capsule causing inhibition of the quadriceps muscle.[34] On the other hand, increased stifle flexion during weight bearing may be chosen by the dog to avoid maximal extension of approximately 150° (or 30° of flexion)[58] in an effort to avoid tibial subluxation.

However, EMG has not been performed in conjunction with kinematics to validate these speculations of quadriceps inactivity in dogs. During stance phase in the normal dog, stifle angles vary between 120-140° (or 40-60° of flexion)[67], at which time the quadriceps muscle contracts and can produce strain on the CCL by translating the tibia cranially.[59] In the absence of the CCL, quadriceps contraction presumably causes cranial tibial translation at angles between 135-180° (or 0-45° of flexion).[59]

Altered kinematics, specifically increased stifle flexion and cranial displacement

of the tibia occur during stance phase, causes joint instability and degradation that ultimately leads to OA[9,53,59,73] due to a caudal shift of the medial femoral condyle[74] and increased compression between the femoral and tibial joint surfaces at the beginning of stance phase.[59] The increased stifle flexion in CCL-deficient stifles is thought to change cartilage surface contacts to areas that are not normally in contact during gait.[73] Information pertaining to canine kinematic measures of all four limbs during injury to and after cruciate rupture has not been found, but may provide novel information pertaining to compensatory gait during the subclinical, acute and chronic phases of CCLR.

Kinetic Measures of Gait Analysis

Ground reaction forces (GRF) are defined as forces of interaction between the paw and the ground.[10] Force platforms are used to measure three orthogonal forces, vertical (Fz), craniocaudal (Fy), and mediolateral (Fx), that result from limb contact with a ground surface and are representative measures of weightbearing.[75] Vertical forces are the greatest in magnitude and are a more direct measure of weight bearing. Craniocaudal forces quantify limb forces that affect forward progression. The stance phase is divided into braking (deceleration, negative GRF) and propulsion (acceleration, positive GRF). Braking occurs in early stance and is defined as the impulse resulting from decreased momentum of the dog at paw contact.[53] Propulsion is the opposite; measuring impulse required to increase momentum during push off. Minimal mediolateral shear forces are generated during canine gait thus, mediolateral GRF have the smallest magnitude and the

most variability of the three orthogonal forces and are typically not a useful parameter in canine kinetic studies.[53]

Kinetic studies have reported an expected decrease in PVF, as dogs that sustain a CCLR ambulate with a pronounced lameness (altered gait) on the affected pelvic limb.[6,76,77] Budsberg and colleagues were the first to report a decrease in vertical force and associated impulses (impulse of vertical force indicated as the area under the vertical force versus time curve), as well as a decrease in craniocaudal forces and impulses, in dogs that had undergone acute CCL transection.[77] At 2 weeks post unilateral cranial cruciate transection, other studies report a decrease in PVF of approximately 80% of baseline values, gradually improving to approximately 50% of baseline by 12 weeks post transection.[6,76]

Peak vertical force in the pelvic limbs following monopolar radiofrequency energy (MRFE) technique induce cruciate ligament injury and rupture have shown similar decreases in peak vertical forces as dogs that had undergone acute CCL transection.[78] The MRFE model allows the joint to undergo osteoarthritic changes following injury and subsequent CCL degeneration, allowing for a novel subclinical period of approximately 8 weeks until rupture.[58] PVF for each pelvic limb measured preoperatively and at 4, 8, 12, 16, 26, and 36 weeks after surgery showed significant decreases in PVF between the 8- and 12-week evaluation points, which corresponded to the mean cruciate rupture date, and remained decreased for the remainder of the study.[78] However, no thoracic limb or craniocaudal forces have been analyzed using the MRFE

model of CCLR to investigate the effects of compensatory gait.

Compensatory Gait

Outcomes related to compensatory gait and studies with kinetic methods in CCL-transected dogs show contradictory results.[6,76] O'Connor et al. reported no increase in PVF exerted by either the right or left thoracic limb following unilateral CCLR and PVF values for the contralateral pelvic limb remained unchanged from baseline.[6] A 10% decrease of the ipsilateral forelimb was noted at 2 weeks post operative, but returned to near baseline values by week six.[6] Dogs subjected to the same surgical procedure without any manipulation of the CCL (sham-treated) showed no decrease in PVF for each limb.[6]

On the contrary, results from Rumph and colleagues found the PVF in the CCL transected limb to be 80% less at 2 weeks post operative, with a concurrent increase of ~34% in contralateral pelvic limb.[76] At 6 and 12 weeks post CCLR, a decrease in the cruciate-deficient pelvic limb vertical force accompanied a concurrent vertical force increase in the contralateral pelvic limb ranging from 13-20%, concluding that vertical ground reaction forces in all other limbs were affected by CCLR.[76] Within the thoracic limbs, contralateral impulse of vertical force was greater than the ipsilateral impulse at 12 weeks.[76] However, this study included a 12-week regimen of exercise that may have influenced results by increasing pain and/or lameness in the transected limb.[76]

CCLR causes local pain and mechanical instability.[79] Gait abnormalities in dogs with CCLR result in reduced loading of the injured limb[6,76] and include increased stifle

flexion during stance.[59] These gait alterations are thought to cause increased reliance on adjacent muscles to compensate for the reduced joint stability.[59] Thus, muscles surrounding the stifle are expected to have delayed onset patterns and reduced amplitudes secondary to inflammation and pain within the stifle associated with CCLR.[79] The abnormal muscle contractions of stifle-stabilizing muscles may be due to muscle weakness, as muscle atrophy is known to occur post CCLR[79], and may be the dog's attempt to alter the amount of weight bearing required by a painful, unstable limb. Alterations in muscle activity have yet to be reported in dogs with cruciate-deficient stifles and may provide insight into the pathogenesis of CCLR.

Models of Cranial Cruciate Ligament Disease

There is no single factor accounting for the etiopathogenesis of cranial cruciate disease, as it is likely a multi-factorial process. Factors that may influence CCLR, such as breed, anatomical differences, age, and neutering could alter degenerative changes within the CCL, making the CCL more susceptible to injury and rupture.[80] Certain breeds seem more susceptible to mechanical overload, which may have negative effects on the CCL's structural properties.[80]

Anatomically, some dogs with ruptured CCLs have a steeper tibial plateau angle (~ 24°), suggesting that greater slopes predispose the dog to CCL injury by increasing translational stresses on the CCL as it resists femoral condyle motion on the sloping tibial plateau.[81] Structural properties of the CCL may be affected by age, as tensile strength of the ligament is known to decrease with age.[80] The incidence of CCLR increases with

age, with a peak incidence between 7-10 years. Age-related changes may include loss of ligament fibroblasts, progressive degeneration of the ligament fibroblasts and failure to maintain normal collagen fibers, which increase the risk of CCL rupture.[80] Neutering, especially in female dogs, increases the risk for CCL disease, although causes remain unknown.[80]

Many experimental techniques have been used to model or study the early pathogenesis of OA including denervation, immobilization, chemical irritation (e.g. papain, hypertonic saline), and surgical intervention, such as transection of the CCL.[82] Menisectomy[83] and cruciate ligament transection are the most well described in the literature and CCL transection is the most commonly used canine model for studying the development of OA.[84,85] Pond & Nuki first reported this technique in 1973, stating that transection of the CCL is similar to stifle instability secondary to naturally occurring CCLR and ensuing OA.[85] Modified techniques to the Pond-Nuki model have included visualizing the CCL via a lateral or medial arthrotomy. The CCL is transected in each procedure; however, a wide variability exists in the time of onset, severity and location of osteoarthritic cartilage lesions.[86] Due to this variability, transection of the CCL is not the model of choice when designing a project to study stifle instability and OA development.

In naturally occurring CCL disease, the majority of canine CCL disruption occurs from progressive degeneration leading to elongation and weakening, with eventual complete rupture of the ligament.[79] Recently, a canine model using monopolar radiofrequency energy (MRFE) has been developed that produces a delayed but

reportedly predictable onset of CCL degeneration and ultimate rupture at approximately 8 weeks (mean 55 days, range 48-56 days) post intervention.[58,78] This technique better simulates naturally occurring CCLR than the traditional transection model of CCLR, by producing progressive CCL degeneration and eventual rupture post MRFE-induced injury and inciting inflammatory responses.[58,78] MRFE technique is applied arthroscopically to the dorsal surface of the CCL with a temperature of 70°C and a power of 25 W. The entire dorsal surface of the CCL is treated, after which the fibers appear dull and off-white in color rather than the normal glistening white, fibrous appearance. After rupture, complete ablation of the treated CCL has been reported at necropsy in 15 out of 18 dogs, with few ligament fibers remaining in 3 of 18 stifles treated with MRFE.[78]

We suggest that the MRFE model is currently the technique of choice when designing a study involving the pathogenesis and subclinical evaluation of CCLR. Little is known about the disease mechanism of CCLR, though regardless of the risk factors that precipitate CCLR, loss of the CCL integrity leads to degenerative changes within the stifle, as evidenced in both spontaneous and monopolar radiofrequency energy (MRFE)-induced CCLR.[58,78-80] MRFE treatment incites inflammatory responses thought to contribute to degradation of the cruciate ligament.[78,80] Inflammation and ligament degeneration may develop early in cruciate disease, before the development of stifle instability.[40] Loss of fibroblasts occur from this core region in ruptured CCLs[80], mimicked by MRFE treatment of tissue on the dorsal surface. Few fibers remain intact in MRFE-treated stifles, similar to the limited evidence of ligament fibers in canine stifles

that have undergone spontaneous CCLR. Pathologic findings in the MRFE-treated stifles were consistent with osteoarthritic changes documented in canine models with CCL transection and in natural disease.[78]

Gait analysis and radiographic changes post MRFE are also consistent with established canine CCL transection models of OA. Variability between dogs that exists in CCL transected models is reduced as the MRFE technique demonstrates a consistent pattern of degeneration and rupture of the normal CCL.[78] CCL transection does not allow for subclinical evaluation.

Clinical and Physiologic Measures of CCL Disease

Clinical outcome parameters (e.g., goniometry and thigh circumference) may be used to provide the clinician with objective measures that are easy to use, relatively inexpensive, and provide a quick method that could lead to the early detection and progression of CCL disease. Physiologic measures, such as prostaglandin E_2 analysis, intra-articular pressure measures and gross pathology and histopathology, may not be as readily available or feasible to perform in a clinical setting; however, these outcomes could still identify clinical and physiologic measures associated with the development of CCL disease and subsequent rupture.

Goniometry and Joint Range of Motion

Goniometry can offer a measurement tool used in decision-making for therapeutic interventions and treatment efficacy. Goniometry is an objective measurement of joint angles using a large protractor that is used to quantify ranges of joint motion. Each arm

of the goniometer is aligned parallel to the long axis of the proximal and distal limb segments, with the axis of rotation centered over the joint of interest's center of rotation. Maximal joint range of motion (ROM) in limbs of healthy and sedate dogs have been measured and validated to be similar.[87] Pathologic joints, such as those with OA, may have reduced joint ranges of motion in unsedated dogs due to pain or mechanical limitations.[87]

Three consecutive goniometric measurements are recommended for each joint and the median value is calculated for improved accuracy. The average variability in proximal limb (e.g., shoulder, hip, and stifle joints) measurements is greater than the variability in distal articulations (e.g., elbow and tarsus).[87] This difference may be due to a larger amount of soft tissue surrounding proximal joints and poorly localized bony landmarks as compared with distal joints. Additional research is needed to provide objective joint angle measurements in dogs with orthopedic disease.[87] Because of the presence of pain and lameness associated with CCLR[79], it is expected that both maximal flexion and extension ROM in the stifle will decrease post CCLR.

Thigh Circumference

Circumferential measurements of the thigh are an indirect measurement of muscle mass. Muscle atrophy is an inevitable result of CCLR due to disuse and neurogenic muscle atrophy.[59,79] Even after immediate joint stabilization post CCL transection, muscle mass of the thigh musculature continues to decrease for as long as 5 weeks post surgery.[88] Atrophy produces muscular weakness which reduces the protective muscular

activity that normally provides stability to the knee, ultimately increasing joint loading.[37] Morphometric characteristics of pelvic limb musculature has been evaluated via dual-energy X-ray absorptiometry (DEXA), which found atrophy primarily localized to the quadriceps muscle group associated with deficiency of the CCL.[89]

Variables possibly affecting thigh circumference measures include sedation, limb position, consistency of landmarks, and the presence of hair. Circumference of the thigh is typically determined at points 50% and 70% of thigh length, measured from the greater trochanter to lateral fabella. Clipping hair or sedation does not show a significant effect on measurements; however, position of the limb does affect outcome measurements. Flexion of the stifle results in expansion of the muscle bellies, which increases circumference measurements, thus a neutral stifle angle is recommended. This technique is sensitive enough to detect changes in muscle atrophy two weeks after stifle surgery.[90] Thigh girth circumference measurements are a reliable and easy method of measuring muscle mass that has not been reported in dogs with MRFE-induced subclinical CCL injury and rupture.[90]

Pain & Lameness

Rupture of the CCL leads to clinical signs of pain and lameness.[5] Therefore, subjective pain scores are typically used in veterinary medicine to evaluate pain behaviors (e.g., vocalization, lameness, response to palpation), physiologic parameters (e.g., heart and respiratory rate, blood pressure), posture (e.g., sternal or lateral recumbency, sitting, standing) and/or activity (e.g., resting, anorexia, inappetance,

restless) that are believed to be associated with discomfort.[91] Pain is difficult to assess objectively, as animals are not able to communicate quality or quantity of pain.

Few chronic pain scales exist, as most are developed for acute, postoperative pain evaluation, such as the visual analog scale, the University of Melbourne Pain Scale, the numerical rating scale, and the Glasgow Composite Measure Pain Score.[91] The Colorado State University Veterinary Medical Center Acute Pain Scale is the recommended pain scale as it is a composite derived from several pain scales mentioned above that includes not only psychological and behavioral pain signs (temperament, level of comfort) and response to palpation, but also body tension that is not incorporated in the other scales.[91]

Subjective lameness scores are a common outcome assessment tool used to evaluate changes in weight bearing associated with lameness. Intermittent lameness is a typical presentation of dogs with cruciate deficiency. Stifle instability and subsequent lameness is associated with gradual degeneration of the CCL. Many weeks or months may pass after this initial onset of mild, intermittent lameness, until marked lameness develops, typically due to the presence of meniscal injury or complete CCLR.[79]

Lameness evaluation must be done at a walk and trot, as different gait velocities may reveal subtle changes in gait and signs of lameness. The trotting gait may accentuate a mild lameness, as increased force is placed on the affected limb at higher speeds.[90]

Joint Effusion

The pathological mechanism for rupture of the CCL is not well understood.[79] CCL rupture is associated with increased inflammation of the synovium causing pain and

degenerative changes in the chondrocytes and articular cartilage matrix of the injured CCL[80,92]; however, it is not confirmed whether this inflammation precedes actual CCLR.[40] Inflammation within the synovium contains matrix metallopreoteinases (MMPs) which may contribute to collagen degradation predisposing dogs to cruciate disease.[80] Progressive degenerative changes in the mid-substance (or core region) of the CCL and epiligamentous region (a thin layer of connective tissue covering the ligament) occur over a period of time.[80] However, the exact mechanism which causes the significant loss of ligament fibroblasts in the CCL core is unclear.

Joint effusion that occurs following CCL transection may inhibit quadriceps contraction, as in humans[59]; however this theory has not been confirmed in dogs. In humans, normal knee joint ROM causes changes in intra-articular pressure and deformation of the joint capsule that is detected by mechanoreceptors in the joint capsule, specifically Ruffini endings.[93] Excessive joint fluid in the knee activates these sensory fibers causing reflex inhibition of the quadriceps muscle.[93] Up to a 50% inhibition of quadriceps muscle contraction is evoked by intra-articular injection of 60 ml of saline into the human femorotibial joint.[93]

Chronic stifle effusion leads to chronic periarticular fibrosis, especially at the medial aspect of the joint capsule.[79] Capsular thickening may aid stabilization of the femorotibial joint over time.[79] Therefore, reduction of acute inflammation is imperative to alleviate joint pain and effusion, increase muscle activation, prevent permanent fibrosis of the joint capsule, and minimize or prevent the development of OA.[94] Measurements of

joint effusion subclinically and post CCLR important to determine if increased pressure may contribute to alterations in muscle activity.

Cranial Drawer Sign and Tibial Compression Test

The cranial drawer test is used to assess rupture of the CCL. One hand is positioned over the bony landmarks of the distal femur and patella while a second hand grasps the tibia and fibular head.[56] A positive cranial drawer test is indicated by cranial displacement of the tibia on the femur with the stifle joint is held in slight flexion to determine the presence of a partial or complete rupture of the CCL.[56] Assessed with the stifle in both flexed and extended positions, a partial CCLR may only elicit a positive drawer sign in flexion due to disruption of only the craniomedial band of the CCL.[56] In the normal stifle, a slight laxity is palpated during the initial induced craniocaudal motion followed by an abrupt stop as the remaining intact CCL becomes taut. A partial or complete CCL tear elicits a softer endfeel, as the periarticular soft tissue structures are recruited to limit end range of motion.[79]

The tibial compression test subjectively measures instability created by cranial tibial thrust, or cranial force created during weightbearing and by quadriceps contraction, that cause compression of the femoral condyles against the tibial plateau and cranial displacement of the proximal tibia.[56] With the dog lying in lateral recumbency, one hand holds the distal aspect of the cranial femur and the index finger extended over the patellar ligament and positioned on the tibial tuberosity while a second hand flexes the hock. Cranial displacement of the index finger and tibial crest indicates a positive tibial

compression test. These tests are relatively easy to perform and provide an important role in the diagnosis of CCLR.[78]

Intra-articular Pressure

Production and regulation of synovial fluid within a joint are influenced by hydrostatic and osmotic pressure gradients existing between capillaries, synovial cavity and the interstitial space.[95] Changes in synovial fluid volume are reflected by changes in intra-articular hydrostatic pressure. Synovial lining cells secrete synovial fluid that is moved to the joint cavity. Hydrostatic pressure is one factor that controls synovial fluid movement and is dependent upon fluid volume, joint position, muscle contraction, joint capsule compliance and joint movement.[95]

In normal, healthy joints subatmospheric pressures measuring between -1 to -17 mmHg are found at neutral joint angles ranging between 80°-120°[95] and intra-articular pressure (IAP) levels change throughout normal range of joint motion.[96] After infusion of paraffin oil from 0-5 ml into the canine femorotibial joint, no change in IAP was noticed for each volume injected between 125° to 110° of stifle extension. However, as stifle flexion increased from 110° to 50°, an increase in IAP caused a decrease in the total range of motion of the stifle joint, as joint tissue elastance, or stiffness, was increased with a given volume. Similarly, when synovial fluid volumes are increased and the stifle nears full extension, IAP increases significantly.[95] Thus, measuring IAP in mid-range of stifle flexion is imperative to achieve a consistent reading.

In injured or diseased joints, effusion changes the relationship between pressure

and volume as synovial fluid dynamics are altered and effusion implies a net inflow or accumulation of fluid into a joint. Synovitis, inflammation of the synovial membrane, can occur in any form of joint soft tissue damage, which increases the amount of synovial fluid and subsequently increases IAP.[97] Increased synovial fluid volume also disrupts the normal close association of opposing articular surfaces causing increased joint instability.[97-99] Lust and colleagues injected hyaluronic acid into the coxofemoral joint of a normal population of Labrador Retrievers and radiographs revealed subluxation of the femoral head, indicating hip joint instability, which was accompanied by an increase in IAP.[99] Increased IAP causes joint instability in the equine metacarpophalangeal joint due to high intra-articular fluid volumes.[97,98] However, these studies measured IAP in nonweightbearing positions, not taking into account joint movement, position and muscle contraction or its effect on joint fluid volumes. Routine joint injections produce a mild degree of synovitis, however, increased synovial fluid volume secondary to synovitis may be just a single factor contributing to joint instability.[99]

In injured or osteoarthritic joints, increased IAP may cause limited motion, pain, and decreased neuromuscular control, thus leading to muscle atrophy and weakness.[96,100] This increase in IAP, results in arthrogenic muscle inhibition, which is a reflex inhibition of the muscles surrounding an affected joint that normally stabilize and protect the joint from injury.[100] Ferrell et al. infused stifle joints in sedated dogs at various joint angles and IAP and measured sensory fiber discharge.[96] They found that the maximum discharge rate of the medial articular nerve occurred with the stifle in full extension and

full flexion and the minimum discharge rate occurred with the stifle at intermediate joint angles (60° to 110°). An increase in neural activity was seen at intermediate positions with higher infused volumes of fluid. They concluded that elevated IAP produced altered reflex activity, which changed joint afferent discharge patterns. In effusive joints, mechanoreceptors in the stifle joint capsule function as a negative feedback system on pelvic limb musculature to limit or prevent hyperflexion and hyperextension joint movements, therefore protecting the joint from further injury.[96] Measuring IAP in conjunction with muscle activity may determine if increased joint effusion has any effect on alterations in muscle activity in dogs.

Prostaglandin E_2 Analysis

Found in large quantities in OA joints, prostaglandin E_2 (PGE_2) can provide an objective measure of synovitis.[94,101] Damage to intra-articular structures post CCLR can be attributable to the inflammatory process that ensues.[101] Inflammation, a clinical result of CCLR, can produce long-term detrimental effects in a joint via production of inflammatory mediators, degradative enzymes and inflammatory cells that lead to articular cartilage degradation. Mediators associated with deleterious effects to joint health (more specifically, articular cartilage) include aggrecanases, prostaglandins, free radicals and cytokines, specifically interleukins (ILs) and tumor necrosis factor-alpha (TNFα), which influence the production and activation of matrix metalloproteinases (MMPs) that contribute to cartilage matrix degradation.[94] Synovitis produces pain, deterioration of articular cartilage and contributes to joint instability.[94] Stimulated by IL-

1 and TNFα, PGE_2 is the most important inflammatory mediator produced in damaged joints.[94] In the joint, PGE_2 causes vasodilation, enhances the perception of pain and degrades and inhibits the synthesis of proteoglycan, decreasing its content in the cartilage matrix.[94]

Gross Pathology & Histopathology

Joint capsule and synovial membrane inflammation, meniscal injury or degeneration, articular cartilage degeneration, and development of periarticular osteophytes result from changes in mechanical stress and inflammatory mediators due to joint instability post CCLR.[79] In response, cartilage loses its integrity, characterized by a loss of cells in the superficial zone, expanding into the middle and deep zones as the severity of cartilage degeneration increases.[11] Surface irregularities may be present in the cartilage, where fibrillations and erosions may exist in all zones.[11] Hypercellularity, cell loss and chondrones (cell clones) are also characteristic of cartilage injury, as is a reduction in the concentration of proteoglycan.[11] Pond & Nuki reported a detailed post mortem evaluation of dog stifles after CCL rupture between 1 and 26 weeks, where articular cartilage gross appearance varied from normal to deep fibrillation.[85] In regions where gross fibrillation does not exist, damage to the cartilage is evidenced by absence of the superficial zone and the chondrocytes immediately adjacent.[85]

Despite a vast amount of information available in the literature to determine OA severity via histologic assessment, many limitations exist in these techniques including the absence of synovial, meniscal and subchondral bone histologic evaluation; scores are

not weighed for various categories (structural, cellular and matrix composition changes); the location or number of sections scored is not standardized; and methods are not validated to functional or clinical outcomes.[11] A recent recommendation for a comprehensive histological assessment of canine OA has been devised.[11] This method provides a global assessment of canine histologic tissues and has been proven reliable when performed by experts in each of three categories including cartilage, osteochondral and synovium.[11]

Macroscopic scoring of cartilage includes measures of the weightbearing surfaces of the medial and lateral femoral condyles and tibial plateaus. Scores range from 0 (smooth surface) to 4 (severe damage).[11] Gross pathology, or macroscopic scoring of synovial tissue should include evaluation of three sections (medial, axial and lateral compartments) from the stifle and scored from 0-5 corresponding to a normal appearance, meaning a smooth, semitranslucent, off-white color to severe, defined as severe discoloration with diffuse thickening/fibrosis and hypervascularity.[11] Macroscopic scoring of menisci divide each medial and lateral meniscus into thirds and include cranial, middle and caudal sections. Scores for each section range from 0-4, with zero indicating no affected areas to 4, describing complete maceration of tissue.[11]

Microscopic histological techniques utilize hematoxylin and eosin (H&E) staining to investigate synovial, meniscal and cartilage characteristics, such as morphology, cell loss, and necrosis. Safranin-O stains cartilage and meniscal tissues red and is counterstained with fast-green (SOFG), to detect the presence of proteoglycans and

observe advanced damage to the tissues, such as structural disintegration, fibrillation and mineralization of the subchondral bone. Microscopic scoring of synovial tissue includes three sections (medial, axial and lateral compartments) from each joint, stained with H&E, and item categories including lining cell characteristics, lining characteristics, and cell infiltration are graded separately.[11] Microscopic scoring of menisci include H&E and SOFG staining of anterior (cranial), middle and posterior (caudal) sections of the medial and lateral menisci. Tissue architecture (H&E), cell and matrix content and morphology (SOFG) and proliferative response (H&E) categories are graded separately for each section.[11] Four cartilage samples are recommended from specific locations of each of four weight bearing compartments in the stifle. Categories including cartilage structure and chondrocyte pathology, stained with H&E, and proteoglycan pathology, stained with SOFG, are graded separately for each sample.[11] Histological evaluation of articular cartilage post MRFE-induced CCL injury and rupture has yet to be reported and associations of the severity of cartilage damage with clinical alterations in muscle activity are important to investigate the relationship between neuromuscular patterns and signs of OA development in dogs.

Radiography

Radiographs are used to aid in the diagnosis of CCLR by ruling out other bony orthopedic or soft tissue abnormalities and to assess the degree of OA present, which provides a baseline for researchers to determine benefits of treatment and to follow progression of disease.[79] Depending on chronicity of CCLR, radiographic findings vary.

In acute CCLR, radiographic changes associated with CCL deficiency include visible distension of the caudal joint capsule and elevation of the infrapatellar fat pad due to joint effusion.[79] In chronic cases, prominent effusion continues and osteophytes may be seen on the trochlear ridge and patellar margins.[79]

Radiographic signs of OA have been described in the contralateral stifle of dogs with CCLR.[7,8] de Bruin and colleagues studied the progression of OA development in the unaffected limb.[41] After 6 and 12 months, osteophyte formation and tibial plateau subchondral sclerosis were identified, but these changes did not progress in a linear fashion, indicating that osteophyte formation did not significantly increase at all sites between 6 and 12 months. They concluded that OA progression is not a continual process.[41] In addition, a poor correlation exists between severity of OA on radiographs and lameness utilizing subjective scoring systems or via force platform analysis.[41] Therefore, use of radiographs alone should not be used to assess clinical outcomes.[102]

Future Directions in CCL Research

To date, no diagnostic or treatment approaches have addressed the direct contribution that muscles have in increasing stifle stability through surgical or conservative approaches to improve dynamic instability, defined as the stifle's inability to remain stable when subjected to rapidly changing forces and moments during activity[18], of the CCLR limb. The majority of research and clinical practice focuses on structural properties and repair of the CCL-deficient limb, with little regard for muscular involvement or functional stability of the stifle.

Surgical management of stifle instability caused by CCLR has predominantly focused on reconstruction of the ligamentous static constraints.[3,4] Intra-articular stabilization often focuses on replacement of the CCL with autogenous tissue; whereas extra-capsular techniques attempt stabilization via the placement of stabilizing sutures within periarticular tissues.[103] Geometry-altering procedures, such as the tibial plateau leveling osteotomy (TPLO) and tibial tuberosity advancement (TTA), attempt to provide dynamic stability, thereby minimizing the need for the static stabilizing role of the CCL.[18,61,104,105]

Unfortunately, little is known about normal pelvic limb muscular activity and forces or how these surgical procedures alter stifle biomechanics and muscle activity. To date, no single surgical procedure can claim superiority over another in stabilizing the stifle, preventing OA, or guaranteeing long-term success in returning dogs to normal function.[106] Therefore, investigation into alternative or intrinsic mechanisms for optimizing stifle stability in dogs with CCL deficiency must be explored.

Knowledge of muscle activity around the stifle is a missing link in understanding appropriate conservative treatment for the CCL-deficient stifle. Understanding alterations in muscle firing and amplitudes associated with pre-clinical CCLR may provide a first step in addressing and possibly preventing CCLR and its sequelae. Surgical management of CCL instability currently focuses on biomechanical modifications or reinforcement of the passive (ligamentous or osseous) structures of the stifle. However, knowledge of pelvic limb flexion and extension angles, ground reaction

forces and simultaneous muscle activity will help to clearly define the role of muscular stabilization. A complete understanding of muscle contributions to stifle stability will provide more objective and validated recommendations to help direct choices in surgical and nonsurgical management of stifle instability associated with partial or complete CCLR. Future studies evaluating current surgical techniques with EMG and stifle kinematics may provide information to determine which approach is indicated to help return the patient to optimal function and prevent long-term OA.

A better understanding of the muscle activity patterns is fundamental in designing effective physical therapy programs that target specific muscles involved in specific phases of stifle joint stabilization. This knowledge would help to develop individualized neuromuscular rehabilitation programs, such as functional training, agility, and perturbation training (unconscious reaction to a sudden movement), that optimize specific aspects of dynamic stifle stability.[18] Abnormal or maladaptive compensatory gait strategies provide an additional target for physical therapy techniques, with the goal of preventing contralateral CCL injury and development of stifle OA, without compromising the ipsilateral limb. Physical therapy protocols should be directed at normalizing muscle activity and joint forces with targeted strengthening and gait training techniques.[31] Targeted physical therapy treatment plans may also be developed with the ultimate goal of reducing short and long term morbidity associated with CCLR.

Purpose of Dissertation

The overall **purpose** of this project is to characterize alterations in EMG activity

in ipsilateral and contralateral pelvic limbs and measure biomechanical, clinical and physiologic parameters in all four limbs in dogs during subacute, acute and chronic phases of CCLR. This goal will be achieved by measuring EMG, kinetics, kinematics, clinical, physiologic and histologic parameters following unilateral MFRE-induced CCL degeneration and subsequent rupture.

Specific Aim 1: Post unilateral induced CCL injury with MRFE and subsequent CCLR, determine muscle activity in the bilateral vastus lateralis, biceps femoris and medial gastrocnemius muscles via surface EMG at six time intervals: (1) pre-injury (baseline), (2 & 3) pre-CCLR, 2 and 4 weeks post MRFE surgery (subclinical phase), (4) 4 weeks post CCLR (acute phase), (5) 8 weeks post CCLR (intermediate phase), and (6) 16 weeks post CCLR (chronic phase). It is *hypothesized* that significant alterations in on/off timing, amplitude and frequency of muscle activity will be observed in all muscles at all post-MRFE injury time points, compared to baseline values.

Specific Aim 2: Identify compensatory gait and associated muscle activity patterns within the contralateral pelvic limb and kinetic and kinematic alterations within bilateral forelimbs at all time points post MRFE-induced injury and CCLR. It is *hypothesized* that alterations in gait will be present in bilateral thoracic limbs, and alterations in gait and muscle activity will be present in the contralateral pelvic limb, similar to the affected limb, during subclinical CCL degeneration and the subsequent acute, intermediate and chronic phases of CCLR.

Specific Aim 3: Follow longitudinal changes in clinical and physiologic outcome

parameters in dogs with unilateral CCLR post MRFE-induced injury. These outcome parameters include: goniometry, thigh circumference, pain scores, lameness, joint effusion, cranial drawer sign, intra-articular pressure, synovial fluid analysis for PGE_2 levels, gross pathology, histopathology, and radiography. It is *hypothesized* that certain clinical and physiologic outcomes will be reliable indicators of CCL injury and rupture.

Outline for Remainder of Dissertation

In **Chapter 2,** investigates compensatory gait and associated muscle activity patterns within the contralateral pelvic limb and kinetic and kinematic alterations in bilateral forelimbs at specific time points post MRFE-induced injury. Compensatory gait patterns are adopted[9,54] post CCLR in an effort to help protect the injured stifle, but at the risk of contributing to alterations in muscle stiffness, increased joint loading and possible rupture of the contralateral CCL.[8,22,41] This chapter will present information on how alterations in muscle activity are related to compensatory gait and its potential contribution to contralateral CCLR, addressing specific aim 1.

Chapter 3 investigates muscle activity recorded by surface EMG of the muscles surrounding the stifle joint post MRFE-induced CCL injury and subsequent rupture. Asynchronous muscle firing is theorized to develop after CCL injury, altering stifle stability and causing increased joint loading, which results in CCLR and subsequent OA development.[31,37] Measuring EMG using the MRFE model of CCLR is a novel approach in the study of CCL disease onset and progression. This chapter will present alterations in muscle activity patterns compared to normal, to determine the role of muscles in

dynamic joint stability in the stifle, addressing specific aim 2.

Chapter 4 describes the correlation between alterations in muscle timing and clinical outcome parameters related to CCLR as to assess the predictive value of these parameters in the phases of CCLR and diagnosis of OA. Outcome parameters include range of motion, thigh circumference, pain, lameness, cranial drawer, joint effusion, intra-articular pressure, synovial fluid analysis, gross pathology, histopathology scores, and radiography, addressing specific aim 3.

Chapter 5 provides a general discussion integrating the research findings from chapters 2-4. This chapter evaluates the extent to which this dissertation has succeeded in improving or advancing our understanding of canine limb biomechanics as related to subclinical CCL pathology and subsequent rupture.

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CHAPTER 2

GROUND REACTION FORCE AND KINEMATIC ASSESSMENT OF COMPENSATORY GAIT IN SUBCLINICAL, ACUTE, AND CHRONIC CRANIAL CRUCIATE LIGAMENT RUPTURE POST MONOPOLAR RADIOFREQUENCY ENERGY INJURY

INTRODUCTION

The application of kinetic gait analysis in veterinary medicine is well-established and includes assessment of musculoskeletal pathology, surgical repair, drug therapies, amputation effects, and neurologic disease.[1] Kinetic and kinematic assessment of gait can provide accurate and efficient measures of temporospatial gait characteristics that are limited through subjective gait evaluation alone.[1] Accuracy of kinetic outcome measures may be affected by subject selection, subject's linear velocity, and shape of the dog related to mass distribution; for example, dogs with less mass have lower peak vertical forces.[1]

Digital instrumented kinematic analysis systems quantitatively measure motion, but because of their expense, these systems are typically limited to academic institutions.[1] Two-dimensional systems are less expensive, but typically require less-accurate manual identification of marker positions and are unable to record out-of-plane movements.[1]

Many three-dimensional systems automatically track reflective marker positions in time and space, reducing human processing error and allowing for software calculations of angular segmental movements.[1] Kinematic analysis is not without factors that may lead to error in reported data such as skin movement and differences in canine morphological shapes, which may lead to differences in marker placement on bony anatomic locations.[1] Despite these sources of error, kinematic research in veterinary medicine is well-established and used in evaluating a wide variety of musculoskeletal diseases such as hip dysplasia and CCLR, as well as joint range of motion during swimming versus walking.[1]

Ground Reaction Force Analysis in Dogs

Kinetic studies are commonly used to describe normal and abnormal canine gait.[2] Due to the pronounced lameness on the affected pelvic limb, kinetic studies in dogs post CCLR have reported an expected decrease in peak vertical force (PVF).[3-5] Budsberg and colleagues reported a decrease in PVF and associated impulse (area under the vertical force versus time curve), as well as a decrease in craniocaudal forces and impulse in dogs that had undergone CCL transection.[5] A decrease in PVF of approximately 80% from baseline was greatest at 2 weeks post transection, with a gradual increase to approximately 60% of presurgical values. PVF never fully reached baseline values over a 12 week period of time.[3,4]

While the majority of studies of CCL injury evaluate forces on the pelvic limbs, few studies address compensatory strategies in the thoracic limbs post CCL-transection. O'Connor et al. reported no increase in PVF exerted by thoracic limbs following unilateral CCL transection and PVF values for the contralateral pelvic limb remained

unchanged from baseline.[3] Conversely, Rumph and colleagues found that PVF in the affected limb was 80% less at 2 weeks post operative, with a concurrent increase in PVF of ~34% in the contralateral pelvic limb post CCL transection.[4] Vertical impulse was increased in the contralateral thoracic limb than in the ipsilateral thoracic limb at 12 weeks post surgery.[4] However, this study included a 12-week regimen of exercise that may have influenced the results. Rumph et. al. stated that daily forced exercise beginning 2 weeks post CCL transection for a period of 10 weeks, may have increased lameness in the surgical limb, causing differing compensatory strategies in the unaffected limbs.[4]

A newer model of induced CCLR using monopolar radiofrequency energy (MRFE), has also shown similar decreases in PVF in the affected limb.[6] Arthroscopic induction of cruciate ligament injury via MRFE is reported to reliably produce CCL degeneration and rupture on an average of 55 ± 2 days post injury.[6,7] This novel technique provides a subclinical window of time in which to assess gait changes during CCL degeneration, but prior to CCLR. Lopez and Markel measured each pelvic limb preoperatively and at 4, 8, 12, 16, 26, and 36 weeks after surgery which showed significant decreases in PVF between the 8- and 12-week evaluation points, corresponding to the mean CCLR date, and remained decreased throughout the study.[6] However, no thoracic limb or craniocaudal forces were analyzed using the MRFE CCL injury model to investigate the effects of compensatory gait as a potential contributor to CCL disease both within the injured limb, as well as the three healthy limbs.

Investigation into the musculoskeletal system's response to the onset of CCL degeneration and subsequent rupture (including contralateral limb adaptations) is needed to better understand the pathophysiology of CCL injury and prevention, and to maximize

the effectiveness of surgical and nonsurgical treatments. MRFE is more similar to naturally occurring CCL disease than the traditional, acute transection model of CCL rupture, as gradual deterioration of the CCL occurs.[6,7] It is expected that no change in vertical or craniocaudal ground reaction forces will be found in the treated limb at either 2 or 4 weeks post MRFE-induced CCL injury during standing and trotting gaits, as indicated by Lopez and Markel.[6] However, as the CCL progressively degenerates, we anticipate an increase in vertical and craniocaudal ground reaction forces will occur in the contralateral pelvic limb and thoracic limbs during the subclinical phase, as the CCL is degenerating, due to unweighting of the affected limb caused by pain and lameness. We expect a decrease in vertical and craniocaudal forces in the treated limb at all time points post CCLR, similar to reports in the literature with other CCLR models,[3-5] with changes in the contralateral pelvic limb and thoracic limbs.

Kinematic Gait Analysis in Dogs

Kinematic analysis of healthy dogs has been evaluated at the walk and trot [8-10] and in dogs post CCL transection.[11-14] These studies report that patterns of stifle flexion and extension movement change within the CCL-deficient limb post rupture, as expected. In the affected limb, DeCamp, et. al., reported increased flexion in the femorotibial joint throughout stance and early swing phase and the stifle failed to extend in late stance.[11] They believed that loss of extension at the end of stance may reduce limb propulsion and provide a protective adaptation to pain and joint instability.[11] In contrast, coxofemoral and tarsal joint angles were more extended during stance phase as a compensation to increased femorotibial flexion.[11] They stated that increased extension in these joints during stance may help to preserve gait by maintaining foot contact and limb propulsion

in the presence of a painful and flexed femorotibial joint.

Recognition of specific kinematic patterns of musculoskeletal pathology may be possible by understanding primary versus compensatory gait, as evidenced by these studies.[11-14] Kinematic variables measured by instrumentation of compensatory gait of all four limbs post MRFE surgery prior to and after cruciate rupture was not found in the literature. The purpose of this study is to identify compensatory gait within the contralateral pelvic limb and in bilateral thoracic limbs at six time points post MRFE-induced CCL injury and rupture. We hypothesize that we will be able to document a gradual progression in lameness and compensatory gait using the MRFE model, as gradual CCL deterioration in the treated limb progresses over time.[15] We expect that alterations in gait will be present in the contralateral pelvic limb, as well as in bilateral thoracic limbs during subclinical CCL degeneration and post CCL rupture.

MATERIALS & METHODS

Dogs

As determined by power calculations, six female, purpose-bred, hound dogs free of orthopedic and neurologic disease on initial physical and radiographic examination were used in this study. Dog age ranged from 1.0 to 3.7 years (mean ± SD; 1.6 ± 1.0 years) and dogs weighed 15.7 to 29.0 kg (22.2 ± 4.9 kg). Dogs were maintained with free choice food and water access and housed in individual 1.2 x 2.4 m runs for the duration of the study. Thirty minutes of unrestrained indoor group exercise was allotted for each dog daily; otherwise, dogs were taken out of their runs for gait evaluation and for outcome parameter recording.

Study Design

This study was approved by the Institutional Animal Care and Use Committee at Colorado State University and carried out at The Orthopaedic Research Center. This was a randomized, repeated-measures design that assessed changes in kinetic and kinematic parameters in bilateral pelvic limbs with induced unilateral CCL degeneration and subsequent rupture of 6 dogs at baseline, during subclinical CCL degeneration (2 and 4 weeks post MRFE-induced CCL injury), acute (4 weeks post confirmed CCLR), intermediate (8 weeks post CCLR), and chronic phases of CCLR (16 weeks post CCLR).

MRFE Surgical Procedure

All MFRE and arthroscopic surgeries were performed as described by Lopez and Markel[6] at the Colorado State University Veterinary Teaching Hospital by two board certified surgeons skilled in stifle arthroscopy. A surgeon experienced with the MRFE model performed the first five procedures while training a second surgeon in the model. The second surgeon performed the final MRFE procedure. Random assignment of surgery to the left or right pelvic limb was determined prior to the start of the study. All dogs underwent a unilateral arthroscopic visualization and MRFE application to the CCL with the purpose of inducing CCL injury. Premedication included atropine SQ (0.03 mg/kg) and morphine SQ (0.1-2 mg/kg). Induction medication included diazepam IV (0.1 mg/kg) and propofol IV (4 mg/kg) titrated to effect. Dogs were maintained with gas anesthesia (isoflurane) with oxygen as a carrier gas. Monitoring equipment included: electrocardiogram, Doppler for noninvasive blood pressure monitoring, pulse oximetry, capnography and intravenous lactated Ringer's solution (5-10 mL/kg/hr) were administered. Hair was removed from mid-shaft tibia to proximal femur and dogs were

surgically prepped with chlorhexidine scrub followed by a wash with sterile water. A radiofrequency generator (Smith & Nephew, Andover, MA) and TAC-C scoping probe was utilized for MFRE arthroscopic surgery to induce CCL injury. Dogs recuperated in the Critical Care Unit and were returned to their kennels once they had sufficiently recovered from anesthesia. Postoperative analgesia was provided by a single injection of carprofen (4.4 mg/kg SQ). A fentanyl patch (1 mg/pound) was applied to a shaved patch of skin over the thorax region on the day before surgery and removed after 48-72 hours post surgery. Dogs were dosed with oral carprofen (Rimadyl), 2.2 mg/kg, PO BID x 14 days. CCLR was detected by a sudden onset of non-weightbearing lameness, joint effusion, and a positive cranial drawer sign.[6]

Experimental Design

All data collection was performed within the Gait Analysis Center, which is specifically designed for canine kinematic and kinetic gait analysis. Training and acclimation involved teaching dogs to walk on a leash and exposure of each dog to the data collection environment techniques (kinetic and kinematic gait analysis) for at least one month prior to baseline data collection. A second and third data collection occurred 2 and 4 weeks post MRFE to identify subclinical changes prior to CCL rupture. Limb parameters were assessed again at 4 and 8 weeks following CCL rupture (confirmed by the presence of profound drawer and instability) to assess the affects of acute and intermediate CCLR on outcome parameters. The final data collection occurred at 16 weeks post rupture to assess the affects of chronic CCLR on outcome parameters.

Kinetic Data

Three force platforms (AMTI, model OR-6-6, Watertown, MA) mounted in-series

on a level 10-m, concrete, runway were used for measuring kinetic parameters. Kinetic measures were recorded at a velocity of 2.0–2.4 m/sec and an acceleration of ± 0.5 m/sec/sec using five timing lights installed at 0.5-m intervals.

Static Ground Reaction Forces

Individual & Paired Limb Weightbearing

Static weight bearing of all 4 individual limbs was evaluated to assess compensatory weight shifting or limb unloading at all time points. Dogs stood perpendicular to the gait runway and were positioned with one thoracic limb near the center of the two adjacent force plates to measure PVF of each thoracic limb separately. A successful trial was defined as the dog standing stationary in a square stance, facing forward, without moving the head side to side. This method was then repeated for both pelvic limbs to assess pelvic limb weight bearing at all time points. Three successful 10-second trials were recorded for data collection. One second of still data was analyzed from each 10-second trial and averaged to obtain a representative static GRF value. Force platform data were collected at 2,000 Hz and filtered with a 4^{th} order recursive Butterworth low pass filter, with a cutoff frequency of 12 Hz.

Craniocaudal GRFs during weightbearing were analyzed for thoracic and pelvic limb pairs by measuring bilateral thoracic limbs on one plate and bilateral pelvic limbs on another force plate with dogs standing parallel to the runway. Three successful 10-second trials were recorded for each configuration and three seconds of intermediate still data were analyzed from the 10-second trials and averaged to obtain a representative value. All kinetic variables were normalized to body weight.

Dynamic Ground Reaction Forces

Three-dimensional ground reaction forces and associated impulses, time to peak forces and loading and unloading rates were recorded and processed in the vertical (Fz) and craniocaudal (Fy) directions using Acquire (Sharon Software, Inc., Owosso, MI) at a sampling frequency of 200 Hz. Data from 5-10 successful trials from each limb were collected and analyzed. A trial was considered successful if the entire paw strike of one pelvic limb landed near the center of each of 3 force platforms, within the defined velocity (2.0–2.4 m/sec) and acceleration (± 0.5 m/sec/sec) parameters. Stance duration was defined as the time from paw strike (foot contact) to the time of paw off. Craniocaudal forces were divided into braking (deceleration of the center of mass) and propulsion (acceleration of the center of mass) phases. Average loading rates (i.e., rate at which the limb is loaded from the start of stance phase to the point of peak force) and unloading rates (i.e., rate at which the limb is unloaded from the point of peak force to the end of stance phase) were calculated. All dynamic kinetic variables were normalized to body weight.

Kinematic Data

An eight camera, optical motion analysis system (Motus 9.0, Vicon Motion Systems, Inc., Centennial, CO) with spherical reflective markers (3.5 cm in diameter; Vicon Motion Systems, Inc.) were used to record three-dimensional coordinate data of bilateral thoracic and pelvic limbs. Kinetic and kinematic data were synchronized and parameters were collected simultaneously from both thoracic and pelvic limbs within one stride cycle. Thoracic and pelvic limb joint centers of rotation for established lateral bony landmarks were identified and spherical reflective markers were attached to the skin using cyanoacrylate glue. Joint angles were calculated according to convention for

sagittal plane analysis.[8,10] Joint centers of rotation for the thoracic limb included: dorsal scapular spine, acromion process (representing the scapulohumeral joint), lateral epicondyle of humerus (cubital joint), ulnar styloid process of the carpus (carpal joint), distolateral aspect of the fifth metacarpal bone. Pelvic limb joint centers of rotation included the cranial border of the iliac crest, greater trochanter of the femur (coxofemoral joint), between the lateral epicondyle of the femur and the fibular head (femorotibial joint), lateral malleolus of the distal tibia (tarsal joint), and the distolateral aspect of the fifth metatarsal bone. A marker placed on the occiput was also included to orient the researcher to the specific anatomical locations listed above during 3D reconstruction with analysis.

A Bosch Dinion camera was used to capture video images of each trial to verify limb contact with the force platforms. The instantaneous location of the reflective markers at each joint was calculated using Motus software (Vicon Motion Systems, Centennial, CO). Three-dimensional kinematic data were recorded at a frequency of 200 Hz, filtered using a Butterworth low pass filter with a cutoff frequency of 6 Hz, and analyzed using Motus software (Vicon Motion Systems, Inc., Centennial, CO). Data from 6 successful, 10-second trials (3 right limb leading and 3 left limb leading) were recorded within a 2.6 x 1.5 x 1.0 m calibrated (typical accuracy = 0.7 mm) capture volume. A successful trial was defined as when all markers could be identified within the stride. Previously described standing trials to measure static standing joint angles were recorded with dogs placed in a square stance, standing stationary for three 10-second trials. Three seconds of still data were selected and analyzed and the mean static standing joint angle measurements were reported for all limbs.

During dynamic gait, mean joint angle measurements for stance and swing phases during trotting for the shoulder, cubital, carpal, coxofemoral, femorotibial and tarsal joints were analyzed. Three trials for each dog were averaged. Trial averages of all dogs were pooled. The mean maximum joint angles during stance, the mean minimum joint angle during stance and swing and the percentage of stride of the maximum stance and minimum swing joint angles were calculated for the entire stride at all time periods. The mean ranges of motion for each joint during stance and swing were calculated within dogs and pooled across all dogs. Stride length and duration for the thoracic and pelvic limbs was determined by tracking the metacarpal and metatarsal markers for one stride.

Swing and stride durations were calculated from the 2,000 Hz force platform data. Stride duration was calculated as the time from paw strike to subsequent paw strike of the same limb, whereas swing phase duration was calculated as the time of paw off to the time of paw strike (foot contact). Pilot data demonstrated less than a 1% difference in stance duration calculations between force platform and kinematic software programs, thus stance duration from force platform data and swing duration from kinematic data were expressed as a percentage of the total stride. Paw strike event was defined as occurring at 0% of the stride and the subsequent paw strike of the same paw was defined as 100% of the stride.

Statistical Analysis

A mixed model, repeated measures analysis of covariance (ANCOVA) was performed to assess differences in kinetic and kinematic parameters between the thoracic limbs and the pelvic limbs over six time periods. Independent variables included age, surgical limb (right vs. left) and time factors (Baseline; 2 and 4 weeks post MRFE; 4, 8,

16 weeks post CCLR) related to MRFE-induced CCL injury and eventual CCL rupture. Dependent variables included kinetic and kinematic outcome variables. Significant differences were determined at $p \leq 0.05$. Least square means were used for individual comparisons among the interactions of time * treated limb.

Overlapping strikes, or overlap as the thoracic paw left the ground and the ipsilateral pelvic paw struck the force platform, existed in 4 out of 5 dogs, as indicated by a nonzero value between thoracic and pelvic limb vertical ground reaction forces. This overlap was carried throughout each time point to varying degrees and was analyzed in the ANOVA using an 'overlap' covariate defined by a '0' for no overlapping strikes on the force platforms or a '1' for trials that contained overlapping strikes. A second covariate called 'weeks to rupture' was included in the ANOVA and defined as the dog sustaining a CCLR within the first week post operative (early = '0'); CCLR at 2-6 weeks post-operative (mid = '1'); CCLR occurring at $\geq$ 7 weeks post surgery (late = '2').

RESULTS

Complete CCLR was detected via palpation in all 6 dogs at varying times post MRFE. Two dogs ruptured their CCL within the first week post MRFE (one treated by the MRFE experienced surgeon; and one treated by the less experienced surgeon); 3 dogs ruptured their CCL 6 weeks post MRFE; and 1 dog ruptured 15 weeks post MRFE. Data from one dog that ruptured the CCL within the first week post MRFE was eliminated from trotting kinetic and kinematic analysis, as she would only ambulate with a hopping gait and not the required trot. Data from this dog were included in static weightbearing and standing trials only.

Kinetic Variables - Static

Individual Thoracic Limb Weightbearing – Static Stance

Dogs bore a similar amount of weight on each thoracic limb at all time points during static standing (Figure 1). No significant differences were found when comparing each thoracic limb to its respective baseline value or between the thoracic limbs at all time points.

Individual Pelvic Limb Weightbearing – Static Standing

No significant weightbearing differences of each pelvic limb were found at baseline or at pre-CCLR time points during static standing. However, at time points post CCLR, weightbearing was significantly different ($P < 0.0001$) between the treated pelvic limb and the contralateral pelvic limb (Figure 2). Weight bearing on the treated limb at 4, 8 and 16 weeks post CCLR decreased by as much as 12% body weight compared to the untreated limb. Conversely, untreated limb values increased by up to 12% body weight. Differences between baseline values and all time points post CCLR for pelvic limbs were significant at 4 ($P < 0.0018$), 8 ($P < 0.0001$) and 16 ($P < 0.0256$) weeks post CCLR.

Craniocaudal Weightbearing Limb Pairs – Static Stance

Significant differences were found between baseline values and 4 weeks post CCLR ($P < 0.03$) for the thoracic limb pairs and between baseline and 4 weeks post MRFE ($P < 0.03$) for the pelvic limb pairs (Figure 3). Baseline thoracic limb pair percent total weight bearing was 67%, with an increase to 71% at 4 weeks post CCLR. Baseline pelvic limb pair percent weight bearing was 34%, increasing to 38% at 4 weeks post MRFE. Thoracic limb pair percent weight bearing increased from baseline and pelvic limb pair percent weight bearing decreased from baseline at all time points post CCLR,

however, not all were found to be significant.

Kinetic Variables – Trotting

Thoracic Limb Kinetic Variables at a Trot

Stance duration, peak force, impulse and time to peak force for braking, propulsion and vertical forces, and loading and unloading rates were not significant between the thoracic limbs. Similarly, no significant differences were found when comparing each thoracic limb to baseline values at all time points.

Pelvic Limb Kinetic Variables at a Trot

Peak force, impulse and time to peak force for braking, propulsion and vertical forces, and loading and unloading rates, were significantly different between the treated and untreated pelvic limbs at 4, 8 and 16 weeks post CCLR, respectively. Stance duration, velocity, braking impulse, time to peak braking forces, time to PVF at 8 weeks post CCLR and time to peak propulsion force at 16 weeks post CCLR were not significantly different (Tables 1-4).

Kinematic Variables

Average Standing Joint Angles

Static joint angles were not significantly different between the treated and untreated scapulohumeral, cubital or carpal joints of the thoracic limb. Likewise, coxofemoral, femorotibial or tarsal joints of the pelvic limb were not significantly different at any time point throughout the study.

Thoracic Limb Kinematic Variables at a Trot

No significant differences were found between the ipsilateral or contralateral thoracic limbs for kinematic variables, including stance or swing duration or percentage

of stride during stance or swing phase at any time point. Only swing duration was significantly decreased in the thoracic limb on the ipsilateral side of the treated pelvic limb at 4 weeks (203 ± 8; P = 0.02) and 8 weeks (204 ± 8; P = 0.02) post CCLR, when compared to baseline (225 ± 8) (Figure 4).

Pelvic Limb Kinematic Variables at a Trot

Percentage of Stride

No differences in temporal data, including swing or stride duration or percent stride of stance or swing, were observed before or after CCLR in the treated versus untreated pelvic limb. However, all time points post CCLR significantly increased for the percentage of stance phase (P < 0.03) in the untreated limb and a significant increase was found at 8 and 16 weeks post CCLR in the treated limb (P = 0.02 and P = 0.04, respectively). A significant decrease was found at all points post CCLR for the percentage of swing phase (P < 0.02) in the untreated limb, when compared to baseline values (Figure 5). A significant decrease in the percentage of swing phase (P = 0.03 and P = 0.02, respectively) in the treated limb, when compared to baseline values, was found at 8 and 16 weeks post CCLR (Figure 6).

Trial Averaged Trotting Joint Angles During Stance and Swing Phase of Gait

There were no differences in the average dynamic joint angles for both thoracic and pelvic limb was seen between the treated and untreated sides during the stance phase or swing phase of gait. However, with a decrease of 7°, a significant difference (P = 0.0361) was found in the femorotibial joint of the treated pelvic limb during stance only at 8 weeks post CCLR, compared to baseline values (Figure 7). By observation, femorotibial joint angles appeared to decrease at all time points during the first half of

swing phase, however, these values were not significantly different from baseline, with P values ranging from P = 0.4490 to P = 0.7128. Large variability in femorotibial joint angles during swing, especially in dogs 1 and 3, contributed to these insignificant findings (Appendix A).

Femorotibial joint angles in the untreated limb during stance phase significantly decreased ($P < 0.0018$) by up to 15° at all time points post CCLR when compared to baseline values (Figure 8). No significant differences in femorotibial joint angles were found in the treated or untreated pelvic limbs during swing phase of gait when compared to their respective baseline values.

No significant differences were seen in tarsal joint angles during stance or swing phase in the treated pelvic limb. Though decreased by 8° at 4 weeks post CCLR ($P = 0.1218$) and decreased by 7° at 16 weeks post rupture ($P = 0.1925$), tarsal joint angles in the untreated limb during stance phase significantly decreased ($P = 0.0493$) by 10° only at 8 weeks post CCLR, compared to baseline values (Figure 9). Significant decreases in tarsal joint angles by up to 10° were seen in the untreated pelvic limb during swing phase at 4 ($P = 0.0458$) and 8 ($P = 0.0037$) weeks post CCLR (Figure 9).

Maximal and Minimal Joint Angles During Stance Phase and Swing Phase of Gait

The maximal joint angles during stance and the minimal joint angles for both thoracic and pelvic limbs during swing phase did not show significant differences between the treated and untreated limbs throughout the study. However, a significant decrease ($P < 0.002$) in the minimal femorotibial joint angle by up to 19° during stance phase in the untreated limb compared to baseline was found at all points post CCLR; while the treated femorotibial joint angle significantly decreased ($P = 0.017$) by 9° at 8

weeks post CCLR (Figure 10).

A significant difference ($P < 0.01$) in the minimum joint angle during stance was found for the tarsal joint (Figure 11). An increase in tarsal joint extension of 25°, 19°, and 14° was found, respectively, at 4, 8 and 16 weeks post CCLR in the treated pelvic limb when compared to the untreated pelvic limb (Figure 11). Significance ($P = 0.02$) was shown at Week 4 (125 ± 5°) post CCLR in the treated limb, when compared to baseline (112 ± 4°), with an increase in tarsal joint extension of 13°. Significance was found at Week 4 (100 ± 5°; $P = 0.01$) and 8 (100 ± 4°; $P = 0.01$) post CCLR both with an increase in tarsal joint flexion of 14° in the untreated pelvic limbs when compared to the baseline untreated limb value (114 ± 4°).

Maximal joint angles in the untreated femorotibial joint during stance significantly decreased ($P < 0.02$) from baseline at all time points post CCLR; whereas, the treated femorotibial joint significantly decreased ($P = 0.02$) from baseline at 8 weeks post CCLR (Figure 12). Similar findings were found in the tarsal joint for the maximum joint angles during swing. The maximal tarsal joint angle in the untreated limb significantly decreased from baseline ($P < 0.02$) during swing phase at all time points post CCLR; whereas, the treated femorotibial significantly decreased from baseline ($P = 0.02$) at 8 weeks post CCLR (Figure 13). No difference in coxofemoral joint angles were noted between treated and untreated pelvic limbs at any time point or when compared to their respective baseline values.

Maximal and Minimal Joint Angles as a Percentage of Stride for Stance and Swing Phase

No difference was found in the percentage of stride between the treated and untreated thoracic or pelvic limb for the maximum joint angles during stance phase.

However, percent of stride for the untreated tarsal joint significantly increased from baseline (41.6 ± 1.3%) at 4 (44.7 ± 1.6%; P = 0.049), 8 (46.1 ± 1.4%; P = 0.002), and 16 (45.7 ± 1.3%; P = 0.003) weeks post CCLR (Figure 14).

Percent of stride for the minimal femorotibial joint angle during swing significantly increased (P = 0.01) by up to 4% in the untreated limb for all time points post CCLR when compared to the treated limb (Figure 15). The percent of stride for the untreated pelvic limb during swing at 4, 8 and 16 weeks post rupture was 71 ± 1.0°, 73 ± 1.0° and 72 ± 1.0°, respectively (Appendix B); whereas, the percent of stride for the treated pelvic limb during swing at the same time points was 68 ± 1.0°, 69 ± 1.0° and 68 ± 1.0°, respectively (Appendix B). The percent of stride for the untreated pelvic limb significantly differed (P = 0.02) from baseline (69.7 ± 0.9°) at 8 weeks post CCLR (72.9 ± 1.0°), though increased at all time points post rupture.

Mean Range of Motion

A difference in range of motion (ROM) for the coxofemoral (Figure 16) and tarsal (Figure 17) joints was significant during stance phase in the treated limb; however, no significance was found for any joint during swing phase. ROM at the hip during stance increased in the treated limb at 4 (P = 0.020) and 8 (P = 0.004) weeks post cruciate rupture, compared to the untreated contralateral pelvic limb (Appendix C). Hip stance ROM for the untreated limb at 2 (P = 0.03) and 4 (P = 0.02) weeks post MRFE and 8 weeks post rupture was significantly different than the baseline ROM value for the untreated limb. Treated limb hip ROM during stance increased at 4 (P = 0.04) and 8 (P = 0.04) weeks post CCLR when compared to baseline.

Tarsal joint range of motion during stance decreased in the treated limb at 4

weeks ($P < 0.0001$), 8 weeks ($P < 0.0001$) and 16 weeks ($P = 0.0010$) post cruciate rupture, compared to the untreated contralateral pelvic limb (Figure 17). When compared to the untreated pelvic limb baseline tarsal joint ROM value, all ROM values post CCLR significantly increased ($P < 0.0002$; whereas, treated pelvic limb ROM at 4 and 8 weeks post CCLR significantly decreased ($P < 0.017$) when compared to the treated limb baseline mean ROM value (Appendix C).

Mean femorotibial joint range of motion was not significant between the treated and untreated limb at any time point; however, the mean femorotibial range in the untreated limb was found to significantly increase ($P < 0.04$) at all time points post CCL rupture, when compared to the baseline, presurgical values (Figure 18).

Stride Length and Stride Duration

No significant difference in stride length was found between the treated and untreated thoracic or pelvic limbs at any time point throughout the study. However, stride length significantly decreased in both the thoracic ($P < 0.03$) and pelvic ($P < 0.05$) limbs at all time points post CCLR when compared to their respective baseline values (Figures 19-20). Stride duration was not significantly different between the treated and untreated limb, or when compared to baseline.

DISCUSSION

This study evaluated compensatory changes in gait post MRFE surgery and subsequent CCL rupture in the thoracic and pelvic limbs. An interesting finding in this study was an increase in the mean femorotibial joint flexion angle in the untreated limb during stance phase at a trot following CCLR. These results contradict reports in the literature that show an increase in femorotibial flexion of 5-14° at a walk in the CCL-

deficient limb during stance post CCL transection.[11,12,14,16] Less pronounced femorotibial flexion of approximately 1-8° was found at the trot.[14] A second study found an increase in femorotibial flexion of approximately 8° at a walk.[16] These studies investigated kinematics of CCL insufficiency using a transection model of CCLR versus the MRFE model of CCLR used in our study. Kinematic differences may exist between the two models as the transection model produces an acute loss of femorotibial stability versus the MRFE model, which depicts a more naturally-occurring disease by producing gradual degeneration of the CCL and allowing the dog to adjust movement strategies.

We hypothesized that gradual changes in kinetic and kinematic parameters would be seen at subclinical time points. In thoracic limbs, this hypothesis was proven for only one outcome parameter at all time points. A shorter swing duration existing in the ipsilateral thoracic limb at 4 and 8 weeks post rupture may be explained by the dog attempting to shift body weight onto the untreated contralateral limbs faster. Kinetic data did not confirm our hypothesis in the pelvic limbs during subclinical time points, as no significant kinetic changes were found. However, kinematic outcome parameters confirmed our hypothesis during subclinical time periods in coxofemoral joint range of motion only, which was significantly decreased in the untreated limb, as a possible compensation for increased flexion angles at the femorotibial and tarsal joints.

Femorotibial flexion angles of 135-180° are known to cause increased strain on the CCL during stance.[14] Femorotibial joint flexion angles in the untreated limb increased from approximately 145° at baseline to 130-135° (more flexed) post CCL rupture in our study. The increased flexion would lessen the strain on the CCL in the untreated limb, which was a possible adaptation to prevent CCL injury. However, this

increased flexion in the untreated limb may contribute to increased incidence of CCLR and OA in the same limb over time by shifting the normal femorotibial contact areas to regions of thinner cartilage that are not suited to withstand the increased loads.[17-20] This may also possibly lead to CCL strain and increased failure in the contralateral limb, [21] which occurs in 37% to 48% of dogs within 16 months after the initial CCLR.[22,23]

Outcomes as reported in different kinematic studies may be controversial or opposite depending on the method. Vilensky et. al., manually identified anatomic locations and digitized a single stride from a treadmill gait to determine joint angles.[12] DeCamp and colleagues used Fourier transformation to model dynamic joint angles[11], which is only capable of assessing if kinematic waveforms are dissimilar, but not where those differences are located within the stride cycle.[24] Korvick et. al., used spatial linkage to determine three-dimensional kinematics with surgically implanted invasive bone plates that could have artificially affected femorotibial kinematics.[14] In addition, they evaluated kinematics at a single time point only (7 weeks post CCL transection).[14] Tashman's group analyzed only 200 msec, or 60-70% of the stance phase, due to limitations in viewing the full gait cycle using radiographic stereophotogrammetric analysis.[16] Despite variations in study methods, it may be possible that differences between the current study and those reported above are related to the mechanism of injury to the CCL. Progressive degeneration of the CCL using an MRFE model may simply produce different kinematic patterns than the transection model of canine OA, which produces complete and immediate destabilization.

Colborne noted that gait asymmetries existed in one orthopedically sound Labrador Retriever at a trot, with coxofemoral, femorotibial and tarsal angles decreasing

by 10°, 5° and 3° degrees, respectively, between the left and right pelvic limbs.[25] Differences in limb placement may also account for asymmetries in segment angles.[25] We acknowledge that experimental error due to inconsistencies in reflective marker placement or skin movement over centers of joint rotation may account for our different findings.[25] However, care was taken to ensure consistent marker placement at each data collection session.

Static standing did not produce a difference in thoracic limb vertical forces at any time point or in pelvic limb vertical forces at 2 and 4 weeks post MRFE on the affected limb. This could be a result of positioning these dogs in a square stance at the time of data collection, rather than allowing each individual dog to choose their preferred stance, which may vary greatly. Based on clinical observations, it appears that dogs with pelvic limb lameness may shift the affected limb cranial in an attempt to unweight the painful limb. By unweighting the affected limb and shifting forward, static forces on the remaining three unaffected limbs may be altered during stance. As expected, static vertical force at all time points post CCLR were significantly decreased in the treated limb by 66-79% when compared to the untreated pelvic limb, which was most likely due to pain and instability.[3,5]

At a trot, stance phase was significantly increased in the untreated and treated pelvic limbs at all time points post CCLR. The swing phase decreased for both pelvic limbs after CCLR. These results support the concept that by increasing the amount of time during which forces can be exerted in stance phase results in decreased peak loads on the musculoskeletal system.[26]

A sharp decrease was seen in kinetic variables at 4 weeks post CCL rupture in the

treated limb, gradually increasing in weight bearing over 16 weeks, possibly as a result of subsiding inflammation and the formation of scar tissue that forms on the medial aspect of the joint that increases stability.[27] These findings support the tendency for dogs to decrease weight bearing on the affected limb due to pain and instability once the CCL has ruptured and reflects the unwillingness of dogs to load their limbs in both time (time to peak force and limb loading rates) and magnitude of vertical and craniocaudal forces.[3-5]

No differences in thoracic or contralateral pelvic limb kinetic parameters were found. This outcome supports the work of O'Connor, et. al. who failed to show compensatory loading at 6 and 12 weeks post CCL transection in thoracic limbs or in the contralateral pelvic limb post CCL transection.[3] These kinetic outcomes do not support our hypothesis that a difference in weight bearing exists in vertical and craniocaudal forces post MRFE surgery as the CCL is degenerating, but are consistent with findings in the literature for time points post CCLR.[3-5] These findings coincide with the history of a CCLR diagnosis in a clinical setting. Intermittent lameness is a common complaint from owners; however, dogs don't typically present to the veterinarian until a marked lameness is established, usually indicating that CCLR has occurred.[15]

No change in standing joint angles may be due to the square standing position dogs were placed in during data collection, rather than allowing the dog to choose its own stance. However, to reduce variability in the way each dog chose to stand, dogs were placed in a consistent standing position.

Though no difference in stride length was found between treated and untreated limbs at all 6 time points, stride length significantly decreased from baseline values at all time points post CCLR for both the treated and untreated thoracic and pelvic limbs.

Stride length values at a trot are consistent with reported data in the literature for normal dogs[10] and dogs post CCLR.[11,28] With any pelvic limb lameness, a decrease in stride length may occur.[28] These data show that as stride length decreased on the affected limb, stride length on the contralateral limb and bilateral thoracic limbs also decreased to maintain a symmetrical gait pattern.

Our study showed no difference in joint range of motion in the treated femorotibial joint; however, the coxofemoral joint increased and the tarsal joint decreased their ranges of motion. The coxofemoral joint changes may be a compensation for the decreased tarsal range, which produces a longer, more rigid limb in an effort to avoid cranial displacement of the tibia on the femur during weightbearing. DeCamp reported increased extension of the coxofemoral and tarsal joints during stance in the CCL transected limb, with increased femorotibial flexion, in an effort to maintain foot contact and limb propulsion in the presence of a painful, unstable joint.[11]

Increased mean femorotibial and tarsal range of motion in the untreated limb existed at all time points post CCLR when compared to baseline. As the femorotibial joint flexes, gravitational forces bring the tarsal joint range into a more flexed position during stance. This is also evidenced by the significant decrease in the minimum joint angle during stance at all time points post CCLR in these two joints. Due to this increase in tarsal range of motion, an increased duration of dorsiflexion exists, which causes an increase in the time for the percent of stride to reach its maximal joint angle. In other words, it takes up to 5% more of the stride for the tarsus to reach its maximum extension angle. Similarly, due to the increased femorotibial range of motion, an increased time is needed to reach the minimal joint angle during swing, as evidenced by the increase of up

to 4% of swing phase. To compensate for the increase in ROM at the femorotibial and tarsal joints, the hip decreases its total range of motion. These changes in joint range are seen at 2 and 4 weeks post MRFE in all pelvic limb joints, though only significant at the coxofemoral joint for these two time points.

With a functionally shorter contralateral pelvic limb on the untreated side, the ipsilateral tarsal joint must increase flexion to allow the foot to clear the floor. Similarly, with a decreased range of motion at the coxofemoral joint producing a more rigid treated limb, the untreated limb compensated by increasing tarsal joint flexion. These concepts are verified in both limbs that show a significant increase in tarsal joint angles during swing at 4 and 8 weeks post rupture.

The number of weeks to CCLR ranged from 1-15 weeks post MRFE-induced CCL surgery, which is a significant limitation of this study. MRFE surgery has been previously reported to reliably produce CCLR at approximately 8 weeks post surgery.[6] However, with the exception of 2 dogs that ruptured within the first week, our timeline of the number of the weeks to CCLR ranging from 6-15 weeks, which coincides with a typical clinical presentation of intermittent lameness, with complete rupture reported to occur several months after the initial onset of lameness.[15,27] Activity level and size of dog contribute to variations in the forces acting on the CCL, which may explain the variability in time to CCLR.[27]

Two dogs that ruptured their CCL prematurely within the first week may be explained by several factors. A second surgeon performed the MRFE on one dog, possibly contributing to differences in the MRFE technique applied. However, a review of the surgical tapes did not reveal a difference in the amount of energy delivered to the

CCL due to poor impedance or low temperature and all dogs had comparable treatment times. Of the two dogs that ruptured early during week one post MRFE surgery, one was the largest, weighing 29 kg, and the other, the smallest of the research dogs, weighing 16 kg, but the most active. The least active dog, weighing 25 kg, ruptured at 15 weeks post MRFE surgery. Though, all dogs were treated equally, individual temperaments dictated various activity levels that may explain the premature and extended times to rupture. This discrepancy of different weeks to rupture was taken into consideration in all statistical analyses by adding an additional covariate, which proved to not have an effect on results.

This study analyzed the relationship between lameness secondary to MRFE-induced CCL injury and rupture and compensatory loading. Kinetic results from this study are similar to those reported in the literature; however, kinematically, our results differ. The transection model of CCL rupture, with immediate femorotibial destabilization, produces large between-dog kinematic variability in joint angle magnitude and timing.[14,16] Results of our study showed large variability in time to CCLR and different compensatory kinematic gait patterns. Identifying the most recommended method to produce CCLR and clinical correlations of the method with the clinical progression of CCLR has yet to be determined.

TABLES AND FIGURES

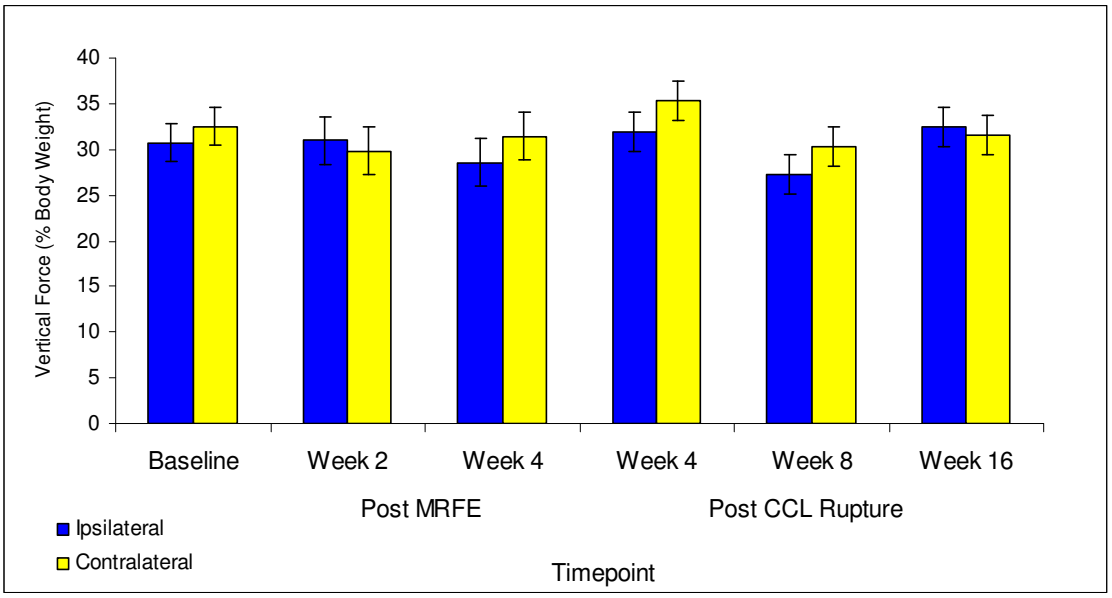

Figure 1 – Average vertical forces (% body weight) for thoracic limb static stance at all time points. Dogs bore a similar amount of weight on each thoracic limb at all time points during static standing.

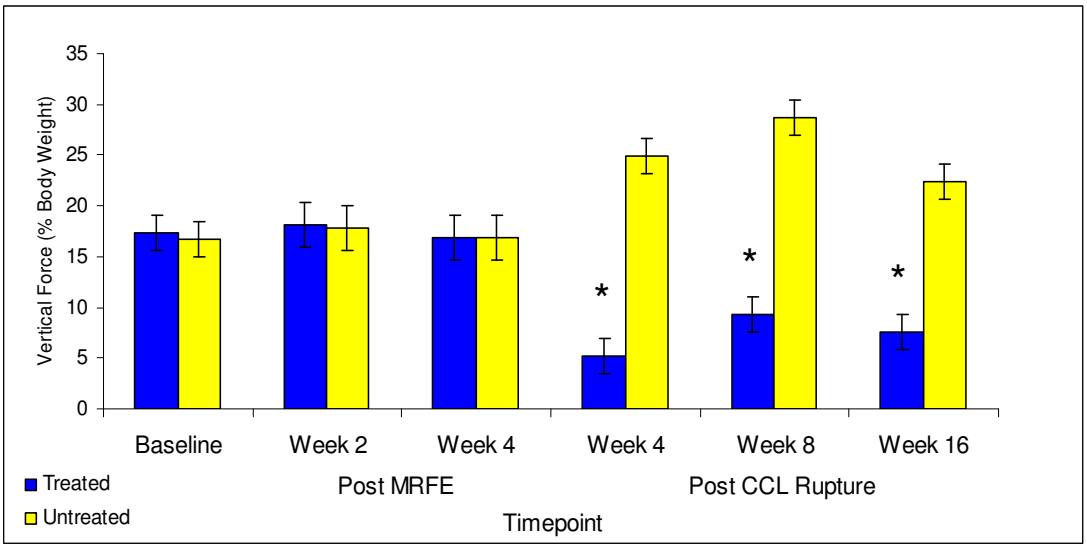

Figure 2 – Average vertical forces (% body weight) for pelvic limb static stance at all time points. No significant differences in weightbearing were found between pelvic limbs at baseline or at pre-CCL rupture (subclinical) time points during static standing. At all time points post CCL rupture, weightbearing was significantly less ($P < 0.0001$) for the treated pelvic limb when compared to the untreated pelvic limb. * Significantly different compared to the untreated pelvic limb value.

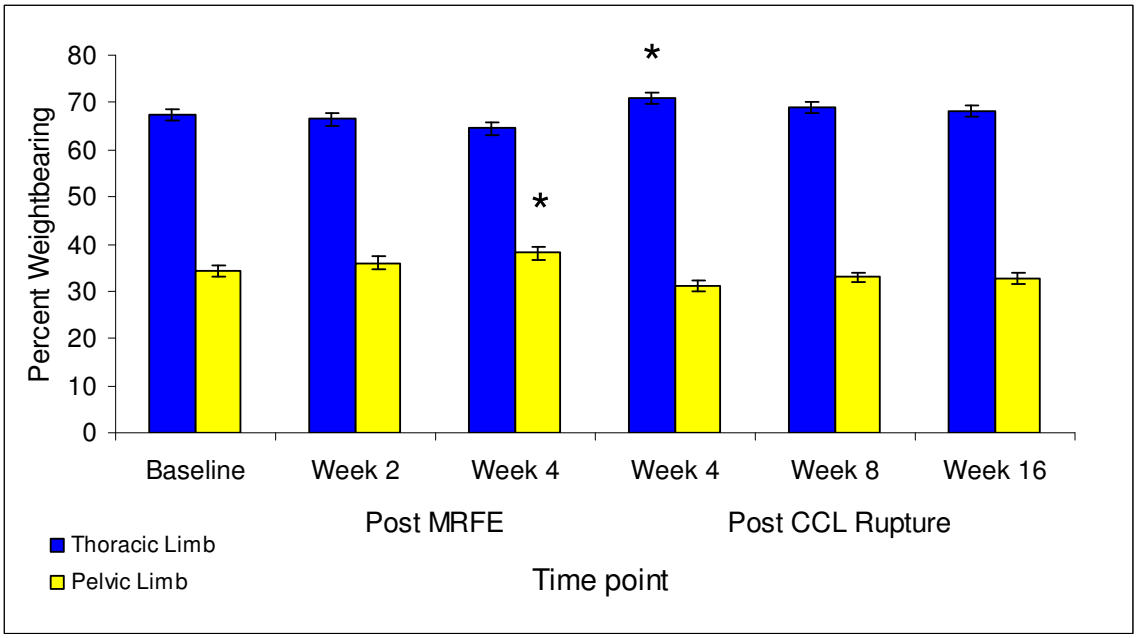

Figure 3 – Craniocaudal weightbearing for thoracic limb and pelvic limb pairs during static stance at all time points. Baseline thoracic limb pair weight bearing was 67%, with a significant increase to 71% at 4 weeks post CCL rupture. Baseline pelvic limb pair weight bearing was 34%, increasing to 38% at 4 weeks post MRFE. * Significantly different from respective baseline values.

TABLE 1 – Treated and untreated pelvic limb vertical force and impulse data post MRFE surgery and CCL rupture.* (N = 5)

Time Points	Peak Vertical Force (% body weight)		Vertical Impulse (% body weight * Sec)	
	Untreated	**Treated**	**Untreated**	**Treated**
Baseline	75.0 ± 3.3	73.0 ± 3.3	8.4 ± 0.41	8.3 ± 0.41
Post MRFE				
Week 2	71.1 ± 3.7	70.3 ± 3.7	8.3 ± 0.46	8.4 ± 0.46
Week 4	73.2 ± 3.7	68.5 ± 3.7	8.6 ± 0.46	8.1 ± 0.46
Post CCL Rupture				
Week 4	74.0 ± 4.2	30.4 ± 3.7†‡	9.6 ± 0.52	3.7 ± 0.45†‡
Week 8	70.6 ± 3.6	37.0 ± 3.6†‡	5.6 ± 0.45‡	4.8 ± 0.45†‡
Week 16	66.4 ± 3.6	44.8 ± 3.2†‡	9.1 ± 0.45	5.6 ± 0.40†‡

* Means ± standard error of the mean (SEM). † Significantly ($P < 0.05$) different compared to the untreated pelvic limb. ‡ Significantly ($P < 0.05$) different compared to baseline, presurgical values.

TABLE 2 – Treated and untreated pelvic limb time to peak vertical force and loading rate data post MRFE surgery and CCL rupture.* (N = 5)

Time Points	Time to Peak (msec)		Loading Rate (N / msec)		Unloading Rate (N / msec)	
	Untreated	**Treated**	**Untreated**	**Treated**	**Untreated**	**Treated**
Baseline	84.5 ± 2.9	83.9 ± 2.9	1.7 ± 0.12	1.7 ± 0.12	-1.4 ± 0.08	-1.3 ± 0.08
Post MRFE						
Week 2	88.2 ± 3.3	88.5 ± 3.3	1.6 ± 0.14	1.6 ± 0.14	-1.3 ± 0.09	-1.2 ± 0.09
Week 4	85.2 ± 3.3	86.3 ± 3.3	1.7 ± 0.14	1.6 ± 0.14	-1.3 ± 0.09	-1.2 ± 0.09
Post CCL Rupture						
Week 4	85.0 ± 3.5	96.0 ± 3.2†‡	1.7 ± 0.16	0.6 ± 0.14†‡	-1.2 ± 0.11	-0.7 ± 0.09†‡
Week 8	90.6 ± 3.1	94.6 ± 3.1‡	1.6 ± 0.13	0.8 ± 0.13†‡	-1.2 ± 0.09	-0.7 ± 0.09†‡
Week 16	91.5 ± 3.1	97.2 ± 2.8†‡	1.6 ± 0.13	0.9 ± 0.12†‡	-1.2 ± 0.09	-0.8 ± 0.08†‡

* Means ± standard error of the mean (SEM). † Significantly ($P < 0.05$) different compared to the untreated pelvic limb. ‡ Significantly ($P < 0.05$) different compared to baseline, presurgical values.

TABLE 3 – Treated and untreated pelvic limb craniocaudal force and impulse data post MRFE surgery and CCL rupture.* (n = 5)

Time Points	Peak Forces (% body weight)				Impulse (N * Sec)			
	Braking		Propulsion		Braking		Propulsion	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
Baseline	-7.6 ± 1.3	-6.2 ± 1.3	12.1 ± 1.4	11.6 ± 1.4	-0.23 ± 0.08	-0.16 ± 0.08	0.89 ± 0.10	0.87 ± 0.10
Post MRFE								
Week 2	-6.0 ± 1.3‡	-6.1 ± 1.3	11.0 ± 1.5	11.5 ± 1.5	-0.22 ± 0.08	-0.20 ± 0.08	0.84 ± 0.11	0.87 ± 0.11
Week 4	-5.6 ± 1.3‡	-5.7 ± 1.3	12.4 ± 1.5	12.1 ± 1.5	-0.17 ± 0.08	-0.11 ± 0.08	0.99 ± 0.11	0.98 ± 0.11
Post CCL Rupture								
Week 4	-4.7 ± 1.4‡	-2.5 ± 2.3†‡	13.8 ± 1.6	6.3 ± 1.5†‡	-0.14 ± 0.08	-0.06 ± 0.08‡	1.20 ± 0.13‡	0.57 ± 0.11†‡
Week 8	-5.9 ± 1.3‡	-3.0 ± 1.3†‡	13.1 ± 1.5	8.0 ± 1.5†‡	-0.21 ± 0.08	-0.05 ± 0.08‡	1.12 ± .011	0.70 ± 0.11†
Week 16	-5.5 ± 1.3‡	-3.3 ± 1.3†‡	11.8 ± 1.5	7.7 ± 1.4†‡	-0.23 ± 0.08	-0.10 ± 0.08	0.99 ± 0.11	0.63 ± 0.10†‡

* Means ± standard error of the mean (SEM). † Significantly ($P < 0.05$) different compared to the untreated pelvic limb. ‡ Significantly ($P < 0.05$) different compared to baseline, presurgical value.

TABLE 4 – Treated and untreated pelvic limb craniocaudal time to peak vertical force data post MRFE surgery and CCL rupture.* (N = 5)

Time Points	Time to Peak (msec)			
	Braking Time to Peak		Propulsion Time to Peak	
	Untreated	Treated	Untreated	Treated
Baseline	15.9 ± 2.6	14.3 ± 2.6	121.1 ± 7.4	121.6 ± 7.4
Post MRFE				
Week 2	17.0 ± 3.0	16.8 ± 3.0	125.6 ± 7.8	128.1 ± 7.8
Week 4	16.2 ± 3.0	14.9 ± 3.0	125.2 ± 7.8	123.2 ± 7.8
Post CCL Rupture				
Week 4	13.6 ± 3.4	13.8 ± 3.0	131.4 ± 8.1	114.9 ± 7.8†
Week 8	19.3 ± 3.0	13.8 ± 3.0	138.5 ± 7.7	125.9 ± 7.7†‡
Week 16	22.6 ± 3.0	17.7 ± 2.6	139.5 ± 7.7	132.4 ± 7.4‡

* Means ± standard error of the mean (SEM). † Significantly ($P < 0.05$) different compared to the untreated pelvic limb. ‡ Significantly ($P < 0.05$) different compared to baseline, presurgical values.

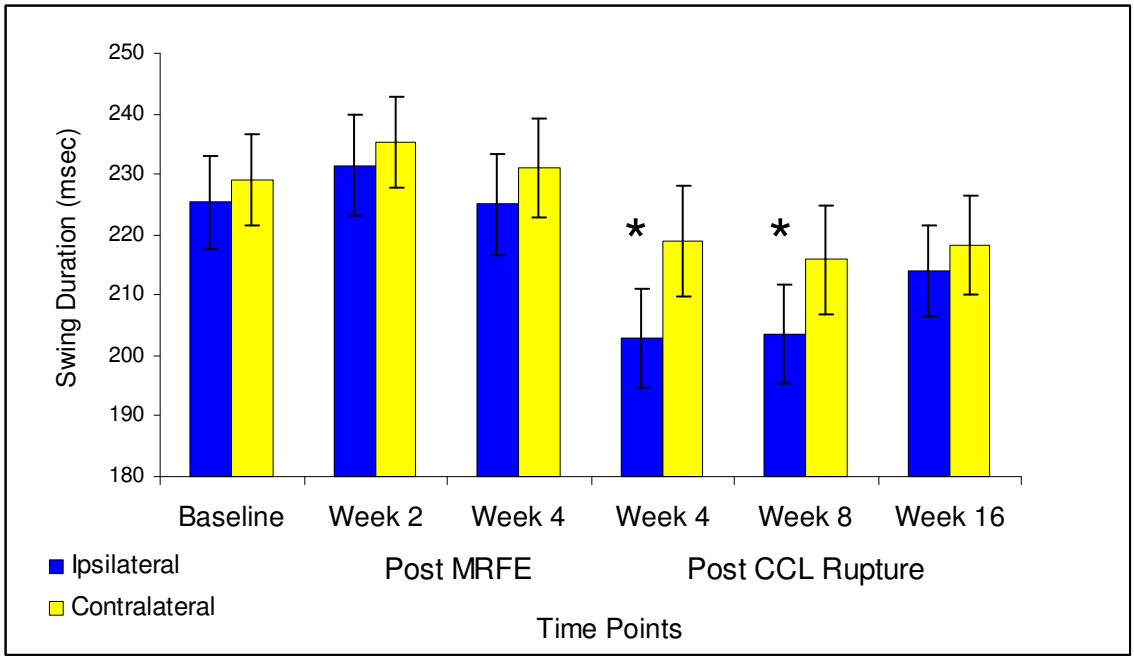

Figure 4 – Swing duration (msec) for the ipsilateral and contralateral thoracic limb at all time points. The ipsilateral thoracic limb at 4 (203 ± 8; P = 0.02) and 8 weeks (204 ± 8; P = 0.02) post CCL rupture significantly differed compared to baseline (225 ± 8) * Significant differences exist compared to baseline values.

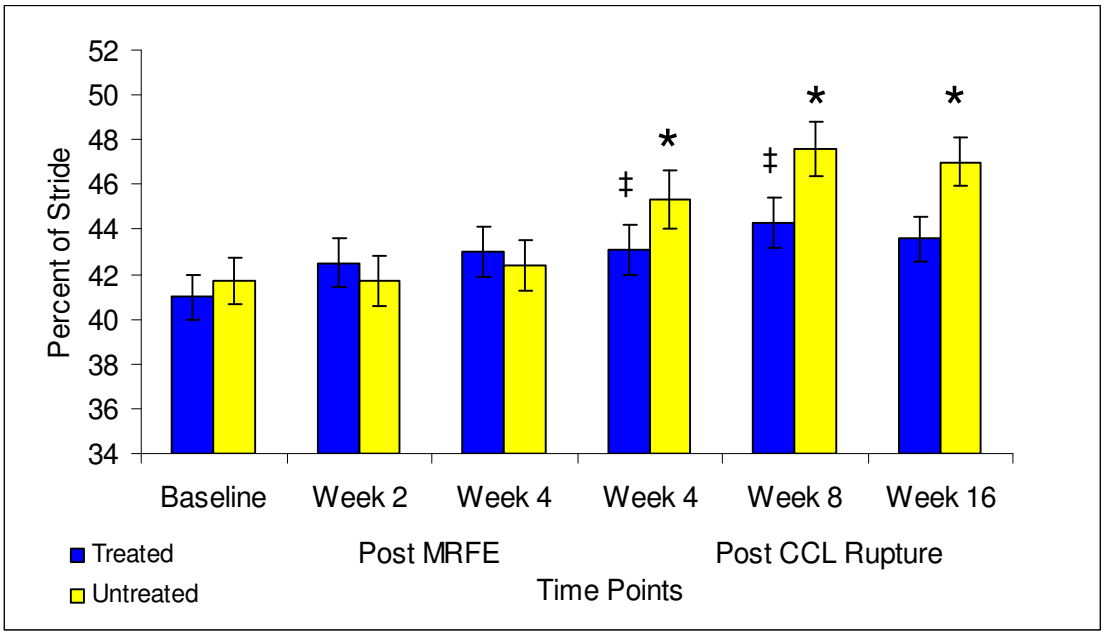

Figure 5 – The percentage of stride for the treated and untreated pelvic limbs during stance phase. ‡ Significantly increased at 8 and 16 weeks post CCL rupture for the treated limb, compared to baseline values. * Significant differences compared to baseline values.

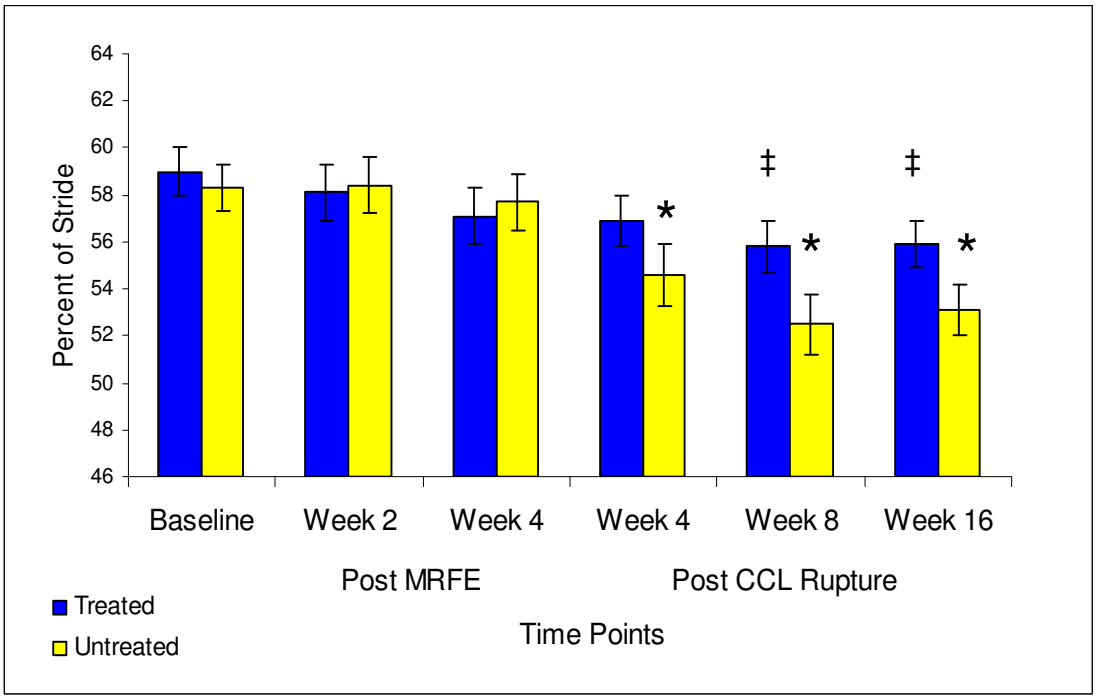

Figure 6 – The percentage of stride for the treated and untreated pelvic limbs during swing phase. ‡ Significantly decreased at 8 and 16 weeks post CCL rupture in the treated limb, compared to baseline values. * Significant differences compared to the baseline values.

Figure 7 – Trial averaged femorotibial joint angles in the treated pelvic limb. The solid black vertical line indicates the transition between stance and swing phase of the stride. With a decrease of 7° in the stifle flexion angle, a significant difference was found at 8 weeks post CCL rupture, compared to baseline values. '2M' and '4M' refers to the number of weeks post MRFE surgery, prior to CCL rupture. '4C', '8C', and '16C' refers to the number of weeks post CCL rupture.

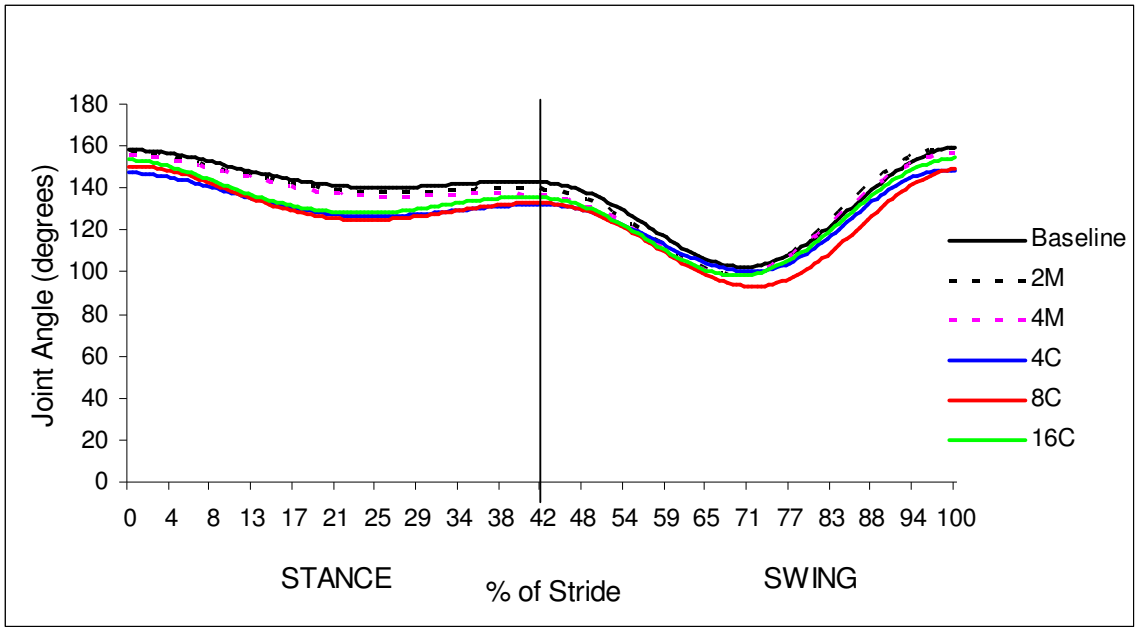

Figure 8 – Trial averaged femorotibial joint angles in the untreated pelvic limb. The solid black vertical line indicates the transition between stance and swing phase of the stride. A significant decrease ($P < 0.0018$) of up to 15° was shown at all time points post CCL rupture, compared to baseline values. '2M' and '4M' refers to the number of weeks post MRFE surgery, prior to CCL rupture. '4C', '8C', and '16C' refers to the number of weeks post CCL rupture.

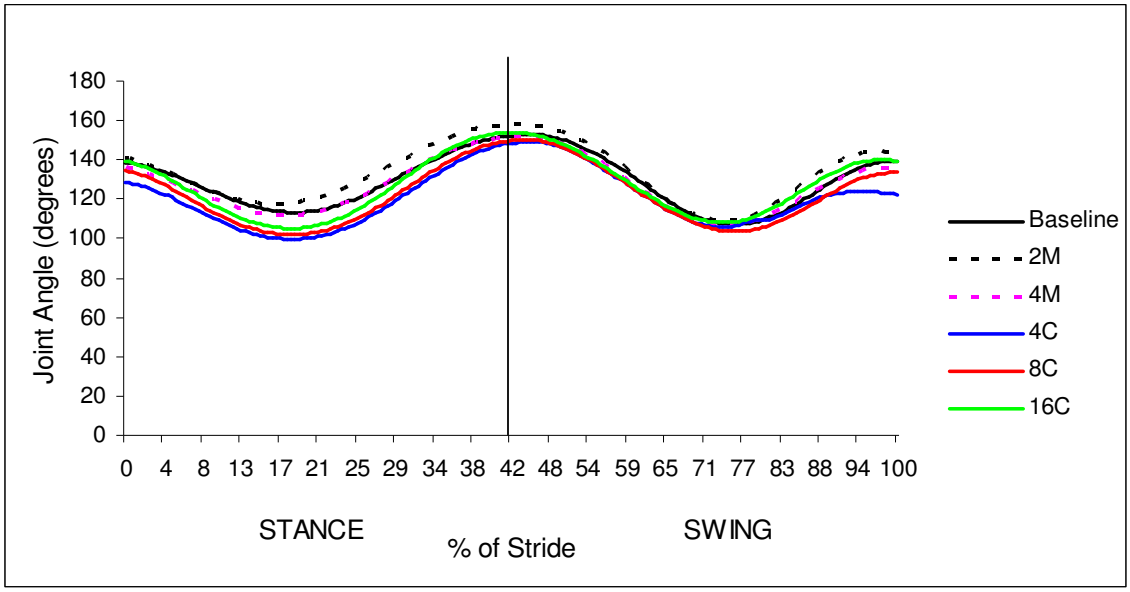

Figure 9 – Trial averaged tarsal joint angles in the untreated pelvic limb. The solid black vertical line indicates the transition between stance and swing phase of the stride. Though decreased by 8° at 4 weeks post CCL rupture (P = 0.1218) and decreased by 7° at 16 weeks post rupture (P = .1925), tarsal joint angles in the untreated limb during stance phase significantly decreased (P = 0.0493) by 10° only at 8 weeks post CCL rupture when compared to baseline values. Significant decreases in tarsal joint angles by up to 10° were seen in the untreated limb during swing phase at 4 (P = 0.0458) and 8 (P = 0.0037) weeks post CCL rupture. '2M' and '4M' refers to the number of weeks post MRFE surgery, prior to CCL rupture. '4C', '8C', and '16C' refers to the number of weeks post CCL rupture.

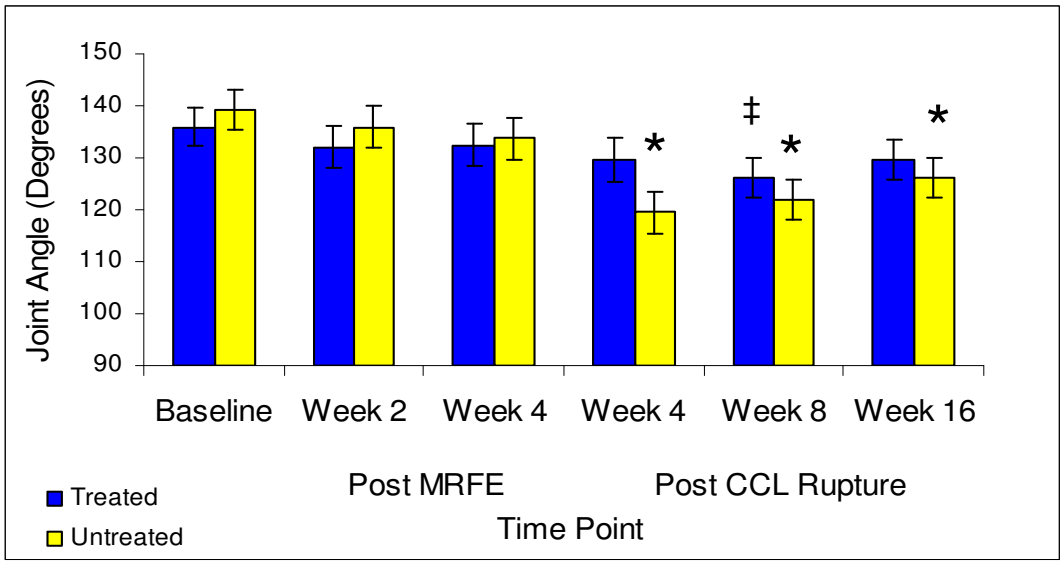

Figure 10 - Minimal femorotibial joint angle during stance phase at all time points.
* Significant decrease of up to 19° in the untreated limb compared to baseline at all points post CCL rupture. The femorotibial joint angle in the treated limb significantly decreased by 9° at 8 weeks post CCL rupture. ‡ Significantly different compared to baseline values.

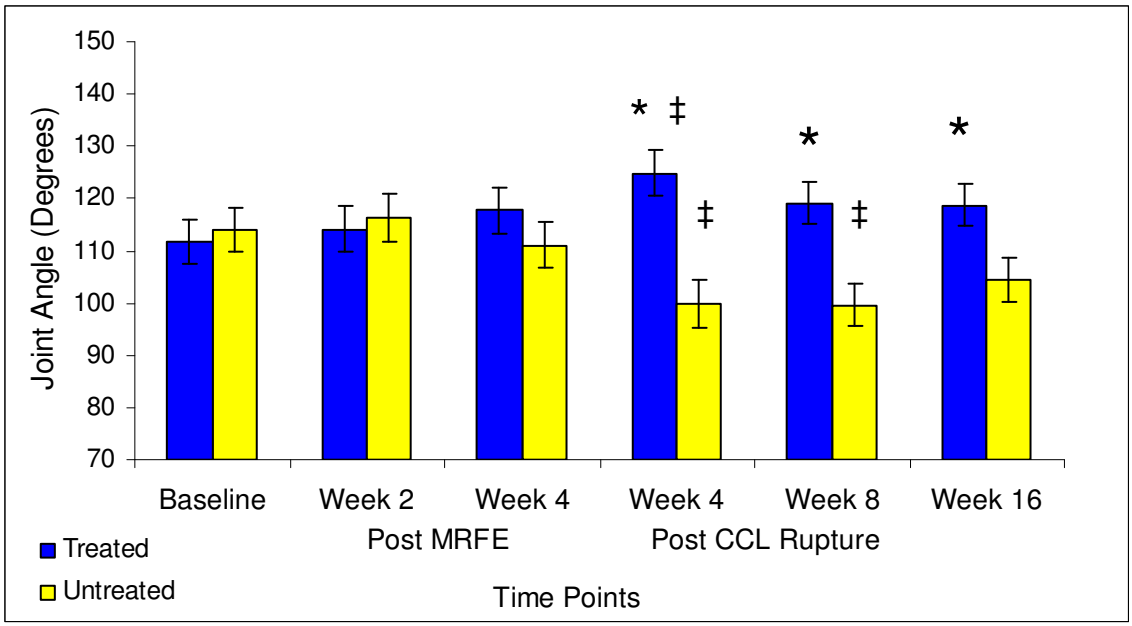

Figure 11 – The minimal tarsal joint angle during stance phase at all time points. Increase in the tarsal joint extension angle of 25°, 19°, and 14° at 4, 8 and 16 weeks post CCL rupture (CCLR) in the treated pelvic limb, compared to the untreated pelvic limb. * Significance was shown at Week 4 post CCLR in the treated limb, when compared to baseline with an increase in the tarsal joint extension angle of 13°. Significance was found at Week 4 and 8 in the untreated pelvic limbs post CCLR, both with an increase in the tarsal joint flexion angle of 14° when compared to the baseline untreated limb value. Significantly different compared to the untreated limb. ‡ Significantly different compared to their respective baseline values.

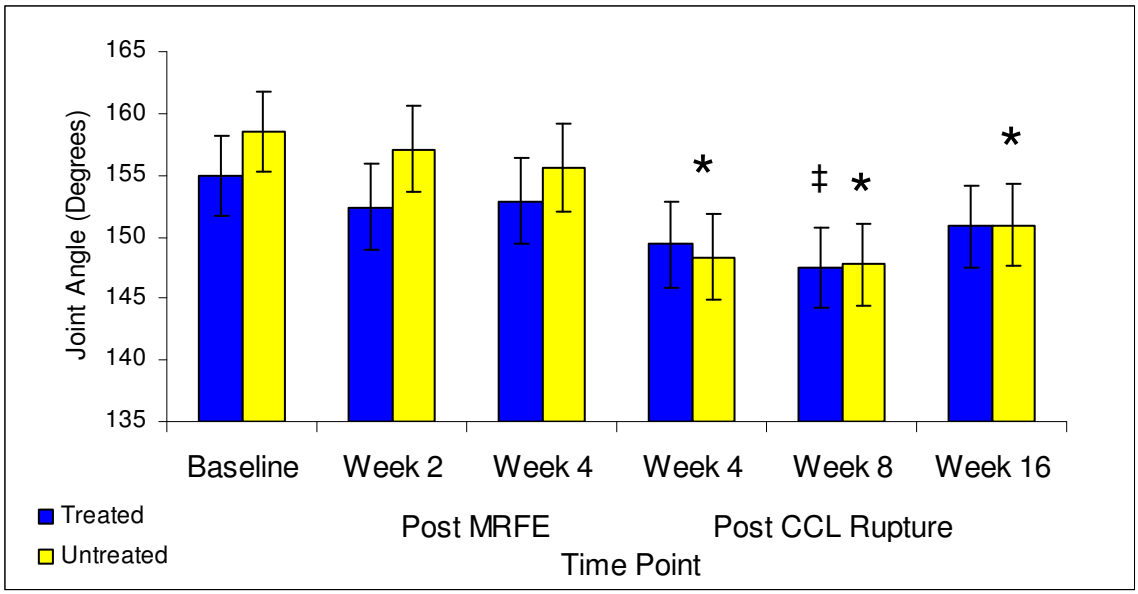

Figure 12 - Maximal femorotibial joint angle during stance phase at all time points. * Significantly decreased from baseline at all time points post CCL in the untreated limb; whereas, the treated femorotibial joint significantly decreased from baseline at 8 weeks post CCL rupture. ‡ Significantly different compared to their respective baseline values.

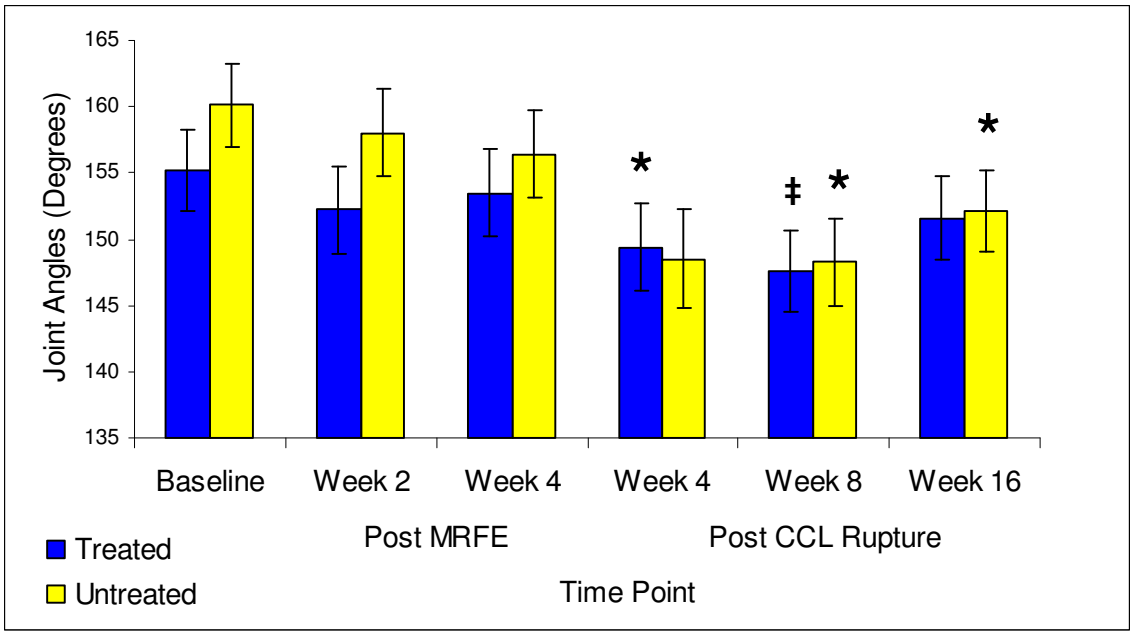

Figure 13 - Maximal tarsal joint angle during swing phase at all time points. * The maximal tarsal joint angle in the untreated limb significantly decreased from baseline during swing phase at all time points post CCLR; whereas, the treated femorotibial significantly decreased from baseline at 8 weeks post CCLR. ‡ Significantly different compared to their respective baseline values.

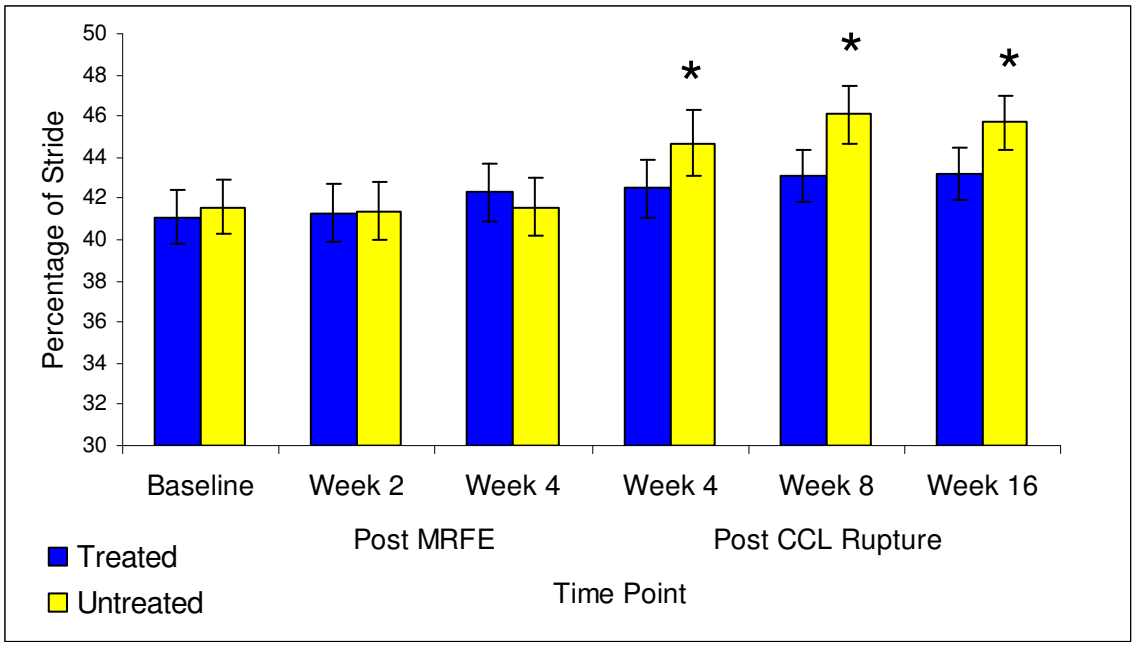

Figure 14 – Percent of stride for the tarsal joint during stance phase at all time points. * Significantly increased from baseline at 4, 8, and 16 weeks post CCL rupture. Significantly different compared to the untreated limb.

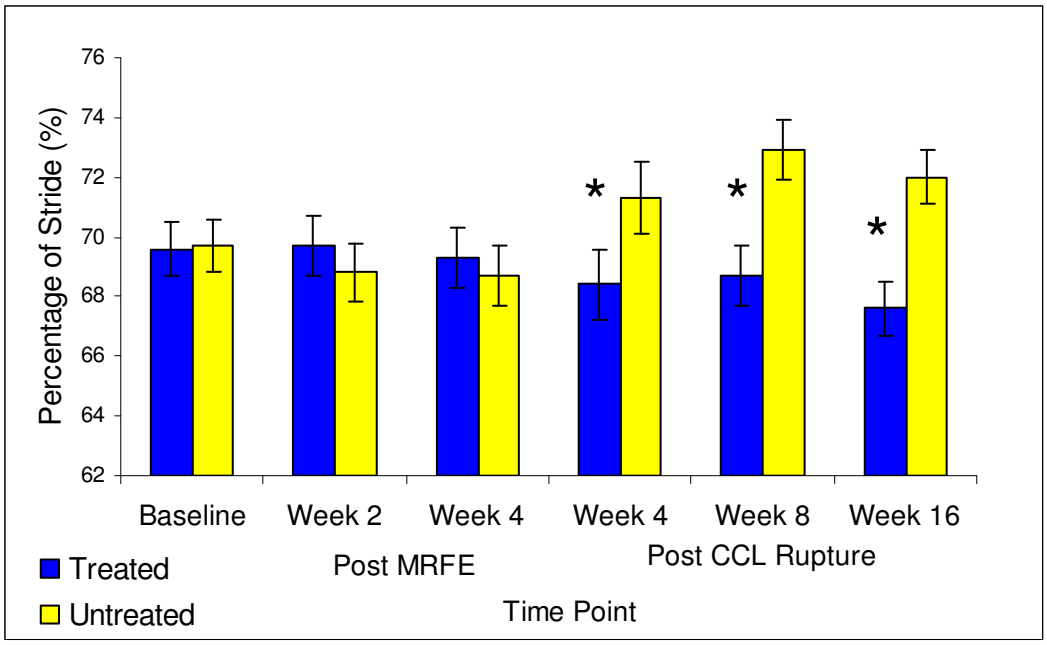

Figure 15 – Percent of stride for the minimal femorotibial joint during swing phase at all time points. * Significantly increased by up to 4% in the untreated limb for all time points post CCL rupture, compared to the treated limb. Significantly different compared to the untreated limb.

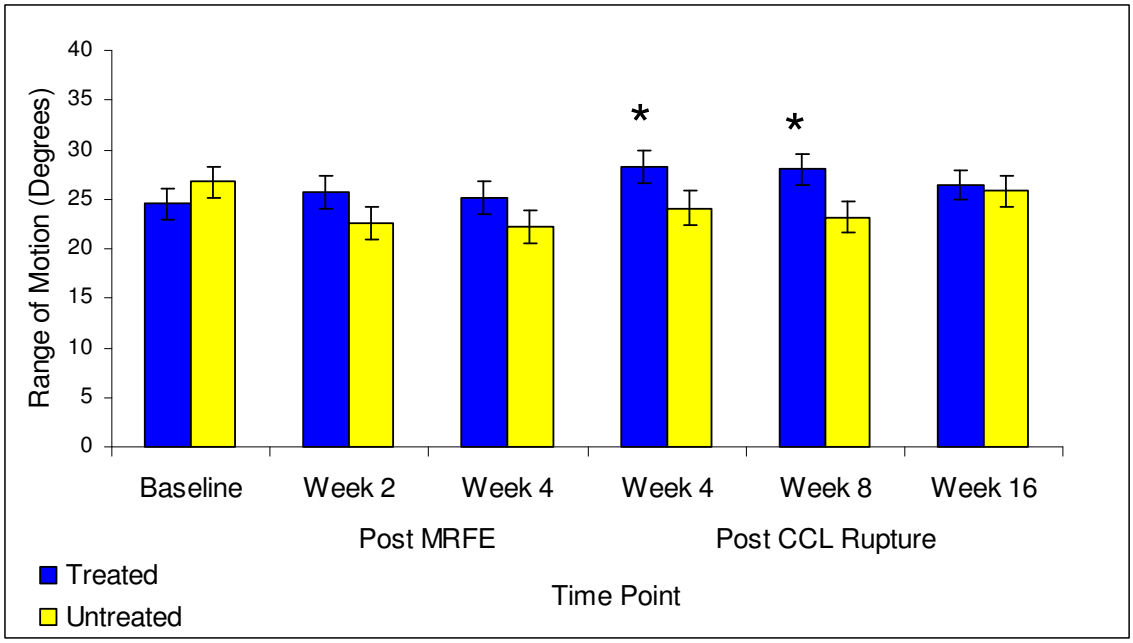

Figure 16 – Mean range of motion for the coxofemoral joint during stance phase at all time points. * Significant increase in the treated limb at 4 and 8 weeks post cruciate rupture, compared to the untreated. Significantly different compared to the untreated limb.

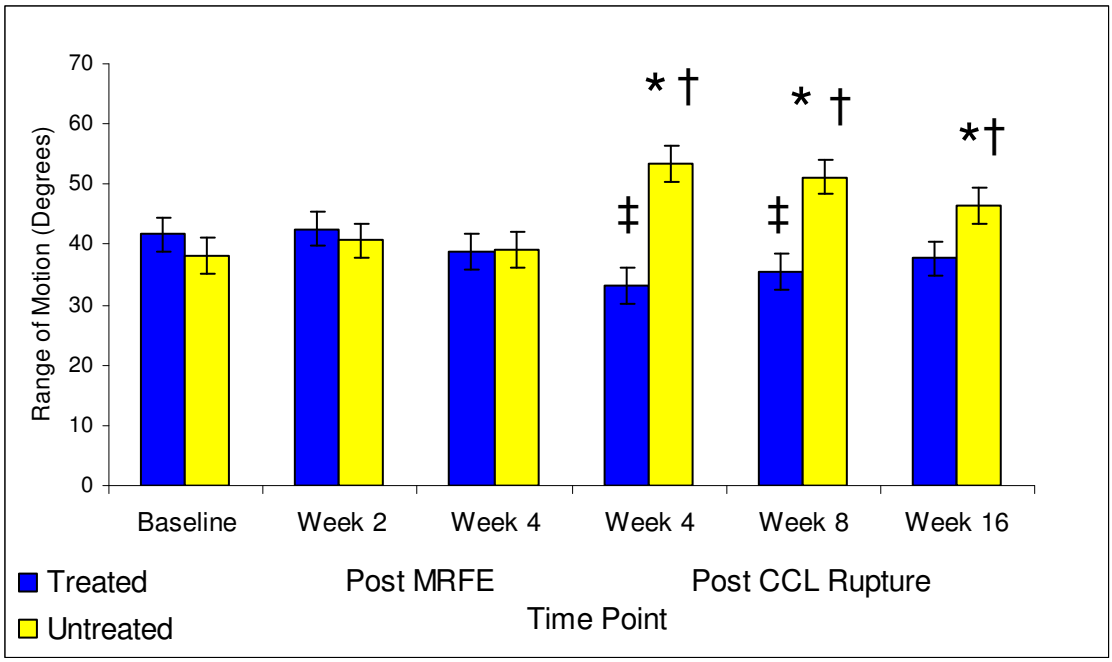

Figure 17 – Mean range of motion (ROM) for the tarsal joint during stance phase at all time points. * Significant increase in the untreated limb at 4, 8 and 16 weeks post cruciate rupture, compared to the treated contralateral pelvic limb. All ROM values in the untreated limb post CCL rupture significantly increased from baseline; whereas, the treated pelvic limb ROM significantly decreased at 4 and 8 weeks post CCLR compared to its respective baseline value. * Significantly different compared to the treated limb. †, ‡ Significantly different compared to their respective baseline values.

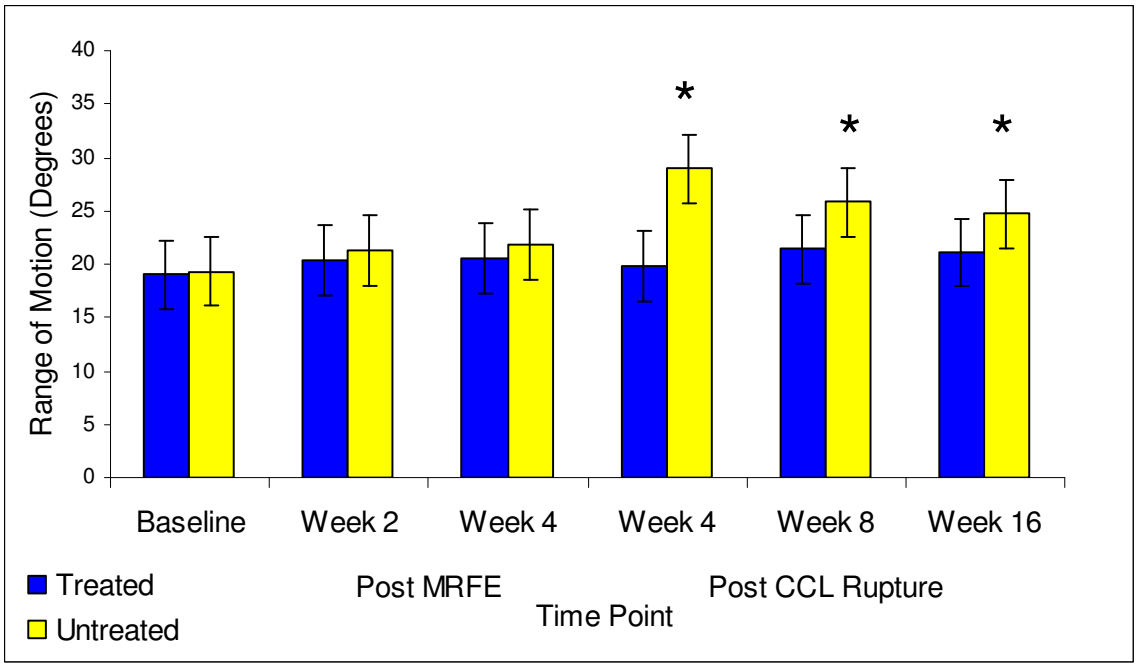

Figure 18 – Mean range of motion for the femorotibial joint during stance phase at all time points. * Significantly increased at all time points post CCL rupture, compared to the baseline values. * Significantly different compared to baseline values.

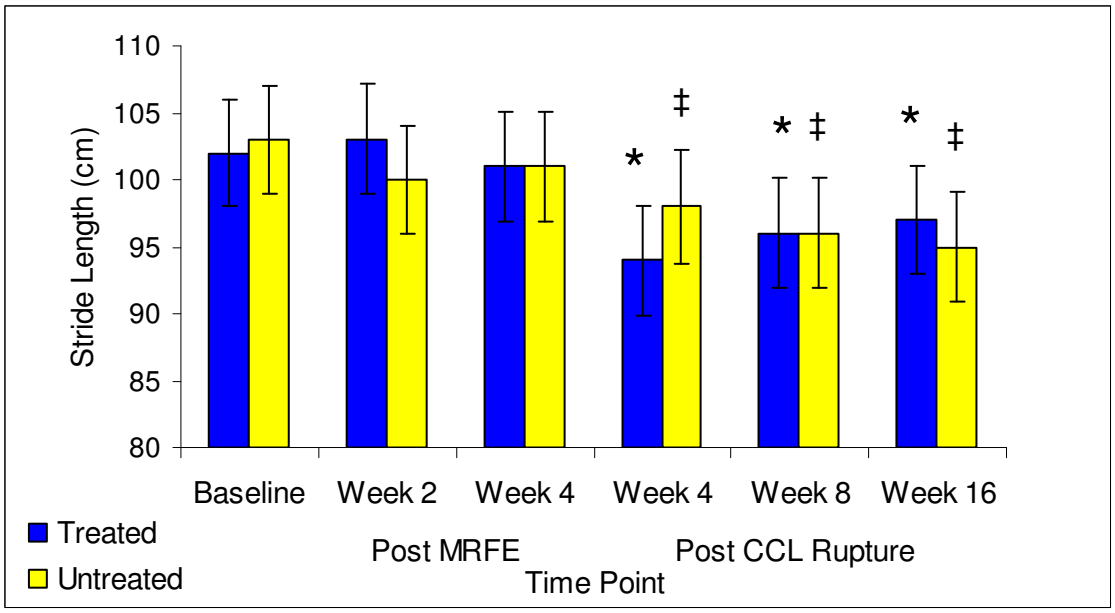

Figure 19 – Mean thoracic limb stride length (cm) at all time points. * Significantly decreased in the treated limbs at all time points post CCL rupture compared to baseline values. ‡ Significantly different compared to baseline values.

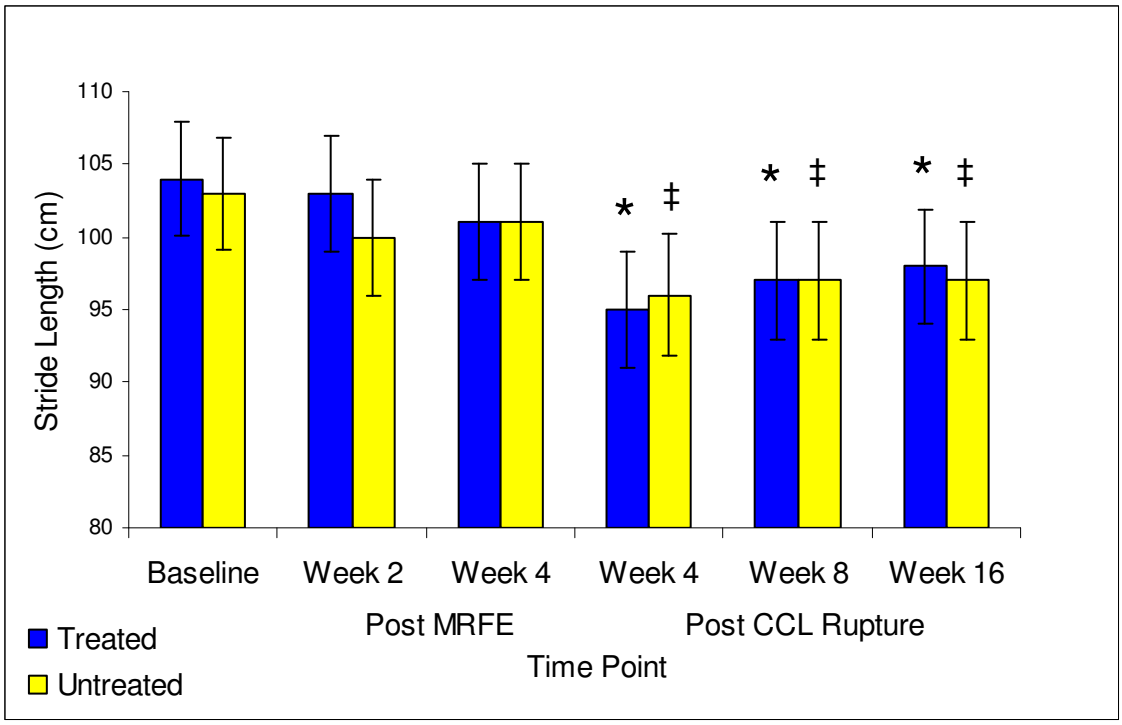

Figure 20 – Mean pelvic limb stride length (cm) at all time points. * Significantly decreased at all time points post CCL rupture in the treated limbs compared to baseline values. ‡ Significantly different compared to baseline values.

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CHAPTER 3

ELECTROMYOGRAPHY OF STIFLE STABILIZING MUSCLES IN SUBCLINICAL, ACUTE AND CHRONIC CRANIAL CRUCIATE LIGAMENT DISEASE

INTRODUCTION

Kinetic and kinematic gait analysis is frequently used to describe external forces and kinematics of normal and abnormal canine locomotion. However, dynamic EMG measures the activation of the muscle itself. In normal stifle function, muscles play a substantial role in providing joint mobility and stability.[1,2] Muscles contribute considerably to the load imposed on the knee and to joint stiffness caused by contraction of the surrounding muscle which is required to maintain stability.[2,3] Flexor and extensor muscles at the knee function together to produce knee joint movement and loading forces. These applied muscle forces are a major contributor to knee stiffness.[5] Soft tissue restraints, such as menisci, cruciate and collateral ligaments, contribute to stability of the knee when mild to moderate loads are applied.[3,4] Excessive loads applied during vigorous activities, such as stopping and sudden turning, may exceed the intrinsic strength of these tissues.[3] Activated individually, simultaneously, or co-contracted, the normal agonist and antagonist muscle activation produces increased joint compression.[5]

A synergistic relationship exists between the CCL, quadriceps, gastrocnemius and biceps femoris to provide mobility and stability to the canine stifle.[6,7] Cranial

thighmuscles (i.e., stifle extensors) and the gastrocnemius (stifle flexor and tarsal extensor) must be active to prevent stifle flexion and pelvic limb collapse. Whereas, the caudal thigh muscles (i.e., stifle flexors) act to stabilize the stifle by pulling the proximal tibia caudally (preventing cranial tibial translation) and extending the hip to create forward propulsion.[6] One contributing factor to CCL disease is purported to be due to a dynamic imbalance of muscle timing, coordination, forces, and levels of activation surrounding the stifle.[8]

During locomotion, antagonist activation acts to compress the surfaces of the femur and tibia, increasing knee joint stiffness and contributing to coordinated muscle activity required for functional joint stability.[5] Microklutziness, or "micro-incoordination of neuromuscular control not visible to the naked eye", has been hypothesized to represent repetitive impulse loading, which can cause joint damage due to the accumulation of microtrauma.[9] Neuromuscular control of limb motion must occur in a timely and appropriate manner for normal gait to occur. Microklutziness involves decreased ability to control deceleration of a limb and to absorb shock, both of which can contribute to the development of OA.[9]

One technique for investigating neuromuscular function and dysfunction is electromyography (EMG), the measurement of electrical signals produced with a muscle contraction. A muscle contraction is caused by depolarization of a muscle fiber. An action potential propagates along a motor neuron, it activates the muscle fibers of that motor unit at the neuromuscular junction.[10] The post-synaptic membrane of the muscle fiber is depolarized through movement of ions, which generates an electromagnetic field

in the vicinity of the fibers.[10] An EMG recording electrode will detect this voltage change (or potential), which is amplified and recorded.

Interpretation of EMG provides insights into alterations in muscle activity such as muscle activation onset, duration and amplitude. EMG can be used to assess muscle activity during tasks with different biomechanical requirements, such as gait with stability and instability of the stifle.[11] The vastus lateralis, biceps femoris and gastrocnemius muscles were chosen to assess EMG due to their significant role in stifle motion and stability.[7,12] Under normal conditions at a trot, the vastus lateralis becomes active prior to paw strike in an effort to stabilize the stifle and remains active for 70% of the stance phase (32% of the stride) during middle and late stance.[7,13] At the end of the swing phase of gait, the vastus lateralis acts as a stifle extensor in preparation for paw strike.[13] The gastrocnemius, a stifle joint flexor and tarsal joint extensor[7], becomes active after paw strike and remains active for 73% of the stance phase (33% of the stride) at a trot to stabilize the stifle and to keep the tarsus from collapsing.[7] Normally, during stance phase, the combined action of the quadriceps group (stifle extensor) and gastrocnemius (stifle flexor) provide controlled stability to the stifle joint.[7,12] The cranial head of the biceps femoris assists coxofemoral and femorotibial joint extension when the pelvic limb is weight bearing.[7,14] When unweighted, the cranial head of the biceps femoris is also capable of flexing the stifle.[7] The cranial head of the biceps femoris becomes active prior to paw strike (25% of swing phase) in an effort to decelerate the limb's forward movement and remains active for 46% of stance phase (34% of stride) at a trot to stabilize the stifle in extension during stance.[7] At a trot, the caudal portion of the biceps

femoris acts to retract the pelvic limb after stifle stability has occurred[12] and flexes the stifle during the swing phase.[15]

Identifying abnormal EMG characteristics associated with the onset and progression of CCL disease could provide insight into maximizing the effectiveness of surgical and nonsurgical treatments for CCL injury and rupture. By recording activity of muscles via EMG, potential alterations in coordinated muscle activity may be determined. The purpose of this project is to characterize alterations in EMG activity patterns within ipsilateral and contralateral pelvic limbs following unilateral monopolar radiofrequency energy (MRFE)-induced CCL degeneration and subsequent rupture. We hypothesize that significant alterations in muscle onset, activation duration and amplitudes will be measured in the vastus lateralis, biceps femoris and gastrocnemius at all time points post-injury and rupture, compared to baseline values within the treated limb.

MATERIALS AND METHODS

Study Design

This study was approved by the Institutional Animal Care and Use Committee at Colorado State University and carried out at the Orthopaedic Research Center. This is a randomized, repeated-measures design that assessed changes in EMG parameters in bilateral pelvic limbs with induced unilateral CCL degeneration and subsequent rupture of 6 dogs at baseline, during subclinical CCL degeneration (2 and 4 weeks post MRFE-induced CCL injury), acute (4 weeks post confirmed CCLR), intermediate (8 weeks post CCLR), and chronic phases of CCLR (16 weeks post CCLR).

Dogs

Six female, purpose-bred, hound dogs free of orthopedic and neurologic disease on initial physical and radiographic examination were studied. Dog age ranged from 1.0 to 3.7 years (mean ± SD; 1.6 ± 1.0 years) and weighed 15.7 to 29.0 kg (mean 22.2 ± 4.9 kg). They were uniformly maintained with free choice food and water access and housed in individual 1.2 x 2.4 m runs for the duration of the study. Thirty minutes of unrestrained indoor group exercise was allotted for each dog daily; otherwise, dogs were taken out of their runs for gait evaluation and testing and for outcome parameter testing only.

Training and acclimation involved exposing each dog to EMG data collection procedures, including donning of an adjustable body harness[a] to secure the EMG telemetry equipment[b] to during data collection. Monopolar radiofrequency energy (MRFE) induction of CCL injury and kinetic and kinematic parameters used in this study are described in Chapter 2 (pp. 54-59).

Surface EMG data were collected using a 16-channel Noraxon TeleMyo 2400 G2 telemetry electromyography unit[b] from bilateral pelvic limb muscles, specifically the vastus lateralis, gastrocnemius, and caudal portion of the biceps femoris. The mid-portion of the muscle bellies were identified via palpation and hair was removed with a #40 clipper blade and shaved with a single-blade disposable razor. The skin was cleaned and degreased with an isopropyl alcohol-soaked gauze pad to reduce electrical impedance. Cyanoacrylate glue was used to adhere the surface EMG electrodes to the skin. Paired electrodes[c] were positioned 2.54 cm apart over the mid-portion of each muscle belly. The EMG ground electrode was a single 3.8 cm diameter surface electrode placed cranial to the greater trochanter of the right femur. A superficial tracing on acetate

sheets was made marking the location of each electrode prior to removal to ensure similar placement of surface electrodes across collection sessions.

A modified snug-fitting harness[a] was placed over the thoracic and pelvic girdle of each dog to secure the EMG telemetry unit and pre-amplified cables (Figure 1). EMG data collection was synchronized and collected simultaneously with kinematic data using 8 infra-red cameras (Motus 9.0, Vicon Motion Systems, Inc., Centennial, CO) and kinetic data using 3 serial force platforms (AMTI, model OR-6-6, Watertown, MA), as described in Chapter 2. Each dog was trotted down a 10-m runway at a consistent velocity (2.0 – 2.4 m/sec) on a loose leash. EMG data was pre-amplified and collected at 2,000 Hz for 3, 10-second trials. The raw EMG signal was filtered using a high-pass filter at 120 Hz to remove any motion artifacts, zero-meaned, full wave rectified, and low-pass filtered at 12 Hz. EMG data were further processed using Motus software (Vicon Motion Systems, Inc., Centennial, CO) to determine average standing muscle activity and muscle onset, percent of stride of muscle onset, activation duration, and percentage of the total stride for the activation duration, and mean amplitudes for each pelvic limb muscle during one stride at a trot.

Pilot data revealed that muscle activity collected in a lateral recumbency, antigravity position was too low to determine thresholds for muscle onset and offset. Thus, EMG data was collected in standing which required the dog to stand square with both thoracic limbs on one force platform and both pelvic limbs on an adjacent force platform for 3 consecutive, 10-second trials. Three seconds of still data were selected from the 10 seconds and analyzed to determine average standing EMG activity at all time points. Since performing a maximum voluntary contraction in a dog is not possible, the

average standing EMG values at baseline and their respective standard deviations were used to determine muscle onset and offset in trotting trials by multiplying each baseline standard deviation by a multiple of 1 to 6 and adding the baseline standing mean for each individual muscle at each time point. Muscle activation tracings depicting examples of this analysis method for the vastus lateralis at baseline, post MRFE and post CCLR for one representative dog are shown in Figures 2-4. The number of standard deviations used to determine muscle onset and offset were 4, 6, and 4 standard deviations for the vastus lateralis, biceps femoris and gastrocnemius, respectively. Muscle onset times were reported as the average of all dogs combined. Muscle activations of less than 50 msec in duration were not considered when determining muscle onset and offset. Muscle onset times were calculated as a percentage of the total stride duration. Negative time values indicated that the muscle activated prior to paw strike, while positive time values indicated that the muscle activated after paw strike for the stride. Mean results were graphed as the trial average of pooled values across dogs.

Stride duration was calculated as the time from paw strike to subsequent paw strike of the same limb (foot contact). Muscle activation durations were determined as the percentage of the total stride. Peak EMG amplitudes normalized to baseline were reported per stride at each time point. Of the six dogs, one dog was eliminated from EMG analysis, as she would only ambulate with a hopping gait and would not trot as required.

Statistical Analysis

A mixed model, repeated-measures analysis of covariance (ANCOVA) was performed to assess differences in EMG parameters between the treated and untreated

pelvic limbs in 5 dogs over six time periods. Independent variables include age, surgical limb (right vs. left) and time factors (normal, subclinical, acute and chronic) related to MRFE surgery. Dependent variables include EMG outcome variables. Least square means were used for individual comparisons among the interactions of time * treated limb. The covariate of 'weeks to rupture' was included in all analyses and defined as the dog sustaining a CCLR within first week post operative (early = '0'); CCLR at 6 weeks post-op (mid = '1'); CCLR occurring greater than 8 weeks post surgery (late = '2') (See Chapter 2). Significant differences were detected at $p \leq 0.05$.

RESULTS

Static EMG during Stance

Standing EMG mean muscle activity (i.e., EMG recordings while the dog was standing) were significantly decreased in the vastus lateralis of the untreated pelvic limb compared to the treated limb at 8 and 16 weeks post CCL rupture, as well as when compared to their respective baseline values (Figure 5). There was no significant difference found in the standing EMG means for the biceps femoris or gastrocnemius muscles.

Dynamic EMG at a Trot

No significant difference was found for any EMG parameters, including muscle onset as a percentage of stride, activation duration, activation duration as a percentage of stride, or peak amplitude normalized to baseline values, at any time point in this study (Tables 1-3). High variability in EMG data and a low sample size may have resulted in or partially resulted in non-significance, thus the following comments and results are based on qualitative descriptions of graphic representations of the data.

Qualitative Descriptions of Muscle Activity Patterns

Trial Averaged EMG

In the treated limb, it appears that a non-significant delay in activation post MRFE occurred in the vastus lateralis, biceps femoris, and gastrocnemius muscles, with a decrease in activation duration (Figures 6-11). An interesting observation is the loss of a second smaller activation post MRFE in the biceps femoris and gastrocnemius muscles during the swing phase of gait (Figures 8 and 10, respectively). Post CCLR, it appears that these three muscles in the treated limb are activated earlier than baseline, with an increase in activation duration.

In the untreated pelvic limb post MRFE, it appears that there was no change in muscle onset for the vastus lateralis compared to baseline, as it is more difficult to visually determine, and no difference in activation duration appears to exist (Figures 12-17). However, muscle activation appears to occur earlier than baseline in the biceps femoris and gastrocnemius muscles with a slight decrease in activation duration in the biceps femoris and concomitant increase in activation duration in the gastrocnemius (Figure 14 and 16, respectively). Post CCLR, a delay in vastus lateralis onset appears to occur at 4 and 8 weeks post CCLR, with the muscle activating earlier than baseline at 16 weeks post CCLR and increased activation durations at all three time points (Figure 13). An early onset appears to exist in the biceps femoris and gastrocnemius muscles, with a concomitant increase in activation duration (Figure 15 and 17, respectively).

Individual Muscle EMG Activation Pattern Descriptions

The range of outcome data from individual dogs that compose the trial averaged data is discussed in more detail below.

Vastus Lateralis

In the treated limbs at baseline, muscle activation occurred immediately after paw strike (1% of the stride, on average) and remained active for an average of 37% of the stride. During subclinical time periods at 2 and 4 weeks post MRFE and prior to CCL rupture, muscle activation occurred after paw strike, between an average of 5-6% of the stride and remained active for 33% of the stride, on average. In the treated pelvic limb post CCLR, the vastus lateralis activated up to 2% prior to paw strike for the stride and remained active for 39-44% of the stride. The single burst of muscle activity at baseline and post MRFE also appears to change to a multiple burst of muscle activity post CCLR . In the untreated pelvic limb at baseline (pre-surgery), the vastus lateralis activated immediately after paw strike (1% of the stride) and remained active for 35% of the stride, on average. Post MRFE, the vastus lateralis activated 2-3% after paw strike, at 2-3% of the stride and remained active for an average of 35% of the stride. Post CCLR, the muscle activated up to 6% prior to paw strike and remained active for 39-44% of the stride.

Biceps Femoris

In the treated pelvic limb, muscle activation at baseline (pre-surgery) occurred at 13% prior to paw strike and remained active for an average of 46% of the stride. Post MRFE, the biceps femoris activated 6-11% prior to paw strike and remained active for 38-39% of the stride. Post CCLR, the muscle activated between 13-24% prior to paw strike and remained active for 49-57% of the stride. The single burst of muscle activity at baseline and post MRFE also appears to change to a multiple burst of muscle activity post CCLR. In the untreated pelvic limb, biceps femoris activation at baseline occurred

at 10% prior to paw strike and remained active for an average of 44% of the stride. Post MRFE, the biceps femoris activated 8-9% prior to paw strike and remained active for 38-43% of the stride. Post CCLR, the muscle activated between 4-9% prior to paw strike and remained active for 39-46% of the stride.

Gastrocnemius

In the treated pelvic limb, gastrocnemius muscle activation occurred 6% prior to paw strike and remained active for 44% of the stride at baseline. Post MRFE, muscle activation occurred an average of 3% prior to paw strike and remained active for 41-44% of the stride. Post CCLR, the muscle activated 7-9% prior to paw strike for the stride and remained active for 54-59% of the stride. The single burst of muscle activity at baseline and post MRFE also appears to change to a multiple burst of muscle activity post CCL. In the untreated pelvic limb at baseline, the gastrocnemius muscle activated an average of 1% prior to paw strike and remained active for 37% of the stride. Post MRFE, muscle activation occurred an average of 3% prior to paw strike and remained active for 38-41% of the stride. Post CCLR, the muscle activated 3-4% prior to paw strike and remained active for 48-50% of the stride.

Muscle Onset Time as a Percentage of Stride

Muscle onset timing (Table 1) did not show any significant differences at any time point for the vastus lateralis ($P = 0.94$), biceps femoris ($P = 0.17$) and gastrocnemius ($P = 0.54$). In the treated pelvic limb, data shows a non-significant delay in muscle onset post MRFE and an earlier muscle onset post CCLR in all three muscles. In the untreated pelvic limb, a non-significant delay in muscle onset appears to occur in the vastus lateralis and biceps femoris muscles, with an earlier activation in the gastrocnemius post

MRFE. Post CCLR, the untreated limb appears to show an earlier activation in the vastus lateralis and gastrocnemius, with a delay in biceps femoris muscle activation.

Activation Duration

No significant difference was found in activation duration for the vastus lateralis (P = 0.95), biceps femoris (P = 0.94) and gastrocnemius (P = 0.99), when comparing the treated and untreated limbs, or when comparing each limb to baseline values (Table 2).

Activation Duration as a Percentage of Stride

No significant difference was found in activation duration as a percentage of stride in the vastus lateralis (P = 0.88), biceps femoris (P = 0.52) or gastrocnemius (P = 0.93), when comparing the treated and untreated pelvic limbs, or when comparing each limb to baseline, pre-surgical values (Table 3). In the treated limb, the data appears to show a non-significant decrease in activation duration as a percentage of stride in the vastus lateralis and biceps femoris muscles, with no change in the gastrocnemius when compared to baseline values. Post CCLR in the treated limb, increased activation duration appears in all three muscles. In the untreated pelvic limb, no change in activation appears to occur post MRFE in the vastus lateralis, with a decrease in the biceps femoris and increase in activation duration in the gastrocnemius when compared to baseline (Table 3). Post CCLR, a slight decrease in activation duration appears to occur in the biceps femoris muscle, with an increase in the vastus lateralis and gastrocnemius muscles.

Normalized Peak Amplitudes

No significant difference was found in the peak amplitude at any time point (Table 4) in the treated and untreated pelvic limbs of the vastus lateralis (P = 0.93),

biceps femoris (P = 0.52) or gastrocnemius (P = 0.54). In the treated limb post MRFE, a non-significant decrease in the peak amplitude appears to occur in the vastus lateralis and biceps femoris, with an increase in the gastrocnemius. Post CCLR, peak amplitude appears to increase in the vastus lateralis, but decrease in the biceps femoris and gastrocnemius. In the untreated limb post MRFE, a decrease in peak amplitude appears to occur in the vastus lateralis, with inconclusive changes in the biceps femoris and vastus lateralis. Post CCLR, increased peak amplitude appear in the vastus lateralis and gastrocnemius, but decrease in the biceps femoris.

DISCUSSION

Qualitative descriptions of muscle activity patterns of normal pelvic limb muscles measured via fine wire EMG were first illustrated in 1973 by Tokuriki during the stance and swing phase of gait in dogs at a trot.[16] Additional publications followed that described the presence or absence of muscular activity, while no attempt was made to quantify the activity.[7,12,15] More recently, surface EMG was used to describe the activity pattern of the vastus lateralis muscle, in conjunction with ground reaction forces and kinematics, in dogs at a walk.[13]

Qualitative analysis of trial averaged graphs in our study show that CCL injury and subsequent rupture have perceived effects on neuromuscular function; however, no significant differences were found in muscle onset, activation duration or peak amplitudes between the treated and untreated pelvic limbs at all time points. Insignificant changes in EMG outcome parameters for all muscles were likely due to increased EMG variability and a small sample size, as a re-calculation of power utilizing the current

results revealed that a sample of 20 dogs is needed to detect a difference in muscle onset time between treated and untreated limbs.

Subjective observations at time points post CCLR appear to coincide with several significant kinetic and kinematic variables described in Chapter 2. We found that the vastus lateralis became active just after paw strike (~1%) and remained active for 35-37% of the stride at baseline. This observation contradicts Goslow's report stating that the vastus lateralis became active prior to paw strike to stabilize the stifle and remained active for 32% of the stride.[7] The small discrepancy in muscle onset of activity and activation duration may be due to less sophisticated equipment employed by Goslow and colleagues, which included an FM tape recorder to record EMG signals and analyzed the electrical activity for duration (on and off) only, by playing back tape recordings on a strip chart recorder, which limited more precise timing of muscle activity. However, our observation of vastus lateralis muscle timing did agree with that of Bockstahler et. al., who reported that the activity pattern of the vastus lateralis occurred at the beginning of stance as the pelvic limb accepts weight and stays active throughout the majority of stance phase.[13] Their study found a large correlation between vastus lateralis activity and ground reaction forces in 11 normal dogs at a walk, confirming that the vastus lateralis contributes to stifle stabilization during stance as the paw strikes the ground.[13]

In our work during static standing, vertical forces significantly decreased from baseline in the treated pelvic limb, while a compensatory increase in vertical ground reaction forces was found in the untreated limb, as discussed in Chapter 2. However, we found no increase in PVF in the untreated limb at a trot, while the PVF significantly decreased from baseline in the treated limb (Chapter 2). We suggest that the increased

load on the untreated pelvic limb post CCLR was attenuated by increasing the percentage of time that the limb was in stance phase. Biomechanically, this means that the momentum of the contacting limb results in an impulse that is produced more by time and less by force. Our values significantly increased post CCLR by up to 6% from baseline (Chapter 2), consistent with reports in the literature.[17] Earlier muscle onset timing would allow better shock absorption to absorb these higher loads in the untreated limb.[18] Post CCL rupture, the vastus lateralis muscle activated at 2-6% prior to paw strike in both the treated limb and remained active for up to 7% longer than baseline values. The muscle activates sooner and is then able to increase stiffness in preparation for weight acceptance in an unstable joint, as stiffening of the stifle joint due to muscular co-contraction is known to play a role in joint stabilization.[2,18] Hasler and colleagues reported that the contralateral pelvic limb was overloaded post CCL transection, as evidenced by an increase in vastus lateralis EMG magnitude and duration and gastrocnemius muscle and patellar tendon forces measured in vivo.[18]

In addition to increasing time in stance phase, reducing compensatory forces within the untreated limb may be achieved by increasing stifle flexion (as reported in Chapter 2) in the untreated limb post CCLR. With increased stifle flexion in weightbearing, stifle flexion moments are increased[17], which necessitate increased quadriceps activity to maintain trotting.[19] A trend towards increased muscle activity in the vastus lateralis in the untreated limb post CCLR supports this concept, though these results were not significant.

The cranial head of the biceps femoris produces coxofemoral and femorotibial joint extension when the limb is weight bearing.[7,14] Observations in our study appear

consistent with that of others at baseline and 2 and 4 weeks post MRFE surgery.[7,15,16] The cranial portion of the biceps femoris becomes active prior to paw strike to decelerate the limb's forward movement (slows the rate of knee extension) and remains active for 34% of stride at a trot which helps to stabilize the stifle in extension during stance phase.[7,15] Similarly, the medial gastrocnemius, a stifle joint flexor and tarsal joint extensor, is reported to activate prior to or at paw strike and remain active for 33% of the stride at a trot.[7,15,16] Our study observations appear similar in that the medial gastrocnemius activates between 1-6% prior to paw strike and remains active for 37-44% of the stride in the untreated and treated limb at baseline and 2 and 4 weeks post MRFE surgery.

Post CCLR, muscle onset timing and duration for the biceps femoris and gastrocnemius in the untreated limb appear similar to baseline, pre-surgical values. Time of onset for the biceps femoris muscle in the treated limb occurred twice as early prior to paw strike and remained active for up to 11% longer than baseline, in an attempt to stabilize the stifle. In the treated limb, gastrocnemius muscle onset appeared to occur slightly earlier (-7 to -9%) and remained active up to 17% longer than pre-rupture time points, also in an attempt to stabilize an unstable stifle joint. Activation of these stifle flexors are believed to occur in an effort to prevent the cranial displacement of the tibia relative to the femur.[20]

EMG amplitude is not a direct measure of the force of contraction, but rather an indirect measure of the intensity of muscle activity.[21] Injury to the CCL causes weakness in the stabilizing muscles of the stifle, which may be due to muscle atrophy and/or a reduced ability to activate the muscle.[22] Two and 4 weeks post MRFE, our data appear to

show a reduction in the percentage of peak amplitude in the vastus lateralis and biceps femoris of the treated limb, however the onset of muscle timing has not changed. These findings may suggest that a muscle weakness exists in the treated limb secondary to CCL injury. We propose that injury to the CCL causes muscle weakness, as evidenced by muscle atrophy significantly occurring at all time points post CCLR, as discussed in Chapter 4. This concept implies that if muscle weakness is the primary deficit, clinical attempts to identify muscle weakness should be investigated, with the aim of developing muscle strengthening program prior to CCLR.[22] However, if activation deficits are significantly proven to occur post CCLR, future research should direct the focus towards removing inhibitory sources that may prevent or delay muscle activation (e.g., joint effusion) and focus on neuromuscular retraining programs.[22]

Muscle weakness, specifically quadriceps muscle weakness, is considered to be a risk factor in the development of human knee OA.[23] As the primary anti-gravity muscle of the pelvic limb, the quadriceps acts to decelerate the limb during weight acceptance, minimizing impact forces.[23] Weakness may lead to increased impulse loading, predisposing the joint to OA development.[9] Our study showed a decrease in braking forces in the treated limb (Chapter 2), which occurred with vastus lateralis muscle weakness (i.e., reduced peak amplitude) observed post MRFE.

Muscle imbalance, characterized by incoordination and altered muscle firing patterns post CCLR may lead to muscle weakness, which contributes to contralateral CCLR and development of OA in bilateral pelvic limbs, as has been investigated in humans.[24] If muscular weakness is present, protective muscular activity that normally provides stability to the knee may be reduced, which could increase joint loading and

result in CCL rupture.[23] Our study may support the concept that muscle weakness occurred after injury to the CCL, and alterations in muscle activity visually observed post CCLR may contribute to CCLR and OA development in the contralateral limb.

Hasler hypothesized that following CCL transection, increased muscle forces and EMG activity will occur due to compensatory strategies used to stabilize the limb through muscle co-contraction.[18] However, they reported decreased muscle forces (i.e., gastrocnemius muscle and patellar tendon) and magnitude and duration of vastus lateralis EMG activity in dogs at a walk.[18] Our data are similar to reports in the literature that demonstrate a reduction in the magnitude of muscle activity in CCL-deficient limbs.[18] It appears that there was a decrease in peak amplitude in the biceps femoris and gastrocnemius post CCLR at a trot; with an increase in peak amplitude compared to baseline in the vastus lateralis of the untreated limb. This may also be explained as a compensation of the untreated limb to support the increased joint load in an effort to resist collapse of the pelvic limb.

Ragetly, et al. reported a three-fold increase in power generated at the stifle joint of the untreated limb, suggestive of an increased load on the contralateral limb which most likely means an increase in stifle extensor muscle activity to propel the limb forward.[17] Our results seem to show an increase in peak amplitude in the untreated vastus lateralis post CCLR, which coincide with the need for increased quadriceps activity. In humans, it was found that contraction of the quadriceps produced the greatest amount of strain in the anterior cruciate ligament between 15-25° of knee flexion.[25] At push off, with the stifle near full extension, this increased stifle extensor muscle activity may lead to an increase in cranial tibial thrust,[17,26] thought to contribute to the high incidence of

CCL pathology in the contralateral limb.[26,27] Not only does the magnitude of muscle activity appear to be reduced, but the single burst pattern of EMG during stance changes into a multiple burst pattern of muscle activity following CCLR. This more biphasic, inconsistent activation pattern suggests poorly controlled muscle activation patterns which contribute to poorly controlled joint movements.[20] The conflict between excitatory extensor mechanisms attempting to avoid limb collapse and possible inhibitory extensor mechanisms, possibly associated with instability or effusion present in the stifle, are believed to cause this perturbed activation pattern.[20]

Limitations related to the collection and analysis of EMG data exist may have negatively influenced results in this study. Crosstalk, or a signal detected from an adjacent muscle, is an inevitable limitation in using surface EMG. Efforts to reduce crosstalk were employed by choosing large, superficial muscles and by placing the electrodes as close to the muscle belly's midline as possible and using acetate sheets for repeat placement.[28] Instrumentation attached with harnesses to each dog may also have altered gait patterns. For these reasons, dogs donned the instruments, harnesses and kinematic reflective markers for a minimum of one month prior to data collection to acclimatize to the testing procedures. Our study suggests evidence that changes in standing EMG values for the vastus lateralis are due to changes in muscle firing; however, muscle impedance between the electrodes and skin, and possible changes in the size and/or fat content of the muscle were not evaluated. In addition, new electrode preparation and placement was performed at each data collection session limiting the ability to collect the exact same level of muscle activation over time. Standing EMG values were shown to be significantly different post CCLR in the vastus lateralis, also

indicating different levels of activation. However, normalizing EMG data to a maximum voluntary contraction is not feasible in the dog; therefore, standing baseline values were chosen to analyze onset and offset times. Additional studies are needed to improve comparative abilities of EMG in longitudinal studies when maximum voluntary contractions are not practical or possible.

In summary, EMG was used to detect the initiation, duration and magnitude of muscle activity important for understanding the role of dynamic restraints in maintaining joint stability prior to and post CCLR. This study suggests that evaluating kinetic, kinematic and EMG data simultaneously provides a more complete understanding of muscle activity and its relationship to onset and progression of CCL disease. Qualitative observations from this study suggest that CCL injury and subsequent rupture may have an effect on neuromuscular function. Alterations observed in peak amplitude and muscle firing patterns may be a compensation for muscle weakness and decreased stifle stability.

Nonsignificant observations from this study may suggest that alterations in muscle function, specifically changes in peak amplitude, occur while the joint is deteriorating and prior to CCLR. Future studies with larger sample sizes are needed to confirm EMG activity in stabilizing muscles of the stifle, further investigating the role of neuromuscular control in stifle stability. These results may be used to define rehabilitation exercise protocols targeted at improving strength, altering muscle activity, and increasing joint integrity by protecting against or slowing the deleterious effects of osteoarthritis. Rehabilitation research should investigate screening methods targeted at detecting muscle weakness prior to rupture of the CCL and focus on the development of neuromuscular training programs to reduce the risk of contralateral CCLR.

Manufacturer Addresses

a. HelpEmUp Harness, Blue Dog Designs, Denver, CO

b. Noraxon U.S.A., Inc., Scottsdale, AZ

c. Ambu Blue Sensor, 'N' model, electrodes, MVAP Medical Supplies, Inc., Newbury Park, CA

TABLES AND FIGURES

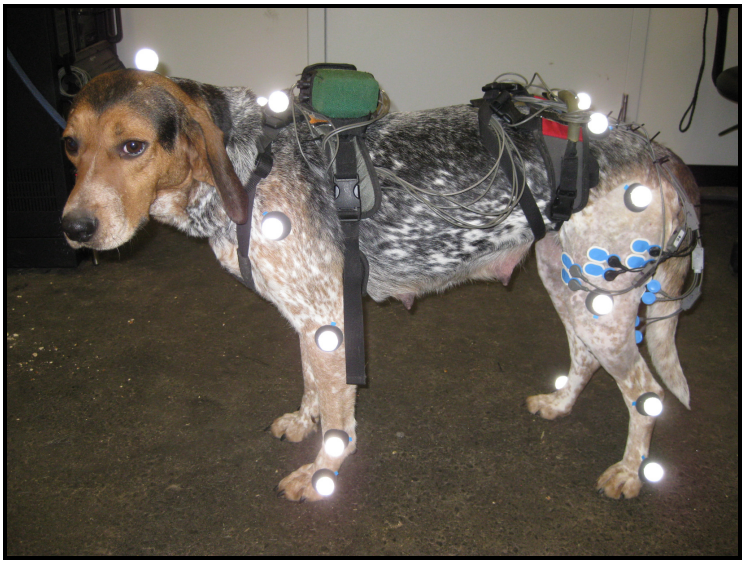

Figure 1: Photograph of a dog prepared for data collection donning EMG equipment and demonstrating kinematic reflective marker attachment sites. Note wireless transmitter (green box) attached to dorsum of the thoracic harness and electrode leads attached to dorsum of the pelvic harness. Note also surface EMG electrode placement on left thigh musculature (blue ovals).

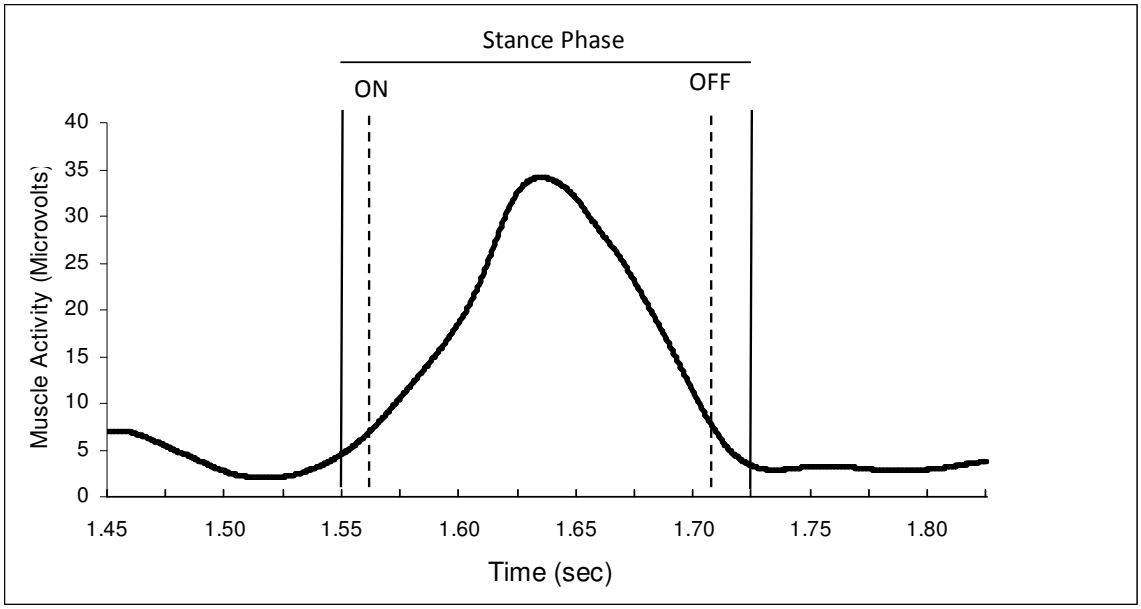

Figure 2: A processed EMG signal at baseline (pre-surgery) of the vastus lateralis muscle in the treated pelvic limb of a single dog at baseline that depicts muscle onset and offset times. Time is reported on the horizontal axis and stance phase (paw strike to paw off) is depicted within the solid black lines. The first dashed vertical line identifies onset of muscle activity and the second dashed vertical line depicts muscle off time. In this example, the muscle activates after paw strike (at 5.7% of stance or 2.3% of the stride) and stays active for a total of 0.15 seconds with a maximum amplitude of 34.1 microvolts.

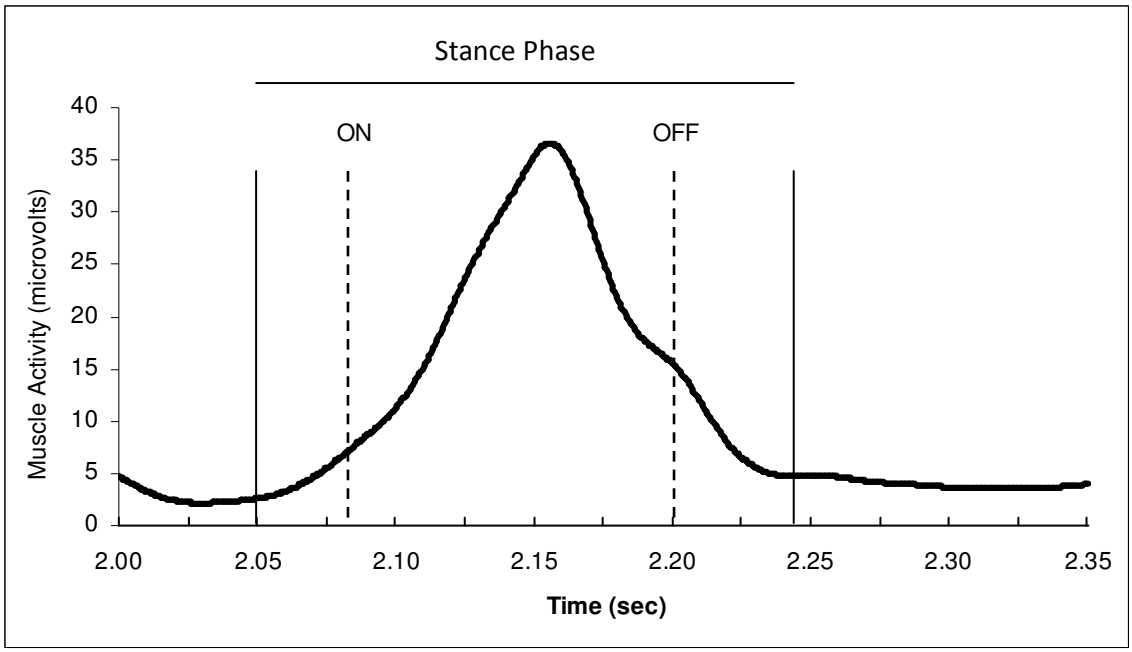

Figure 3: A second processed EMG signal post MRFE of the vastus lateralis muscle in the treated pelvic limb of a single dog that depicts muscle onset and offset times. In this example, the muscle activates after paw strike (at 15.4% of stance or 6.7% of the stride) and stays active for a total of 0.14 seconds, with a maximum amplitude of 36.6 microvolts.

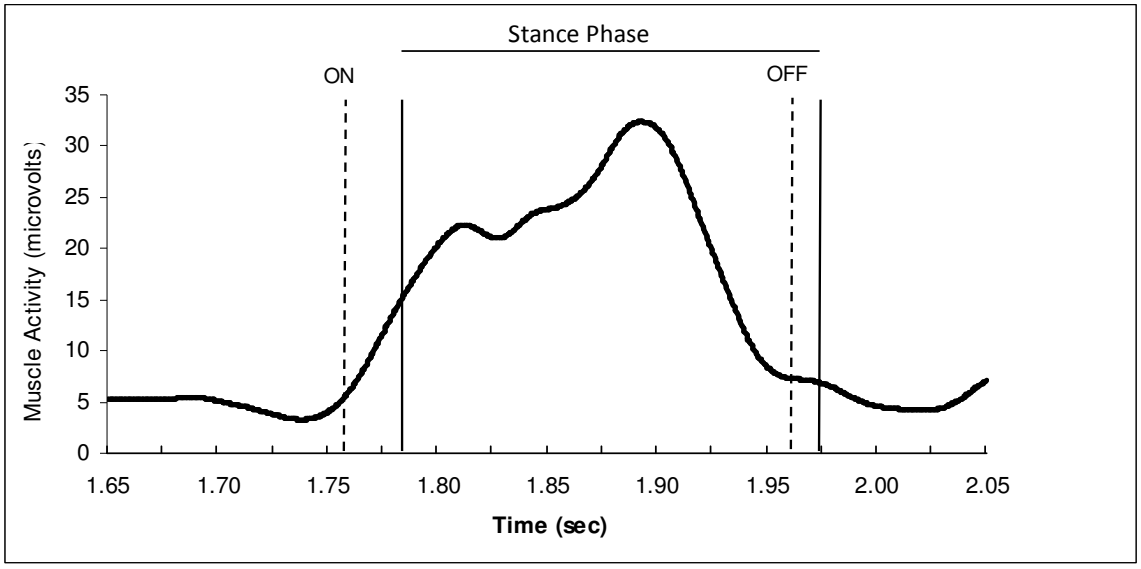

Figure 4: A processed EMG signal post CCL rupture in the vastus lateralis muscle in the treated pelvic limb of a single dog that depicts muscle onset and offset times. The muscle activates 10.3% prior to paw strike (or 4.3% prior to the stride) during the first stance phase and stays active for a total of 0.21 seconds, with a maximum amplitude of 32.4 microvolts.

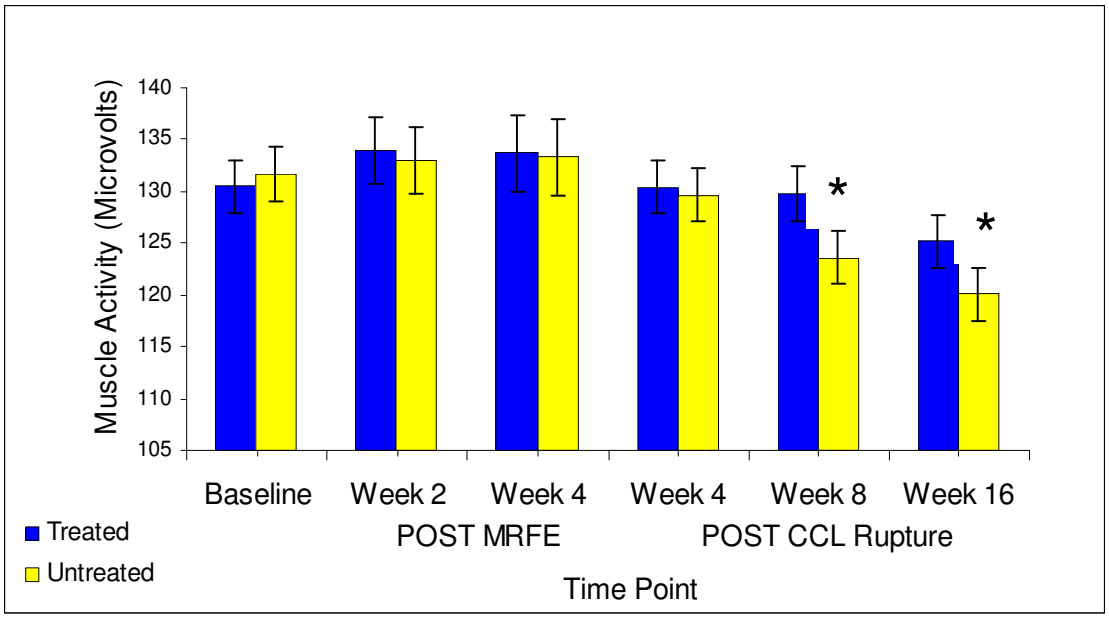

Figure 5 – Mean standing EMG values for the vastus lateralis during stance at all time points. Standing EMG means were significantly decreased in the vastus lateralis of the untreated pelvic limb compared to the treated limb at 8 and 16 weeks post CCL rupture. * Significantly different from the treated limb.

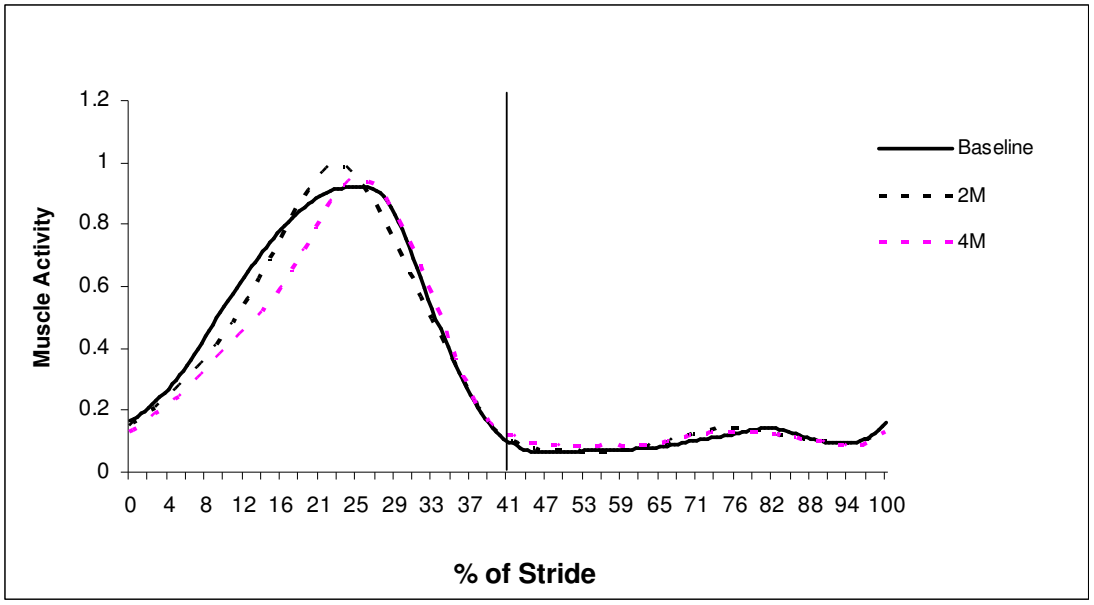

Figure 6: EMG signals of trial averaged vastus lateralis muscles (normalized to maximum value) in the treated pelvic limb at 2 and 4 weeks post MRFE-induced CCL injury. The solid black vertical line indicates the transition between stance and swing phase of the stride. Post MRFE, muscle onset occurs at 5-6% of the stride, indicating an apparent delay compared to baseline, where muscle onset occurs at 1% of the stride. An apparent decrease in activation duration occurs post MRFE, where the vastus lateralis is active for 33% of the stride, compared to baseline where the muscle is active for 37% of the stride. '2M' and '4M'refers to the number of weeks post MRFE surgery, prior to CCL rupture.

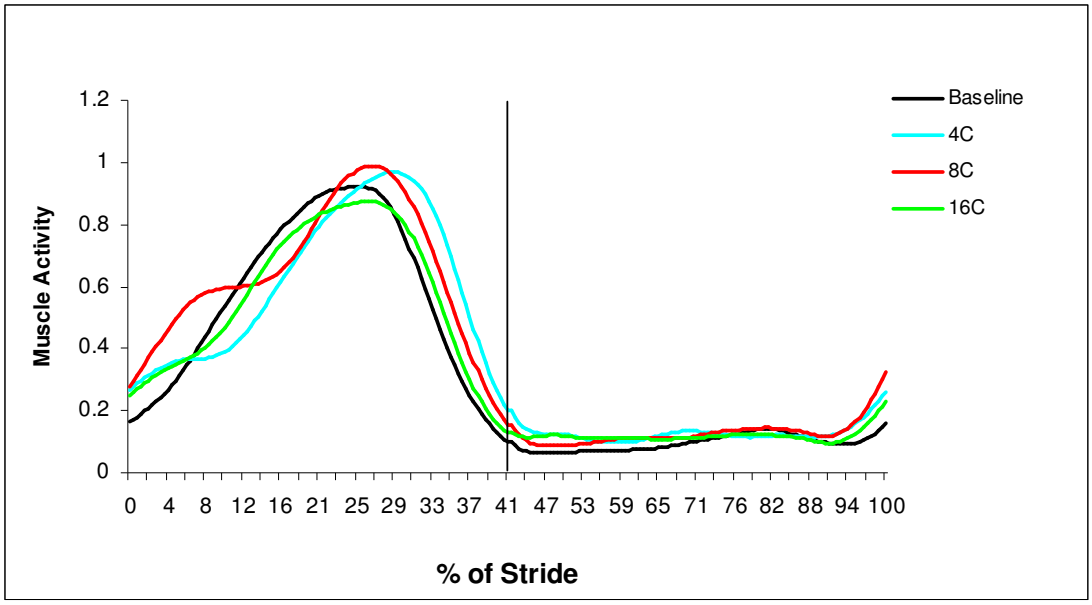

Figure 7: EMG signals of trial averaged vastus lateralis muscles (normalized to maximum value) in the treated pelvic limb at baseline at 4, 8 and 16 weeks post CCL rupture. The solid black vertical line indicates the transition between stance and swing phase of the stride. Post CCL rupture, muscle onset occurs up to 2% prior to initial paw contact on the force platform, indicating an apparent earlier activation compared to baseline, where muscle onset occurs at 1% after paw contact. An apparent increase in activation duration occurs post CCL rupture, where the vastus lateralis is active for up to 44% of the stride, compared to baseline where the muscle is active for 37% of the stride. '4C', '8C' and '16C' refers to the number of weeks post CCL rupture.

Figure 8: EMG signals of trial averaged biceps femoris muscles (normalized to maximum value) in the treated pelvic limb at 2 and 4 weeks post MRFE-induced CCL injury. The solid black vertical line indicates the transition between stance and swing phase of the stride. Post MRFE, muscle onset occurs at 11% (2M) and 6% (4M) prior to initial paw contact on the force platform, indicating an apparent delay compared to baseline, where muscle onset occurs at 13% prior to initial paw contact. An apparent decrease in activation duration occurs post MRFE, where the biceps femoris is active for up to 39% of the stride, compared to baseline where the muscle is active for 46% of the stride. '2M' and '4M'refers to the number of weeks post MRFE surgery, prior to CCL rupture.

Figure 9: EMG signals of trial averaged biceps femoris muscles (normalized to maximum value) in the treated pelvic limb at 4, 8 and 16 weeks post CCL rupture. The solid black vertical line indicates the transition between stance and swing phase of the stride. Post CCL rupture, muscle onset occurs up to 24% prior to initial paw contact on the force platform, indicating an apparent earlier activation compared to baseline, where muscle onset occurs at 13% prior to paw contact. An apparent increase in activation duration occurs post CCL rupture, where the biceps femoris is active for up to 57% of the stride, compared to baseline where the muscle is active for 46% of the stride. '4C', '8C' and '16C' refers to the number of weeks post CCL rupture.

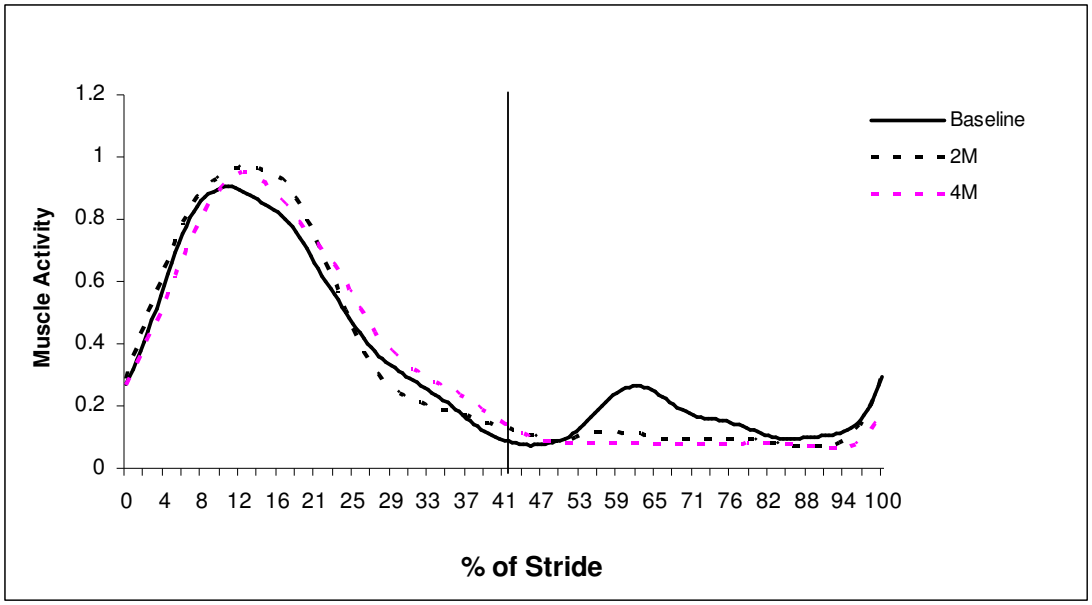

Figure 10: EMG signals of trial averaged gastrocnemius muscles (normalized to maximum value) in the treated pelvic limb at 2 and 4 weeks post MRFE-induced CCL injury. The solid black vertical line indicates the transition between stance and swing phase of the stride. Post MRFE, muscle onset occurs at 3% prior to initial paw contact on the force platform, indicating an apparent delay compared to baseline, where muscle onset occurs at 6% prior to initial paw contact. An apparent decrease in activation duration occurs post MRFE, where the gastrocnemius is active for 41% of the stride, compared to baseline where the muscle is active for 44% of the stride. '2M' and '4M'refers to the number of weeks post MRFE surgery, prior to CCL rupture.

Figure 11: EMG signals of trial averaged gastrocnemius muscles (normalized to maximum value) in the treated pelvic limb at 4, 8 and 16 weeks post CCL rupture. The solid black vertical line indicates the transition between stance and swing phase of the stride. Post CCL rupture, muscle onset occurs up to 9% prior to initial paw contact on the force platform, indicating an apparent earlier activation compared to baseline, where muscle onset occurs at 6% prior to initial paw contact. An apparent increase in activation duration occurs post MRFE, where the gastrocnemius is active for up to 59% of the stride, compared to baseline where the muscle is active for 44% of the stride. '4C', '8C' and '16C' refers to the number of weeks post CCL rupture.

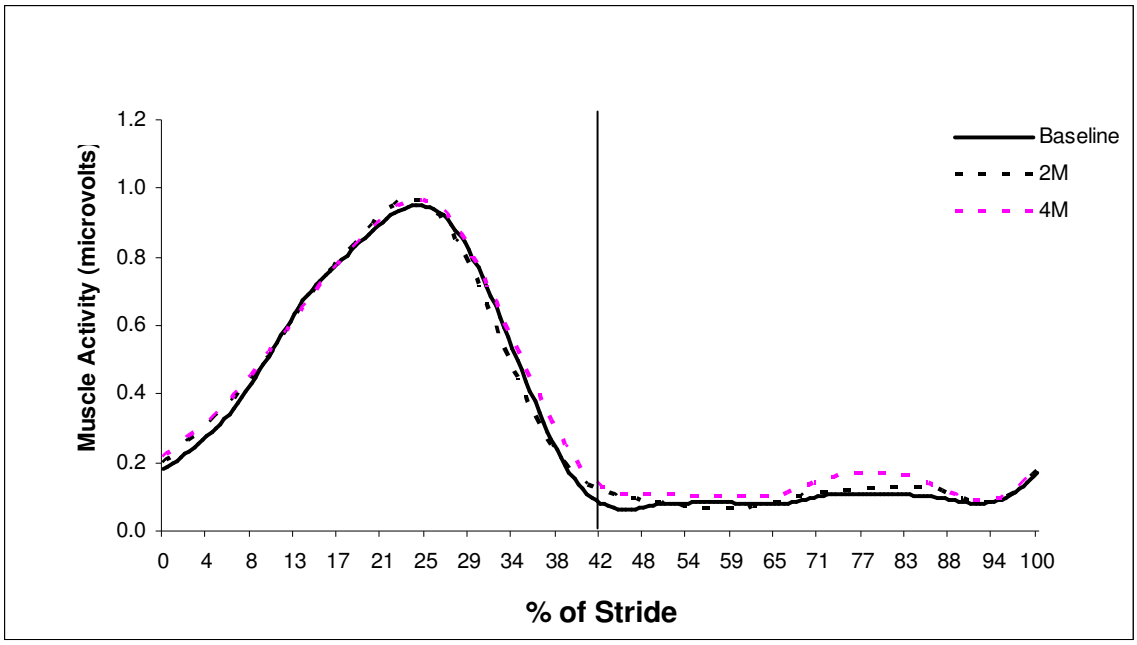

Figure 12: EMG signals of trial averaged vastus lateralis muscles (normalized to maximum value) in the untreated pelvic limb at 2 and 4 weeks post MRFE-induced CCL injury. The solid black vertical line indicates the transition between stance and swing phase of the stride. Post MRFE, muscle onset occurs at 2-3% of the stride, indicating an apparent slight delay in onset time compared to baseline, where muscle onset occurred at 1% of the stride. No apparent change in activation duration occurred post MRFE, where the vastus lateralis is active for 35% of the stride, compared to baseline where the muscle is also active for 35% of the stride. '2M' and '4M'refers to the number of weeks post MRFE surgery, prior to CCL rupture.

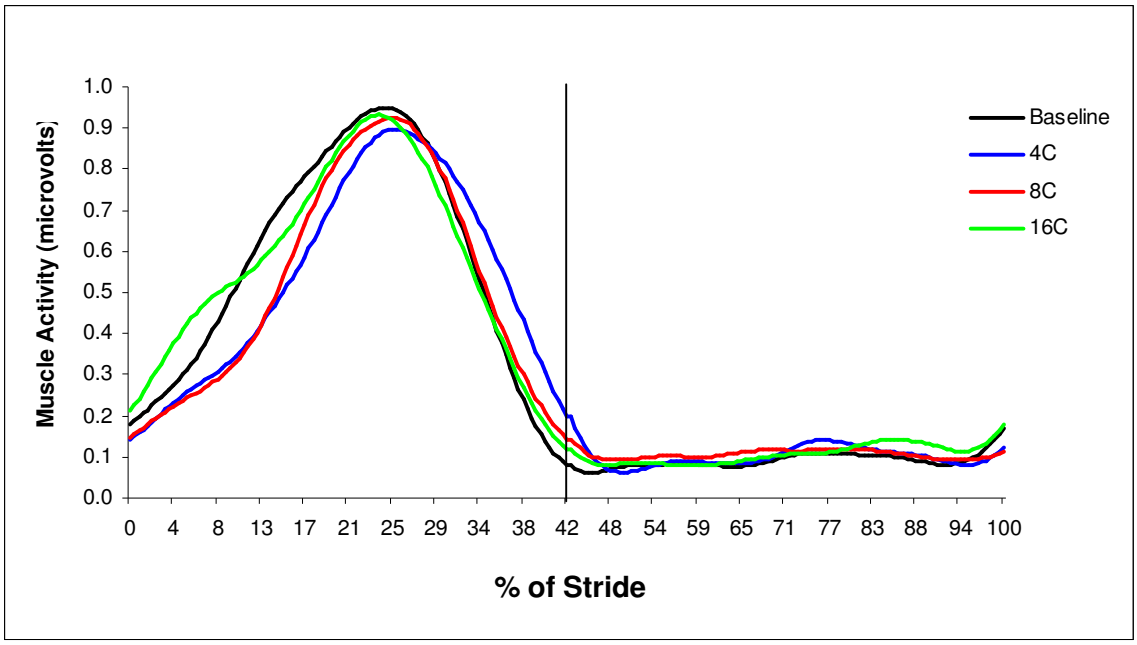

Figure 13: EMG signals of trial averaged vastus lateralis muscles (normalized to maximum value) in the untreated pelvic limb at 4, 8 and 16 weeks post CCL rupture. The solid black vertical line indicates the transition between stance and swing phase of the stride. Post CCL rupture, muscle onset occurs up to 6% prior to initial paw contact on the force platform, indicating an apparent earlier activation compared to baseline, where muscle onset occurs at 1% after paw contact. An apparent increase in activation duration occurs post CCL rupture, where the vastus lateralis is active for up to 44% of the stride, compared to baseline where the muscle is active for 35% of the stride. '4C', '8C' and '16C' refers to the number of weeks post CCL rupture.

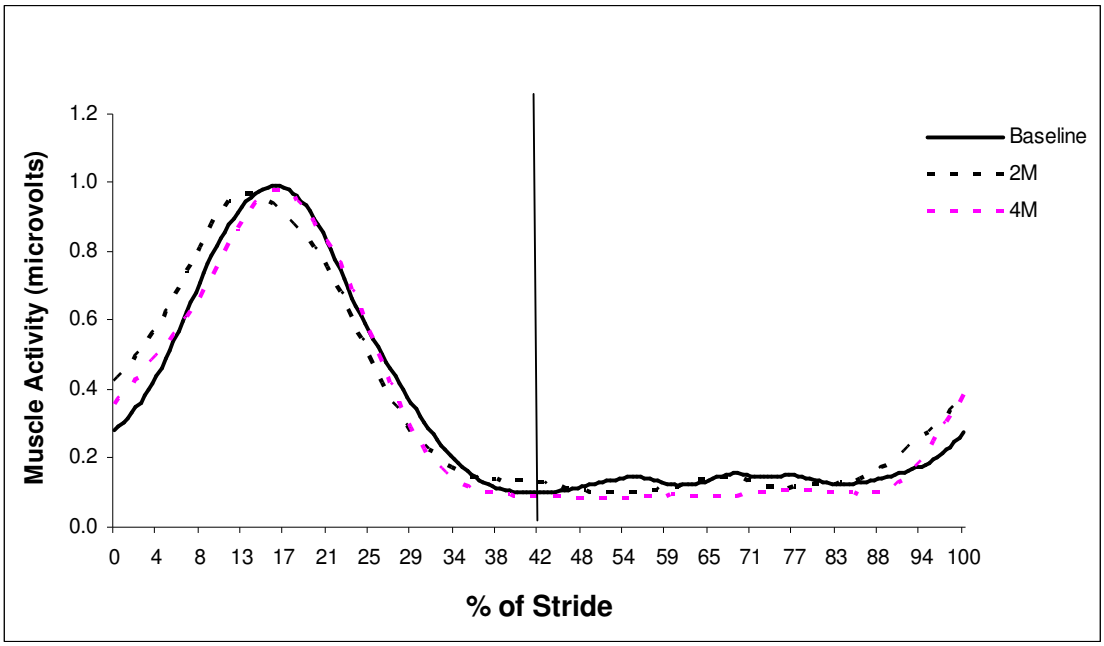

Figure 14: EMG signals of trial averaged biceps femoris muscles (normalized to maximum value) in the untreated pelvic limb at 2 and 4 weeks post MRFE-induced CCL injury. The solid black vertical line indicates the transition between stance and swing phase of the stride. Post MRFE, muscle onset occurs at 8-9% prior to initial paw contact on the force platform, indicating an apparent delay compared to baseline, where muscle onset occurs at 10% prior to paw contact. An apparent decrease in activation duration occurs post MRFE, where the biceps femoris is active for 38-43% of the stride, compared to baseline where the muscle is active for 44% of the stride. '2M' and '4M'refers to the number of weeks post MRFE surgery, prior to CCL rupture.

Figure 15: EMG signals of trial averaged biceps femoris muscles (normalized to maximum value) in the untreated pelvic limb at 4, 8 and 16 weeks post CCL rupture. The solid black vertical line indicates the transition between stance and swing phase of the stride. Post CCL rupture, muscle onset occurs from 4-9% prior to initial paw contact on the force platform, indicating an apparent delay in activation compared to baseline, where muscle onset occurs at 10% prior to paw contact. An apparent decrease in activation duration occurs at 4 and 8 weeks post CCL rupture, where the biceps femoris is active for up to 39-43% of the stride, compared to baseline where the muscle is active for 44% of the stride. However, an apparent increase in activation duration appears to exist at 16 weeks post rupture, where the biceps femoris is active for 46% of the stride, compared to baseline. '4C', '8C' and '16C' refers to the number of weeks post CCL rupture.

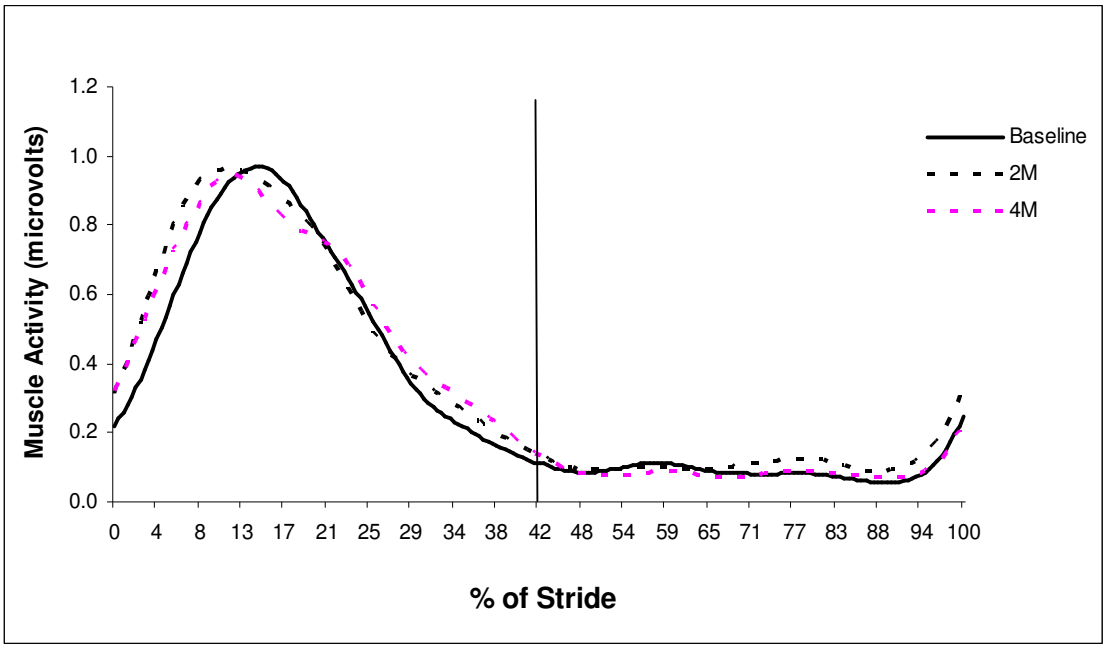

Figure 16: EMG signals of trial averaged gastrocnemius muscles (normalized to maximum value) in the untreated pelvic limb at 2 and 4 weeks post MRFE-induced CCL injury. The solid black vertical line indicates the transition between stance and swing phase of the stride. Post MRFE, muscle onset occurs at 3% prior to initial paw contact on the force platform, indicating an apparent earlier onset compared to baseline, where muscle onset occurs at 1% prior to paw contact. An apparent increase in activation duration occurs post MRFE, where the gastrocnemius is active for 38-41% of the stride, compared to baseline where the muscle is active for 37% of the stride. '2M' and '4M'refers to the number of weeks post MRFE surgery, prior to CCL rupture.

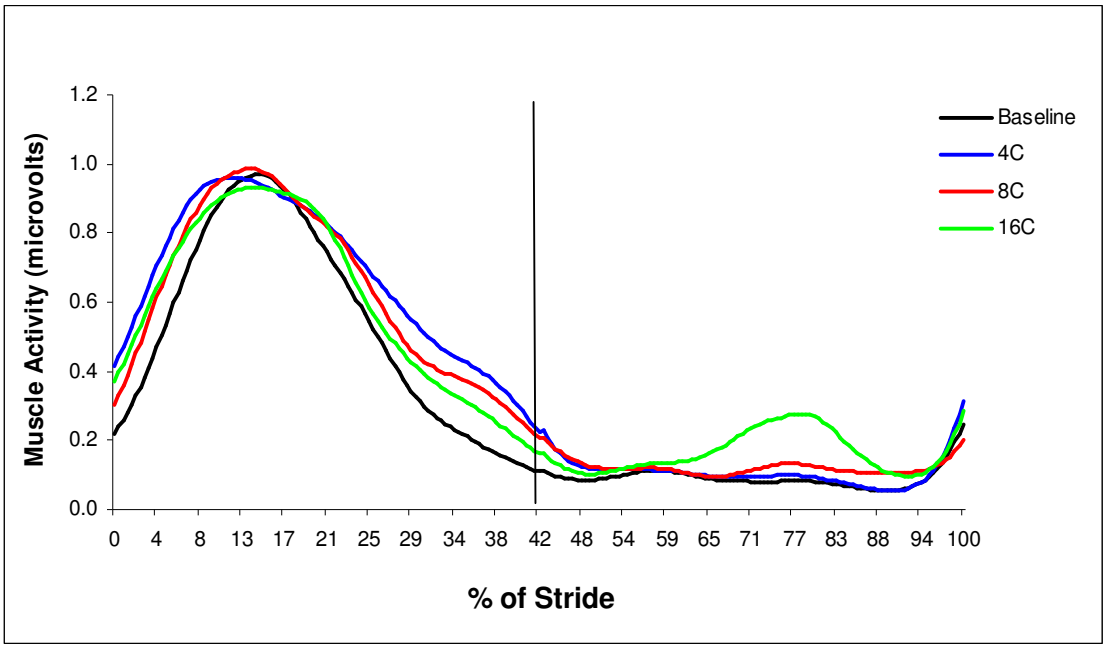

Figure 17: EMG signals of trial averaged gastrocnemius muscles (normalized to maximum value) in the untreated pelvic limb at 4, 8 and 16 weeks post CCL rupture. The solid black vertical line indicates the transition between stance and swing phase of the stride. Post CCL rupture, muscle onset occurs up to 4% prior to initial paw contact on the force platform, indicating an apparent earlier activation compared to baseline, where muscle onset occurs at 1% prior to initial paw contact. An apparent increase in activation duration occurs post MRFE, where the gastrocnemius is active for up to 50% of the stride, compared to baseline where the muscle is active for 37% of the stride. '4C', '8C' and '16C' refers to the number of weeks post CCL rupture.

TABLE 1 – Mean muscle onset timing as a percentage of stride for vastus lateralis, biceps femoris and gastrocnemius muscles in untreated and treated pelvic limbs at all time points (N = 5).

Time Points	Vastus Lateralis		Biceps Femoris		Gastrocnemius	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
Baseline	1.3 ± 3.4	0.8 ± 3.4	-9.9 ± 4.9	-12.9 ± 4.9	-1.2 ± 1.4	-5.6 ± 1.4
Post MRFE						
Week 2	2.0 ± 3.8	4.7 ± 3.8	-7.8 ± 5.3	-11.1 ± 5.3	-2.8 ± 1.6	-3.4 ± 1.6
Week 4	2.7 ± 3.8	6.1 ± 3.8	-9.2 ± 5.3	-6.0 ± 5.3	-3.1 ± 1.6	-3.1 ± 1.6
Post CCL Rupture						
Week 4	-5.9 ± 4.5	-1.5 ± 3.8	-7.7 ± 5.9	-12.8 ± 5.3	-4.2 ± 1.9	-8.9 ± 1.6
Week 8	0.6 ± 3.8	-1.8 ± 3.4	-4.0 ± 5.3	-23.5 ± 4.9	-2.6 ± 1.6	-7.0 ± 1.4
Week 16	-1.9 ± 3.8	0.4 ± 3.4	-9.0 ± 4.9	-13.1 ± 4.9	-4.4 ± 1.4	-7.4 ± 1.6

Positive values indicate that the muscle activates after paw strike and negative values indicate that the muscle activates prior to paw strike, which represents 0% of the stride.

TABLE 2 – Activation duration (msec) for vastus lateralis, biceps femoris, and gastrocnemius muscles in untreated and treated pelvic limbs at all time points (N = 5).

	Vastus Lateralis		Biceps Femoris		Gastrocnemius	
Time Points	Untreated	Treated	Untreated	Treated	Untreated	Treated
Baseline	165.2 ± 20.1	173.6 ± 20.2	205.6 ± 29.5	213.0 ± 29.5	171.8 ± 24.5	210.6 ± 24.5
Post MRFE						
Week 2	169.4 ± 21.9	154.4 ± 21.9	186.6 ± 31.9	180.3 ± 31.9	181.1 ± 27.0	205.3 ± 27.0
Week 4	160.6 ± 21.9	152.1 ± 21.9	194.8 ± 31.9	175.1 ± 31.9	189.3 ± 27.0	205.1 ± 27.0
Post CCL Rupture						
Week 4	192.2 ± 21.9	195.2 ± 21.9	197.0 ± 31.8	226.8 ± 31.8	216.4 ± 27.0	259.4 ± 27.0
Week 8	208.0 ± 20.1	188.4 ± 20.1	229.8 ± 29.5	249.8 ± 29.5	231.8 ± 24.5	263.6 ± 24.5
Week 16	195.2 ± 21.9	178.6 ± 20.1	215.4 ± 29.5	226.8 ± 29.5	221.0 ± 24.5	253.6 ± 24.5

TABLE 3 – Mean activation duration (percentage of stride) for vastus lateralis, biceps femoris, and gastrocnemius muscles at all time points (N = 5).

	Vastus Lateralis		Biceps Femoris		Gastrocnemius	
Time Points	Untreated	Treated	Untreated	Treated	Untreated	Treated
Baseline	35.3 ± 4.3	37.1 ± 4.3	44.2 ± 5.8	45.5 ± 5.8	37.0 ± 5.1	43.9 ± 5.1
Post MRFE						
Week 2	35.1 ± 4.7	32.5 ± 4.7	38.4 ± 6.4	37.7 ± 6.4	38.0 ± 5.7	41.1 ± 5.7
Week 4	34.7 ± 4.7	33.0 ± 4.7	42.8 ± 6.4	38.6 ± 6.4	41.2 ± 5.7	44.4 ± 5.7
Post CCL Rupture						
Week 4	39.0 ± 5.3	43.9 ± 4.7	38.5 ± 7.1	50.8 ± 6.4	48.3 ± 6.5	57.8 ± 5.7
Week 8	41.9 ± 4.7	42.6 ± 4.3	43.4 ± 6.4	56.6 ± 5.8	47.3 ± 5.7	59.4 ± 5.1
Week 16	44.1 ± 4.7	39.4 ± 4.3	46.1 ± 5.8	49.2 ± 5.8	50.1 ± 5.1	53.8 ± 5.1

TABLE 4 –Peak amplitude (normalized to baseline) for vastus lateralis, biceps femoris, and gastrocnemius muscles in the untreated and treated pelvic limbs at all time points (N = 5).

	Vastus Lateralis		Biceps Femoris		Gastrocnemius	
Time Points	Untreated	Treated	Untreated	Treated	Untreated	Treated
Baseline	100.0 ± 22.1	100.0 ± 22.1	100.0 ± 13.3	100.0 ± 13.3	100.0 ± 18.1	100.0 ± 18.1
Post MRFE						
Week 2	98.6 ± 24.4	72.8 ± 24.4	78.3 ± 17.2	82.9 ± 14.9	100.8 ± 22.7	101.5 ± 19.9
Week 4	88.4 ± 24.4	76.3 ± 24.4	101.1 ± 17.2	75.9 ± 14.9	116.8 ± 22.7	116.7 ± 19.9
Post CCL Rupture						
Week 4	109.3 ± 27.7	105.8 ± 24.4	93.8 ± 17.2	65.8 ± 14.9	131.2 ± 22.7	77.8 ± 19.9
Week 8	127.2 ± 24.4	105.5 ± 22.1	91.4 ± 14.9	91.4 ± 13.3	112.2 ± 20.0	106.7 ± 18.1
Week 16	138.5 ± 27.8	96.1 ± 22.1	111.3 ± 17.2	67.0 ± 13.3	95.2 ± 22.8	77.1 ± 18.1

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CHAPTER 4

CLINICAL AND PHYSIOLOGIC OUTCOME PARAMETER MEASURES AND THEIR ASSOCIATION WITH CRANIAL CRUCIATE LIGAMENT INJURY AND RUPTURE

INTRODUCTION

Clinical outcome measures (e.g., goniometry and thigh circumference) can be used to provide the clinician with objective measures that are relatively inexpensive and provide a quick method of gaining insight into the early detection of CCL disease and progression. Physiologic outcome measures, such as prostaglandin E_2 analysis, intra-articular pressure (IAP) and gross and histopathologic evaluation, may not be as readily available or feasible to perform in a clinical setting; however, they may associated with the development of CCL disease and subsequent rupture. They are indicators of the OA disease process and can be used to correlate with clinical signs and symptoms.

Kinetic, kinematic and electromyographic measurements, as described in Chapters 2 and 3, are common tools used when analyzing canine gait prior to and post CCLR. However, these costly and time-consuming research tools may not be feasible for clinicians to purchase or apply on a daily basis in a clinical setting. Several common clinical outcome measures have been selected for evaluation in this project,

which include: goniometry, thigh circumference, radiographs, cranial drawer test, pain, lameness. Several common physiologic outcome measures, have been selected for evaluation in this project, which include: PGE_2, IAP, gross evaluation and histopathologic findings post mortem.

Goniometry and thigh circumference provide simple, noninvasive, inexpensive and relatively quick objective assessments of joint range of motion and muscle mass. Manual goniometric evaluation provides a reliable assessment of passive joint range of motion.[1] Thigh circumference is a reliable, cost-effective and easy method to indirectly measure muscle mass, which has not been reported in dogs following MRFE-induced CCL injury.[2] Decreased muscle mass is an indicator of muscle weakness. Therefore, decreased thigh circumference may provide insight into changes in muscle activity post CCL injury and subsequent rupture.

Radiographs may be used to assess the degree of osteoarthritis (OA) in CCLR patients, which helps to guide clinicians to determine treatment plans.[3] Depending on severity of OA, radiographic findings vary and a poor correlation has been reported between the severity of OA seen on radiographs and lameness utilizing subjective scoring systems and force platform analysis.[4] Therefore, the use of radiographs alone should not be used to assess the clinical effects of OA. Radiographs aid in the diagnosis of CCLR and to rule out other bony or soft tissue abnormalities. Radiographic changes indicating the presence of CCLR may include joint effusion, periarticular swelling, thigh muscle atrophy, and osteophyte formation.[5]

The cranial drawer test is an orthopedic test used to assess structural integrity of the CCL.[5] A positive cranial drawer test, indicated by the presence of cranial

displacement of the tibia relative to the femur, is indicative of a partial or complete rupture of the CCL. The tibial compression test is another orthopedic test used to subjectively assess proximal tibial displacement as the forces associated with weightbearing and by the stifle musculature cause compression of the femoral condyles against the tibial plateau, called a positive cranial tibial thrust.[5] With training or experience, these orthopedic tests are easy to perform and provide an important clinical tool in the diagnosis of CCL injury and rupture.[6]

Rupture of the CCL leads to clinical signs of pain and lameness.[3] Objective pain is difficult to assess, as animals are not able to verbally communicate quality or quantity of pain present. Therefore subjective pain scores are used in veterinary medicine to evaluate pain behaviors (i.e., temperament, level of comfort, vocalization, ambulation), physiologic parameters (heart and respiratory rates, blood pressure), posture (recumbent, sitting, standing) and/or activity (resting, eating, restless) that are believed to be associated with the presence of discomfort.[7] Limited chronic pain scales exist for dogs. The Colorado State University Veterinary Medical Center Acute Pain Scale (Appendix E) is a composite derived from several pain scales that includes not only psychological and behavioral pain signs and response to palpation, but also body tension which is not incorporated in other scales.[7] Therefore, this scale may provide a more complete method for assessment of chronic pain.[7]

Lameness is a typical presentation of dogs with cruciate ligament deficiency. Stifle instability and subsequent lameness is associated with gradual degeneration of the CCL. Many weeks or months may pass after this initial onset of intermittent lameness, until marked lameness develops, typically due to the presence of a meniscal

injury or complete CCL rupture.[3] Lameness evaluations are typically done both at a walk and trot, as different velocities may reveal subtle changes in gait and signs of lameness. The trotting gait may accentuate a mild lameness, as increased force is applied to the affected limb at higher gait speeds.[2] Subjective lameness scores are a common outcome assessment tool used to evaluate changes in weightbearing associated with lameness and CCL injury.

Inflammatory changes in the synovial membrane and synovial fluid are present in dogs with CCL rupture, however, it is not confirmed whether inflammation precedes actual CCL rupture.[8] Therefore, subjective and objective evaluation of joint effusion needs to be done prior to CCLR. Inflammation can also produce long-term detrimental effects in a joint via production of inflammatory mediators, degradative enzymes and inflammatory cells that lead to articular cartilage degradation.[9] The most important inflammatory mediator is prostaglandin E_2 (PGE_2), stimulated by interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNFα). PGE_2 influences the production of MMPs (matrix metalloproteinases) that contribute to cartilage matrix degradation.[9] In the joint, PGE_2 causes vasodilation, alters nociception and degrades and inhibits the synthesis of proteoglycan, decreasing its content within the cartilage matrix.[9] Found in large quantities in OA joints, PGE_2 can provide an objective measure of synovitis associated with CCL disease.[9,10] Whether or not elevations in PGE_2 precede other more commonly used parameters to detect CCL disease is not currently known.

Synovitis, or inflammation of the synovial membrane typically increases the amount of synovial fluid and subsequently increases intra-articular pressure (IAP).[11]

Conceivably, detection of increased IAP could precede detection of palpable joint effusion. In osteoarthritic knees, increased IAP may cause limited joint motion, pain, and decreased neuromuscular control, thus leading to muscle atrophy and weakness.[12,13] Increased IAP results in muscle inhibition, which is a reflex inhibition of the muscles surrounding an affected joint that normally stabilize and protect the joint from injury.[13] In normal joints, IAP levels fluctuate throughout normal range of joint motion.[14] Studies evaluated increasing fluid volumes within a joint via intra-articular injection and measured the corresponding changes in IAP.[12-14] In dogs, as stifle flexion increases past ~90°, an increase in IAP is noted, which causes a decrease in the total range of motion of the stifle joint.[14] Similarly, when synovial fluid volumes are increased and the stifle nears full extension, IAP increases significantly.[14]

CCL rupture and the resultant joint instability leads to degenerative changes in the stifle joint. Despite a large amount of information available in the literature to grade OA severity via histologic assessment, many limitations exist in these techniques, including a lack of standardization for the number and location of sections for scoring and no global joint assessment.[15] A recent recommendation for a comprehensive histological assessment of canine OA has been devised and provides a global assessment of canine histologic tissues and has been proven reliable and includes evaluation of cartilage, osteochondral and synovium.[15] Detailed gross and histological evaluation of articular cartilage following MRFE-induced CCL injury and eventual rupture has yet to be reported. Associations of the severity of cartilage damage with alterations in muscle activity are important to investigate the

relationship between neuromuscular patterns and OA development in dogs with CCL rupture.

The purpose of this study was to assess the reliability of clinical outcomes commonly associated with the development of CCL disease and subsequent rupture. We hypothesized that clinical and physiologic measures, such as goniometry, thigh circumference, radiography, cranial drawer test, pain, lameness, effusion, prostaglandin E_2 analysis, intra-articular pressure, and gross and histopathology will be reliable indicators of CCL injury and rupture.

MATERIALS AND METHODS

Subject descriptive data, surgical technique and monopolar radiofrequency energy (MRFE) are described in Chapter 2. Clinical and physiologic measures were recorded at various points throughout subclinical CCL degeneration (2 and 4 weeks post MRFE-induced CCL injury), and acute (4 weeks post confirmed CCLR), intermediate (8 weeks post CCLR), and chronic phases of CCLR (16 weeks post CCLR). The treated pelvic limb received a MRFE-induced CCL injury with subsequent rupture and the untreated pelvic limb was the nonsurgical, contralateral pelvic limb.

Outcomes were recorded at baseline (pre-surgery). Weekly measures of goniometry, thigh circumference, cranial drawer, pain, lameness and joint effusion were recorded for the duration of the study. IAP and PGE2 measurements were recorded at 4 weeks post MRFE (prior to CCLR). Radiographs and additional IAP and PGE_2 were recorded at 4, 8 and 16 weeks post CCLR. Gross and histopathology

of synovium, menisci and articular cartilage was evaluated to assess signs of OA at study termination.

Passive Joint Range of Motion

Goniometric assessment of the passive range of motion of coxofemoral, femorotibial and tarsal joints in untreated and treated pelvic limbs were performed by one investigator (CA) in laterally recumbent, unsedated dogs using an extendable goniometer[a]. Tarsal joint angles were measured with stifles positioned and stabilized in a neutral (natural resting angle chosen by the dog at baseline) resting position of approximately 115° of flexion.[16] Anatomical landmarks used to measure each angle were consistent with those of a previous study.[1] Maximum flexion and extension movements were measured in triplicate, prior to any invasive techniques and mean values were used for analysis.

Thigh Circumference

To assess thigh muscle mass, femur lengths were measured from the greater trochanter to the distal aspect of the lateral fabella.[17,18] A location equivalent to 70% of the femur length from the greater trochanter was recorded and marked with an indelible marker.[18] Circumferential measures were taken at this location by one investigator (CA) that was used as a consistent landmark for all subsequent weekly measurements. A spring tensioned measuring tape[b] was used to minimize operator and instrument variability. Mean thigh circumference was calculated from 3 consecutive measurements and used for analysis.[18]

Radiography

All radiographs were performed with the animal sedated (acepromazine, 0.10 mg/kg SQ; Medetomidine, 10 mcg/kg IV; Atipamezole, 50 mcg/kg, IM). Radiographs of the thoracic and pelvic limbs were taken at baseline to rule out any pre-existing orthopedic disease. Repeated radiographs of bilateral stifles (craniocaudal and lateral views) at 4, 8 and 16 weeks post cranial cruciate ligament rupture were taken to grade the degree of OA present in the stifle joints. Radiographs were evaluated at 9 anatomic sites for signs of OA and graded on a scale from 0–3 according to the amount of OA at each landmark as follows: 0 = normal (No OA); 1 = mild; 2 = moderate; and 3 = severe.[19] Cumulative scores on each limb were calculated and a mean final score was used in the analysis and compared over time. A board certified radiologist (AV) graded each radiograph for the presence and severity of OA using a previously published subjective radiographic scoring system (Appendix D).[19]

Cranial Drawer Test

With the stifle joint held in slight flexion, the index finger of one hand was positioned on the patella while the thumb and third finger were placed caudal to the fabellae.[5] The second hand positioned the index finger on the tibial tuberosity and the thumb is caudal to the proximal tibia and fibula. Assessed with the stifle both flexed and extended in an unsedated dog, the tibia was glided cranially, while the distal femur was held stationary.[5] Two veterinarians (KH, RP) performed this test and results were recorded as the presence of 0 = no tear, 1 = partial tear or 2 = full tear.[3] Scores were collected and analyzed weekly.

Pain and Lameness

Pain and lameness scores were graded by two veterinarians (KH, RP). The Colorado State University Veterinary Medical Center Acute Pain Scale (Appendix E) was used, which included pain behavior signs, response to palpation, and body tension.[7] Dogs were trotted down a 25-ft hallway with two consistent handlers (CA, AA) to assess lameness according to the following 0-5 lameness scale.[17] Scores were collected and analyzed weekly.

Lameness Scale
0 = Severe non weightbearing lameness
1 = Intermittent non weightbearing lameness
2 = Severe weightbearing lameness
3 = Obvious lameness
4 = Slight lameness
5 = Trots normally

Stifle Joint Effusion

Stifle joint effusion was graded bilaterally (KH, RP) via manual palpation of the stifle joint and recorded according to the following scale.[6] Effusion scores were collected and analyzed weekly.

Joint Effusion Scale
0 = Absent
1 = Mild effusion
2 = Moderate effusion
3 = Severe effusion

Intra-articular Pressure

Both stifles were clipped and prepared with standard sterile preparation techniques for IAP measures of the femorotibial joint. The limbs were positioned in 90° of hip and stifle flexion. The lateral and medial margins of the patellar tendon were identified by palpation. The needle (20 gauge, 2.54 cm) was inserted into the

stifle joint capsule, was held stable for the entire duration of the procedure. IAP measurements were made using an intra-compartmental pressure monitor system[c] by two veterinarians (KH, RP) in triplicate, zeroing the unit between measures. The average of the three measures was used as the final IAP measurement.

The following landmarks were identified to ensure accurate needle placement into each joint capsule and accurate IAP measurements.

Left Pelvic Limb: A lateral approach was used for the left stifle joint. The junction of the distolateral edge of the patella and the lateral femoral condyle was located via palpation. With the bevel of the needle facing the articular surface of the femoral condyle, the needle was inserted at the patellofemoral junction at a 45º angle along the medial edge of the lateral femoral condyle. The tip of the needle followed the contour of the intertrochlear groove, being cautious not to injure the articular cartilage. Approximately 1 cm of the needle remained external to the skin.

Right Pelvic Limb: A medial approach was made into the stifle joint capsule. The distomedial edge of the patella and the medial femoral condyle was identified via palpation. With the bevel of the needle facing upward, the needle was directed vertically along the medial femoral condyle, and directed towards the popliteal lymph node. Caution was taken by slowly inserting the needle along the medial femoral condyle, careful not to disrupt articular cartilage. Confirmation that the needle was through the fat pad occurred by allowing the needle to glide along the femoral condyle articular cartilage as the needle was inserted and positioned in the joint capsule. Once the needle was resting on the articular cartilage, the needle was

redirected distally at a 45º angle, along the contour of the intertrochlear groove. The entire length of the needle was inserted into the stifle joint.

Prostaglandin E_2

Synovial fluid samples were collected from bilateral stifle joints under sedation (acepromazine, 0.10 mg/kg SQ; Medetomidine, 10 mcg/kg IV; Atipamezole, 50 mcg/kg, IM) at the same time radiographs and IAP measures were performed. PGE_2 analysis of synovial fluid was consistent with that of a previous study[10] and concentrations were measured using a commercial enzyme immunoassay kit[d]. Based on occasional small sample volumes, samples were repeated if the coefficient of variation exceeded 17%.

Gross Pathology and Histopathology

Dogs were euthanized with Beuthanasia-D (1-2ml/10#) at study end and gross pathology and histopathology of the synovium, menisci and articular cartilage was performed to grade the presence and severity of OA in the treated and untreated stifles. Necropsy procedures were followed according to a previously reported procedure[15] and summarized in Appendix F. Gross and histopathology samples were collected, stained, and graded by one investigator (CK) according to a previously published scoring system[15] summarized in Appendix G. Mean scores were analyzed according to zone, category and total OA score.

Statistical Analysis

Calculation of mean values of each outcome parameter at each time point and all statistical analyses were performed using a computer software program (PROC MIXED, SAS, 9.2, SAS Institute, Cary, NC). All data were expressed as mean ±

standard error of the mean (SEM). All outcomes were assessed for normality by analyzing residual plots. All parameters were normally distributed, except PGE_2 concentrations and IAP. These data were logarithmically transformed to provide normally distributed data. Since some IAP values were negative, a value of 10 was added to each data point before computing the log transformation.

A mixed model repeated measures analysis of covariance (ANCOVA) was performed on all data (except gross pathology and histopathology) to assess differences in clinical and physiologic outcome parameters between the treated and untreated pelvic limbs in 6 dogs over time. Independent variables include age, MRFE-treated limb (right vs. left) and time factors (Baseline; 4 weeks post MRFE; 4, 8, 16 weeks post CCLR) related to MRFE injury and cruciate rupture. Dependent variables include clinical and physiologic outcome variables. Least square means were used for individual comparisons among the interactions of time * treated limb. A mixed-model ANCOVA was used to detect differences between gross and histologic outcome parameters between the treated and untreated pelvic limbs. The covariate of 'weeks to rupture' was included in all analyses and defined as the dog sustaining a CCLR within first week post operative (early = '0'); CCLR at 6 weeks post-op (mid = '1'); CCLR occurring greater than 8 weeks post surgery (late = '2'). Significant differences were detected at $p \leq 0.05$.

RESULTS

Passive Joint Range of Motion

Goniometric parameters did not show significant change between treated and untreated limbs prior to or post CCLR or when compared to baseline, with the

exception of tarsal joint flexion angles. No significant difference in tarsal flexion angles in the treated limb was seen at any time following MRFE; however, a significant decrease was found at all time points post CCLR (Figure 1).

Thigh Circumference

Thigh circumference measurements were not significantly different between the treated and untreated pelvic limb at any time point post MRFE. However, significant differences were seen as early as 1 week post CCLR between the two pelvic limbs and remained significantly lower in the treated pelvic limb throughout the duration of the study (Figure 2).

Stifle Radiography

Significant differences in stifle radiographic OA scores were seen at 8 and 16 weeks post CCLR, with an increase in signs of OA in treated limbs (Figure 3).

Ligamentous Instability

Significant differences in the results of the cranial drawer test and tibial thrust between treated and untreated pelvic limbs were seen weekly at all time points post CCLR, where cranial drawer test scores were greater in the treated limb (Figure 4). Cranial drawer test scores were significantly different at 6 and 15 weeks post MRFE surgery which corresponded to three of the dogs that ruptured their CCLs at 6 weeks post MRFE and 1 dog rupturing her CCL at 15 weeks post MRFE surgery.

Pain and Lameness

Subjective pain scores did not show significant differences prior to or post CCLR. Lameness scores were significantly higher in the treated pelvic limbs when compared to the untreated pelvic limbs at all time points post CCLR (Figure 5).

Stifle Joint Effusion

Stifle effusion scores significantly increased in the treated pelvic limb compared to the untreated limb in the first 3 weeks following MRFE, and at 6 and 13 weeks post MRFE (Figure 6). Effusion scores increased in the treated stifle joint at 14 (P = 0.08) and 15 (P = 0.08) weeks post MRFE, however, these values were not significant. Joint effusion scores post CCLR were significantly higher in the treated limb compared to the untreated limb at all time points (Figure 6).

Intra-articular Pressure

IAP within the untreated stifle joint were negative (subatmospheric) at baseline and remained so throughout the study. In the treated stifle, IAP significantly increased compared to the untreated stifle 4 weeks after MRFE surgery and remained elevated at all time points post CCLR (Figure 8). The highest mean pressure increase was seen at 4 weeks post MRFE, where the IAP differed by 15 mm Hg between the treated and untreated stifles.

Synovial Fluid PGE_2 Concentration

Synovial fluid sample volumes ranged from a minimum of 10 μL to a maximum of 1,000 μL in the untreated pelvic stifle and a minimum of 150 μL to a maximum of 2.25 mL (2,250 μL) in the treated stifle. The interaction of time * treated was not significant; however, a positive trend was noted (P = 0.09). Concentrations of prostaglandin E_2 in synovial fluid significantly increased in treated stifles when compared to the untreated stifle and to baseline values at all time points post CCLR. PGE_2 concentrations increased over 5 fold at 4 weeks after CCLR in the

treated stifle compared to the untreated stifle, and remained approximately 3-fold higher for the duration of the study (Figure 7).

Gross Pathology

Evaluation of gross pathology revealed that 20-40% of the caudal fibers of the CCL remained intact in treated limbs in 4 of 6 dogs. Of these four dogs, two ruptured their CCL within the first week and two dogs had ruptured CCLs at 6 weeks. The remaining 2 dogs had complete rupture of the CCL, with 0% of the fibers remaining at necropsy. One of these dogs ruptured their CCL at 6 weeks and the other ruptured at 15 weeks post MRFE surgery. Gross pathology scores comparing the treated and untreated stifles were significantly different in all zone, location and total score categories. Gross scores in the treated pelvic stifle had higher scores compared to the untreated stifle, indicating an increased presence of signs of OA at 16 weeks post CCLR (Figures 9-11).

When compared to the untreated stifle, meniscal evaluation in the treated stifle showed significantly higher scores in all zones. Primarily revealing scores of 2 and 3, which indicated incomplete or complete tear(s), respectively, the highest scores were found in the middle and posterior zones of the medial menisci (Figure 9). Macroscopic evaluation of menisci of untreated stifles revealed scores of 0-1, indicating no meniscal pathology to fibrillation only[15], with the exception of a single dog who ruptured their CCL at 15 weeks post MRFE. A score of 2, indicating incomplete tear(s)[15] was recorded in the middle zone of the medial meniscus of this untreated stifle.

Histopathology

Histopathologic outcome parameters did not differ between the treated and untreated pelvic limbs within zone, location or total histologic score categories (Tables 1-2; Figures 12).

DISCUSSION

The purpose of this study was to identify clinical outcomes that correspond to the development of CCL disease and rupture. Several clinical measures, such as goniometry and pain scores, did not show significant differences prior to CCLR. Positioning for goniometric measurements was similar to performing the tibial compression test[3], as the stifle was held in approximately 115° (neutral position) of flexion while the tarsal joint was moved into flexion. Discomfort may have been experienced as the tibia was flexed, causing cranial tibial translation relative to the femur once the CCL had ruptured, indicating a positive tibial compression test.[3] Hock flexion range of motion differed slightly from literature reports of 110°.[16] This study reported a neutral stifle angle of 105° from which goniometric tarsal joint measures were recorded.[16] In our study, stifles were extended by an additional 10° during goniometric measures, possibly lending to a decrease in available flexion range at the tarsus due to limitations in range of motion of passive constraints.

Muscle atrophy, lameness and a positive cranial drawer sign are all clinical signs well documented in the diagnosis of CCLR.[3,5] As expected, thigh circumference decreased and lameness and cranial drawer test scores increased at all time points post CCLR in the treated limb. Thigh circumference measures also decreased slightly following MRFE, however, these values were not significant. No significant difference was seen in lameness scores at any time point post MRFE,

suggesting that clinical signs of lameness in research dogs may be difficult to detect until the CCL actually ruptures. This may be explained by the fact that these research dogs were exposed to minimal exercise regimens daily, rather than frequent leash walks or running free, as many family pets experience. Lameness and cranial drawer tests correspond well to the onset of CCL rupture; however, are not changed in subclinical CCL injury.

Joint effusion, detectable caudal to the patellar ligament is another common finding in dogs post CCLR.[3,5] Our findings suggest that detection of palpable effusion may be one of the earlier and simplest methods for detecting CCL disease and impending rupture. Significant increases in stifle joint effusion corresponded to times immediately following the MRFE procedure. Interestingly, increased effusion was palpable 2 weeks prior to the dog that ruptured at 15 weeks post MRFE; whereas, no detectable effusion was noted prior to the dogs rupturing at 6 weeks. This suggests that joint effusion may be detectable upon palpation prior to CCLR, however, our small sample size (n=1) warrants further investigation.

Pain is a common sign in dogs with CCLR.[3] However, our results did not show any significant change in subjective pain scores prior to or 4 months post CCLR using the CSU score. One reason for this finding may be explained by the fact that the pain scale is primarily designed to assess acute, postoperative pain, as are most pain scales in veterinary medicine.[7] In addition, this pain scale is designed for family pets, not cage-confined research dogs. The primary reason for including a pain scale in this project was to determine if analgesic medication was needed post operatively.

CCLR may produce radiographic changes including thigh muscle atrophy, joint effusion, and osteophyte formation.[5] Radiographic comparison with the contralateral stifle may be useful to evaluate bilateral disease. In our study, the severity of OA on radiographs increased in the treated stifle compared with the contralateral stifle, however, the degree of OA was still considered mild at these time points. Radiographs were also used in our study to assist in the diagnosis of CCLR and to rule out other bony orthopedic or soft tissue abnormalities.[5]

To our knowledge, our study is the first project to measure IAP and electromyography (see Chapter 3) in dogs with CCL injury and subsequent rupture over time, where effects of joint movement, position and muscle activation are considered to determine if increased IAP may influence muscle timing. Synovitis can occur in any form of articular soft tissue damage causing joint effusion.[11] Our results indicate that mean IAP significantly increased post MRFE and CCLR, with the highest change (15 mm/Hg) in IAP occurring at 4 weeks post MRFE surgery. These results suggest that an increase in synovial fluid volume, as indicated by an increase in IAP, occurs due to increased joint effusion in injured and ruptured CCLs, with the highest pressure recorded at 4 weeks post CCL rupture. However, muscle timing results were not significant; therefore, effects of increased IAP on muscle timing are inconclusive. IAP increased at time points when palpable effusion was indistinguishable from the untreated stifles. The IAP may result from undetected effusion or a combination of effusion and decreased capsular compliance. Either way, it is possible that elevated IAP could be the earliest clinically detectable sign of CCL disease, though its clinical practicality is questionable.

Inflammation within the stifle joint is further evidenced by an increase in prostaglandin E2 concentrations in the treated limb. A nonsignificant increase in PGE_2 was found at 4 weeks post MRFE. The highest concentration was found at 4 weeks post CCLR, suggesting that PGE_2 is involved in acute inflammation associated with CCLR.[10] Our results coincided with that of Trumble, et. al., who found a significant increase in PGE_2 concentrations post CCL transection compared to baseline values.[10] Similar to our findings, they found a positive correlation between PGE_2 concentrations and lameness scores and a significant negative correlation between PGE_2 concentrations and PVF, as measured by force platform analysis (see Chapter 2).[10]

Our PGE_2 concentrations, however, were much higher in the treated stifles at 4, 8 and 16 weeks post CCLR, compared with values reported by Trumble, et al., which ranged from 230, 200 and 130 pg/mL, at 2, 10 and 18 weeks post CCLR, respectively.[10] MRFE surgery produces a more gradual deterioration of the CCL and onset of CCLR[6], possibly allowing for a greater accumulation of inflammatory mediators. The use of the MRFE technique itself, as the probe is passed over the entire dorsal surface of the CCL, may also incite an inflammatory response that may contribute to the larger concentration of PGE_2 seen at 4 weeks post MRFE and post CCLR.[6]

Small synovial fluid volume samples did not allow for the analysis of other clinical variables, such as total protein content or white blood cell count.[10] Color and clarity descriptions were typical of an intact stifle (untreated = colorless, clear) and acute trauma (treated = pink, cloudy).[3]

Gross pathology and histopathology scores were used to report the global severity of stifle OA. Signs of OA have been found on weightbearing surfaces of the medial and lateral femoral condyles and tibial plateaus[15], consistent with results from our study when these regions were pooled. The lack of significant results in our study suggests that severity of OA at these sites is similar; thus, when pooled and compared between the treated and untreated pelvic limbs, minimal differences exist. The severity of OA had not progressed enough to determine a significant difference by 16 weeks post CCLR. These findings contrast with other studies that report high variability in the grading and increased severity of OA lesions 12 weeks post CCL transection.[21] However, their study evaluated a single area of cartilage using the Mankin scoring system, which is not as global as the scoring system used in our study.[15]

On a cellular level, histopathology scores tended to be higher in the treated stifles; however, no significant difference between the grade of severity of OA was detected in any histopathologic outcome variables. These results may indicate that cellular damage to the contralateral, untreated limb is similar to that of the treated limb. Similar OA severity was seen bilaterally in the synovium, menisci and articular cartilage may be due to the relatively short duration of this study (16 weeks post CCLR). Progression of OA is known to exist in CCL-transected stifle joints over time[22], especially in the medial femoral condyle and tibial plateau, due to a caudal shift of stifle contact areas and increased peak pressure post CCL transection.[23] The caudal shift of the medial femoral condyle in the unstable limb during weightbearing is believed to occur during cranial tibial translation.[23] These altered mechanics are

thought to shift the normal femorotibial contact areas to regions of thinner cartilage, not normally suited to withstand increased loads, resulting in the development of OA.[24-26] Our study concluded at 16 weeks post CCLR suggesting that this may not have enough time to show a significant difference between zones, locations or total scores in treated and untreated limbs.

In summary, joint effusion corresponded well to subclinical rupture of the CCL. Targeting certain outcome variables, such as joint effusion, can may be used to detect CCL injury in the clinic could assist with development of a prescreening tool to detect subclinical signs of CCL disease. Physiologic outcome measures, such as IAP, PGE_2, and gross and histological assessment are not a feasible routine measure to perform in a clinical setting, but provide valuable information pertaining to in vivo assessment of inflammation and OA severity. Other clinical signs, such as thigh circumference, pain, lameness, goniometry, cranial drawer test and radiography, did not identify subclinical CCL disease or injury, but are best used to confirm eventual CCLR. Formal correlations between outcome parameters and CCL injury are warranted to determine the strength of relationships between these variables and CCL injury and rupture.

Investigator Abbreviations:

AV = Alex Valdes-Martinez, DVM, DACVR

CA = Caroline Adrian, MSPT, PhD Candidate

CK = Christopher Kawcak, DVM, PhD, DACVS, DACVSMR

KH = Kevin Haussler, DVM, DC, PhD, DACVSMR

RP = Ross Palmer, DVM, , DACVS

Manufacturer Addresses:

a. Model 01135, Lafayette Instrument, Lafayette, IN

b. Gulick II, Country Medical Technology Inc., Gays Mills, WI

c. Intra-compartmental pressure monitor system, Stryker, UK

d. C2 Ethyl Solid Phase Extraction Columns, Agilent Technologies, Santa Clara, CA

TABLES AND FIGURES

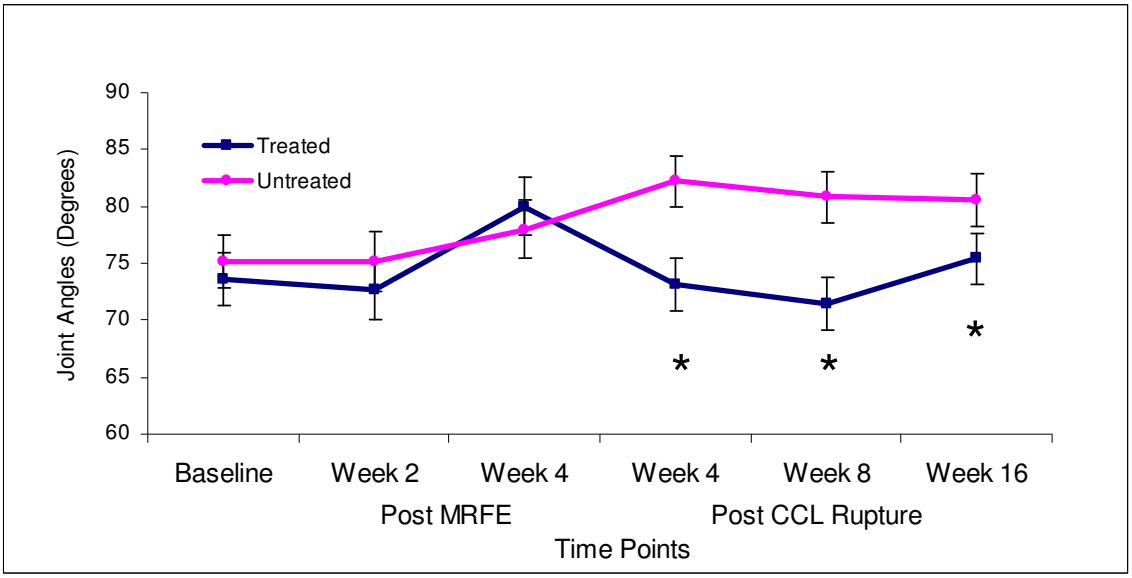

Figure 1 – Mean passive range of motion for treated and untreated tarsal joints across time (N = 6). * Significantly ($P < 0.05$) different from contralateral limb. No significance in tarsal flexion angles were seen at any time post MRFE, but decreased in the treated limb at all time points post CCL rupture.

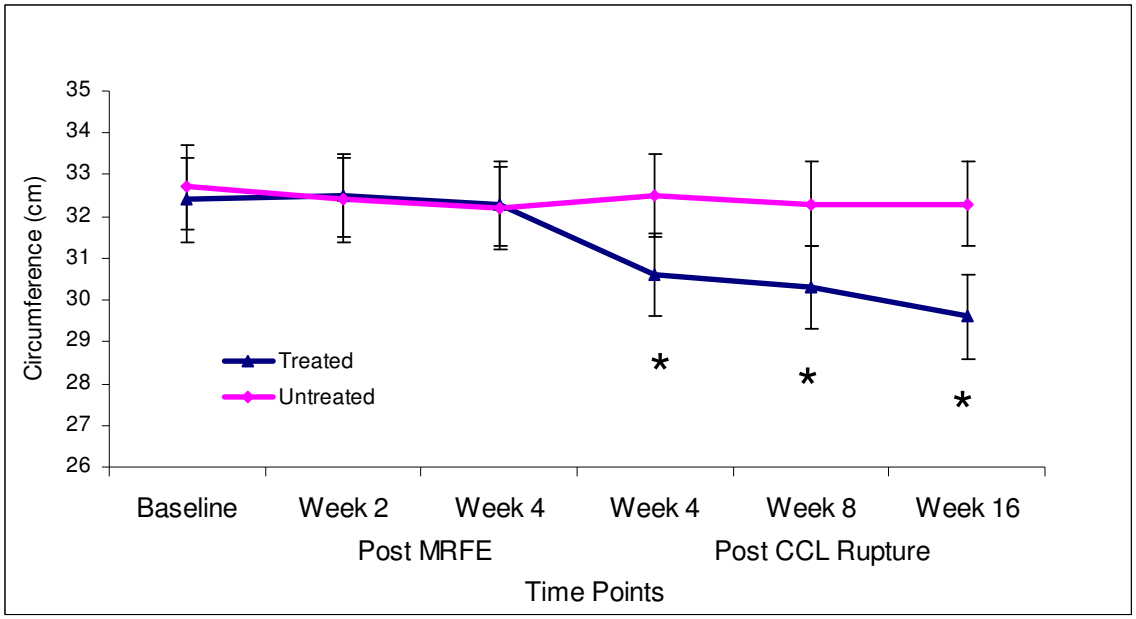

Figure 2 – Mean thigh circumference of treated and untreated limbs across time (N = 6). * Significantly ($P < 0.05$) different from contralateral limb. Thigh circumference measurements were not significant between the treated and untreated pelvic limb at any time point post MRFE, though significantly different at all time points post CCL rupture.

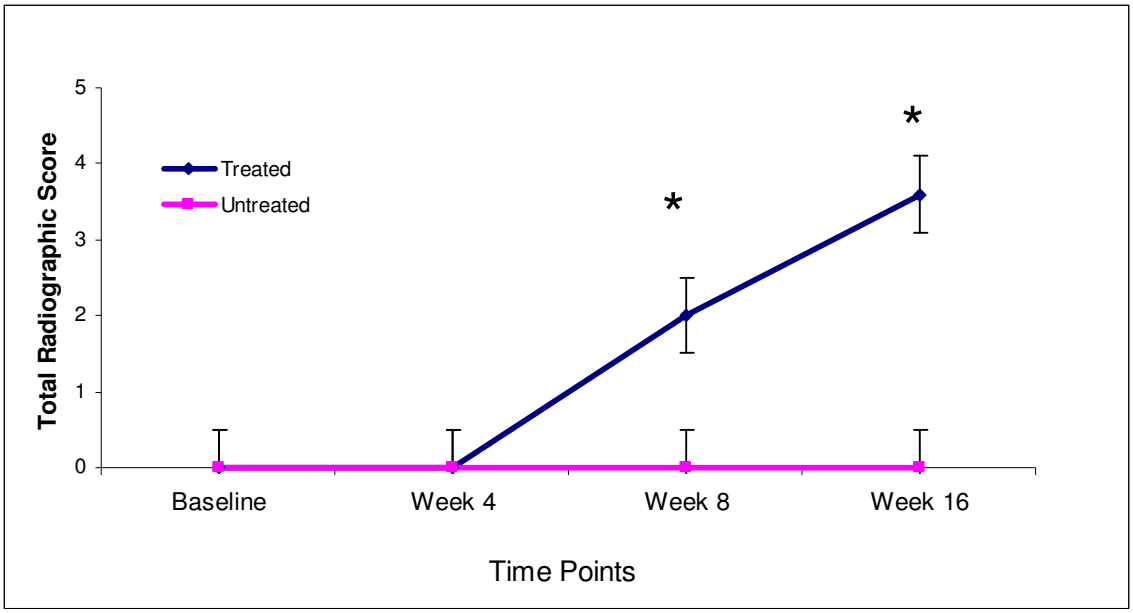

Figure 3 – Mean subjective total radiographic score of treated and untreated pelvic limbs at baseline and at 4, 8 and 16 weeks post cranial cruciate ligament rupture (N = 6). * Significantly ($P < 0.05$) different from contralateral limb. Significant differences in stifle radiographic OA scores were seen at 8 and 16 weeks post CCL rupture.

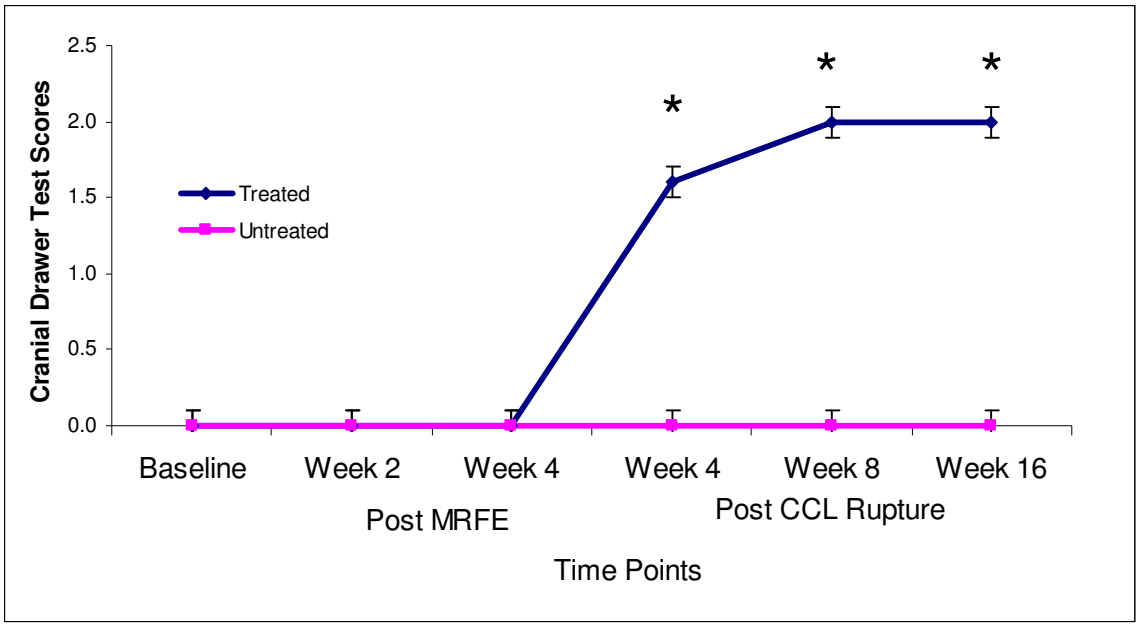

Figure 4 – Mean cranial drawer test scores of treated and untreated pelvic limbs across time. * Significantly ($P < 0.05$) different from contralateral limb. Significant differences in the results of the cranial drawer test between treated and untreated pelvic limbs were seen weekly at all time points post CCL rupture.

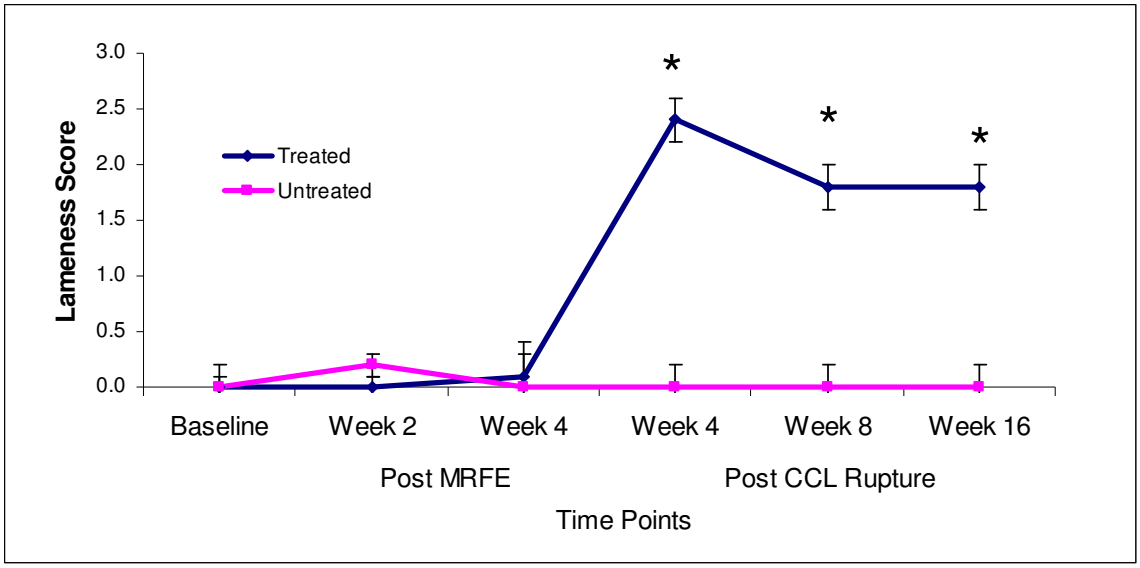

Figure 5 – Mean lameness scores of treated and untreated pelvic limbs across time (N = 6). * Significantly ($P < 0.05$) different from contralateral limb. Lameness scores were significantly different between the treated and untreated pelvic limbs at all time points post CCLR

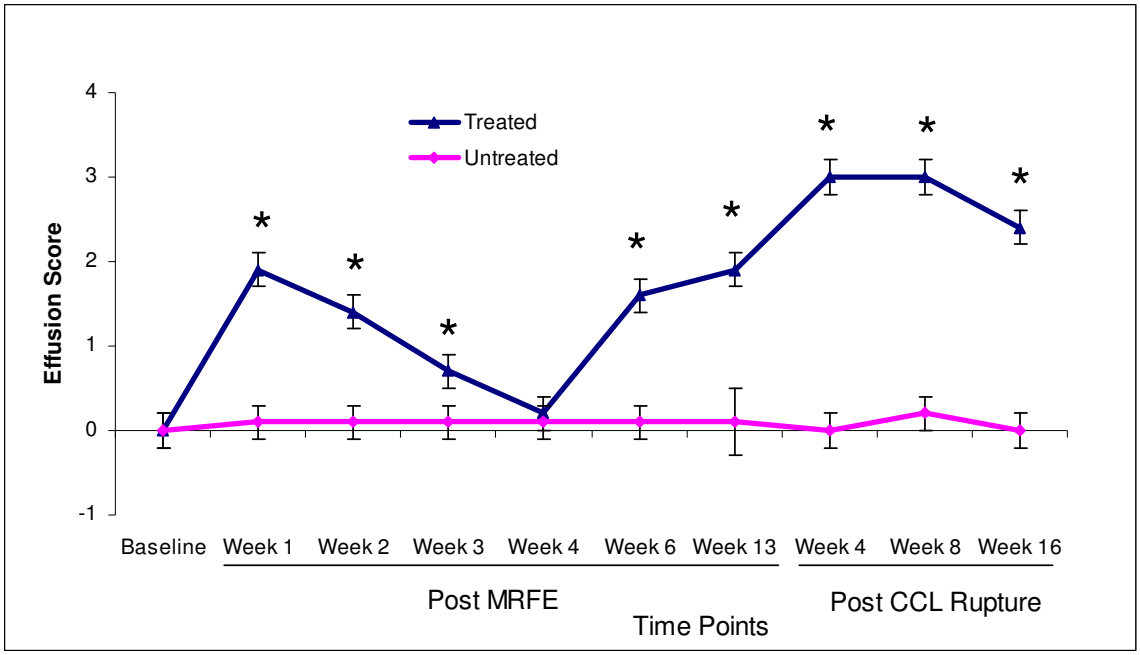

Figure 6 – Mean joint effusion scores of treated and untreated stifles at 10 time points prior to and post CCLR (N = 6). * Significantly ($P < 0.05$) different from contralateral stifle. Stifle joint effusion scores significantly increased in the treated limb compared to the untreated stifle in the first 3 weeks following MRFE, and at 6 and 13 weeks post MRFE. Stifle joint effusion scores post CCL rupture were significantly higher in the treated stifle compared to the untreated stifle at all time points.

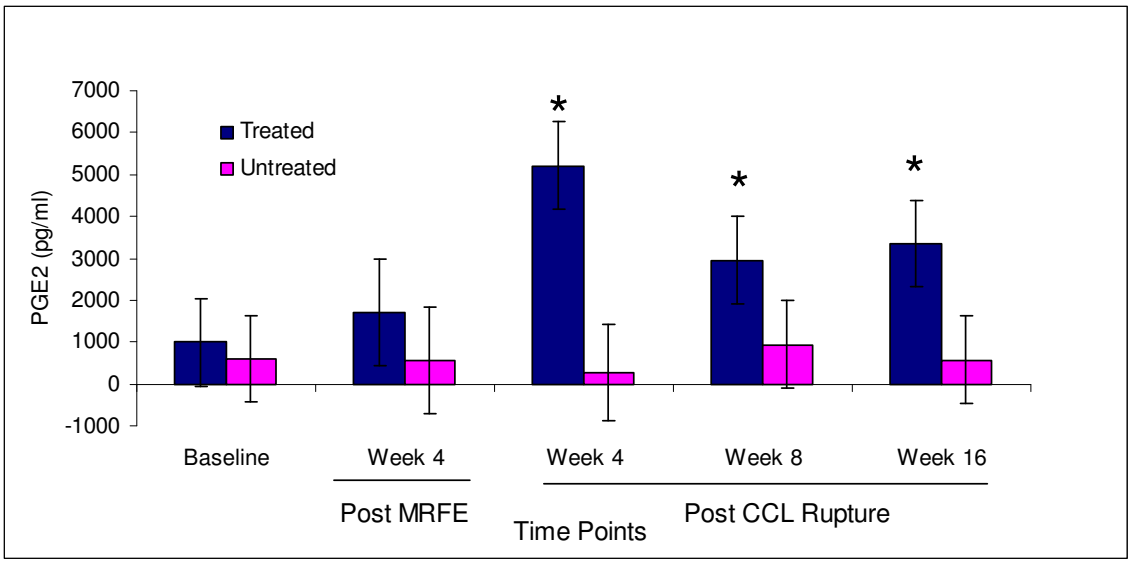

Figure 7 – Mean prostaglandin E_2 (PGE_2) concentrations in treated and untreated stifles over time prior to and post CCLR (N = 6). * Significantly ($P < 0.05$) different from contralateral stifle. Concentrations of PGE_2 in synovial fluid significantly increased when compared to the untreated stifle and to baseline at all time points post CCL rupture.

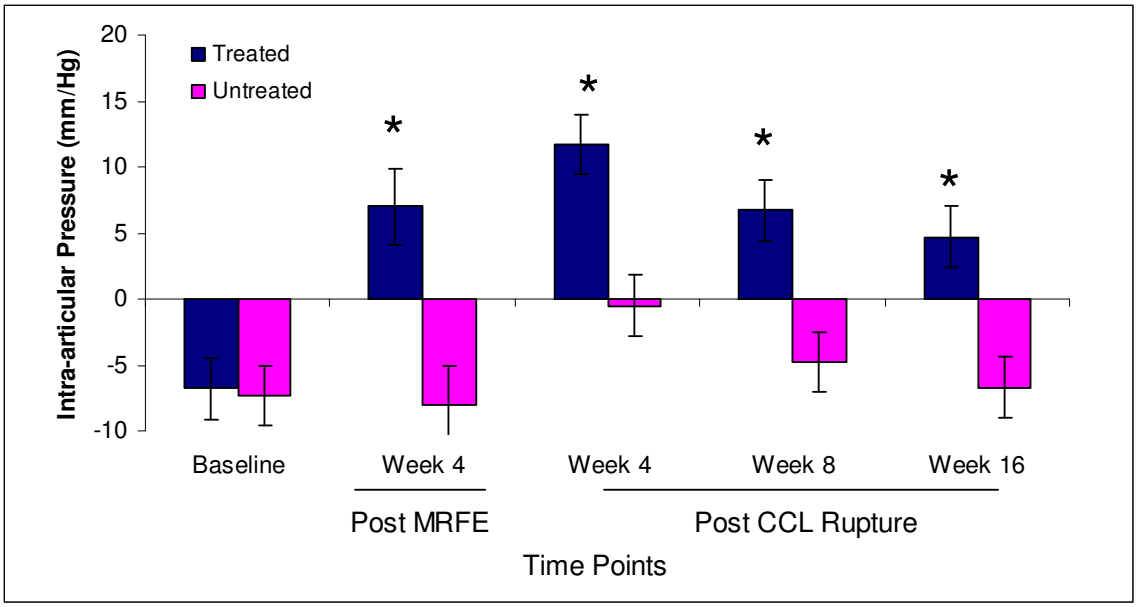

Figure 8 – Mean stifle intra-articular pressures (IAP) in treated and untreated stifles over time prior to and post cranial cruciate ligament rupture (N = 6). * Significantly ($P < 0.05$) different from contralateral stifle. IAP within the stifle was negative at baseline and remained so throughout the study in the untreated stifle. In the treated stifle, IAP significantly increased compared to the untreated stifle 4 weeks after MRFE surgery and at all time points post CCL rupture.

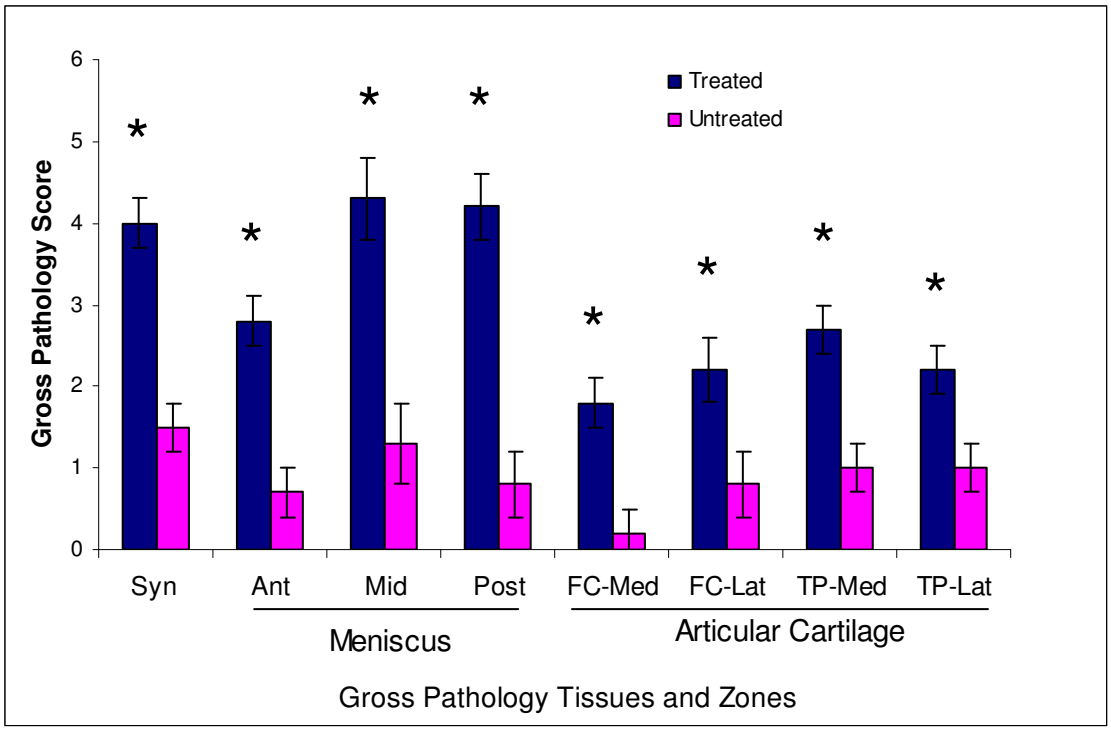

Figure 9 – Mean stifle gross pathology scores in treated and untreated stifles analyzed by zones within tissues. Zone abbreviations include: SYN = synovium; ANT = cranial meniscus; MID = middle meniscus; POST = caudal meniscus; FC-MED = articular cartilage samples from the medial femoral condyle; FC-LAT = articular cartilage samples from the lateral femoral condyle; TP-MED = articular cartilage samples from the medial tibial plateau; TP-LAT = articular cartilage samples from the lateral tibial plateau. * Significantly ($P < 0.05$) different from contralateral limb. Gross pathology scores comparing the treated and untreated stifles were significantly different in all zone categories. Gross scores in the treated stifles had higher scores compared to the untreated stifles, indicating an increased presence of signs of OA at 16 weeks post CCLR.

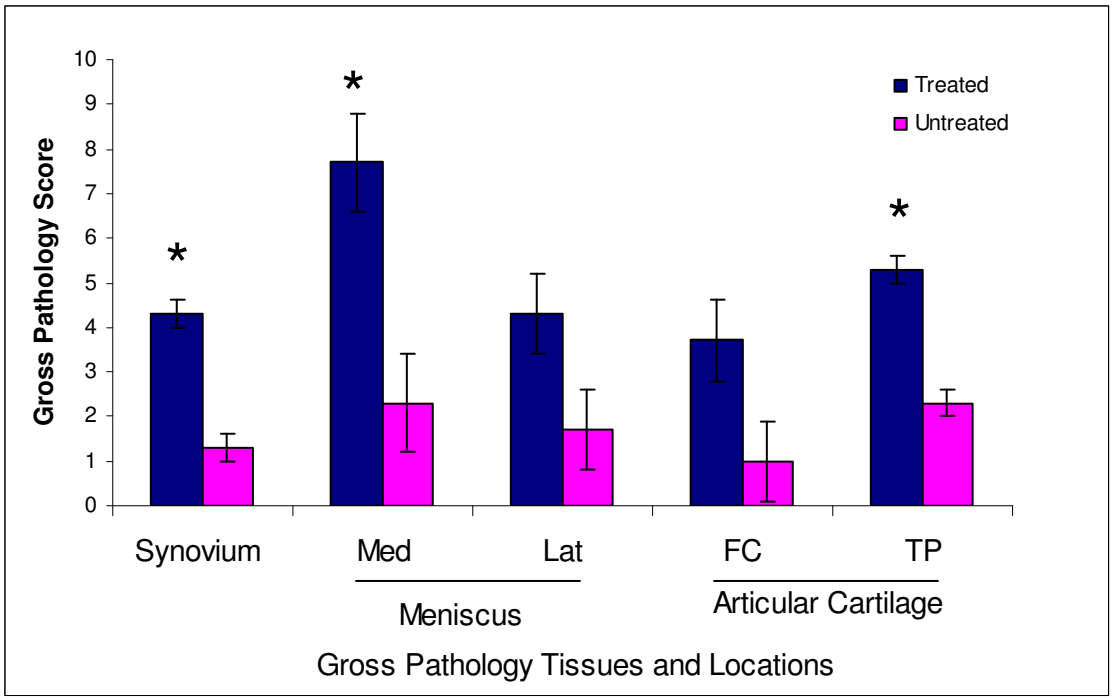

Figure 10 – Mean stifle gross pathology scores in treated and untreated stifles analyzed by location (N = 6). Location abbreviations include: SYN = synovium; MED = medial menisci; LAT = lateral menisci; FC = articular cartilage samples from femoral condyles; TP = articular cartilage samples from tibial plateaus. * Significantly ($P < 0.05$) different from contralateral stifle. Gross pathology scores were significantly different in the synovium, medial menisci and tibial plateaus.

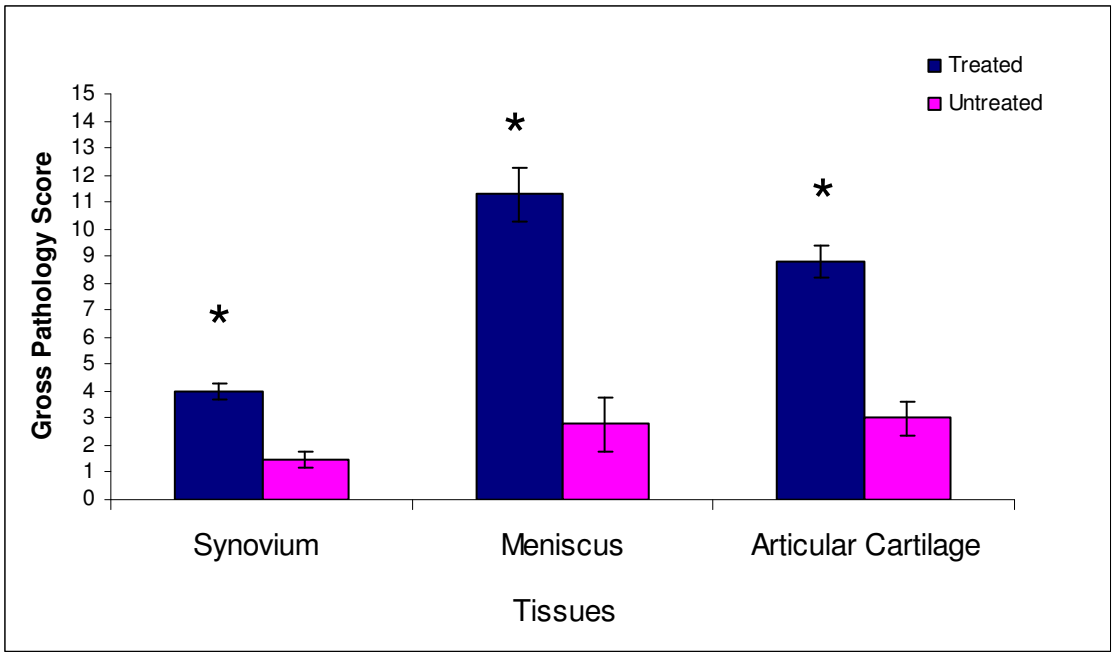

Figure 11 – Mean stifle cumulative gross pathology scores in treated and untreated stifles. * Significantly ($P < 0.05$) different from contralateral stifle. Total scores were significantly different in all tissues.

Table 1 – Mean stifle histopathology scores in treated and untreated stifles analyzed by zone (N = 6). Histopathologic outcome parameters did not differ between the treated and untreated stifles by zone.

Histopathology Scores		
Tissue / Zone	Untreated	Treated
Synovium		
Medial	6.8 ± 2.6	9.5 ± 2.6
Axial	5.2 ± 4.0	12.2 ± 2.9
Lateral	3.9 ± 1.9	7.9 ± 2.5
Mensici		
Medial, Cranial	4.5 ± 1.5	6.5 ± 1.5
Medial, Middle	4.3 ± 1.1	5.3 ± 1.1
Medial, Caudal	4.3 ± 1.1	4.8 ± 1.1
Lateral, Cranial	1.1 ± 1.0	2.7 ± 0.9
Lateral, Middle	2.2 ± 0.8	2.2 ± 0.8
Lateral, Caudal	3.4 ± 0.7	4.3 ± 0.7
Articular Cartilage Pathology		
Medial Femoral Condyle	6.6 ± 2.2	8.6 ± 2.2
Lateral Femoral Condyle	6.5 ± 2.5	10.0 ± 2.5
Medial Tibial Plateau	11.1 ± 3.2	16.8 ± 3.2
Lateral Tibial Plateau	10.2 ± 3.0	13.5 ± 3.0
Chondrocyte Pathology		
Medial Femoral Condyle	15.8 ± 4.8	24.5 ± 4.8
Lateral Femoral Condyle	17.8 ± 4.9	22.5 ± 4.9
Medial Tibial Plateau	22.2 ± 5.8	27.2 ± 5.7
Lateral Tibial Plateau	24.0 ± 4.4	24.7 ± 4.4
Proteoglycan Pathology		
Medial Femoral Condyle	28.9 ± 4.2	22.4 ± 4.2
Lateral Femoral Condyle	29.2 ± 4.4	37.7 ± 4.4
Medial Tibial Plateau	31.3 ± 5.4	33.4 ± 5.4
Lateral Tibial Plateau	39.6 ± 4.2	39.3 ± 4.2

Table 2 – Mean stifle histopathology scores in treated and untreated stifles analyzed by location. Histopathologic outcome parameters did not differ between the treated and untreated stifle by location.

Histopathology Scores		
Tissue / Zone	Untreated	Treated
Synovium		
Lining Cells	5.3 ± 2.0	10.9 ± 2.0
Lining	3.8 ± 2.4	9.4 ± 2.4
Infiltrate	3.6 ± 3.0	7.4 ± 3.0
Mensici		
Medial	13.1 ± 3.2	16.6 ± 3.2
Lateral	6.7 ± 2.1	9.2 ± 2.1
Articular Cartilage Pathology		
Femoral Condyles	13.1 ± 4.5	18.3 ± 4.5
Tibial Plateaus	21.3 ± 5.9	30.3 ± 5.9
Chondrocyte Pathology		
Femoral Condyles	33.6 ± 9.4	46.9 ± 9.4
Tibial Plateaus	46.2 ± 10.1	51.9 ± 10.1
Proteoglycan Pathology		
Femoral Condyles	58.1 ± 7.5	60.1 ± 7.5
Tibial Plateaus	70.9 ± 9.2	72.7 ± 9.2

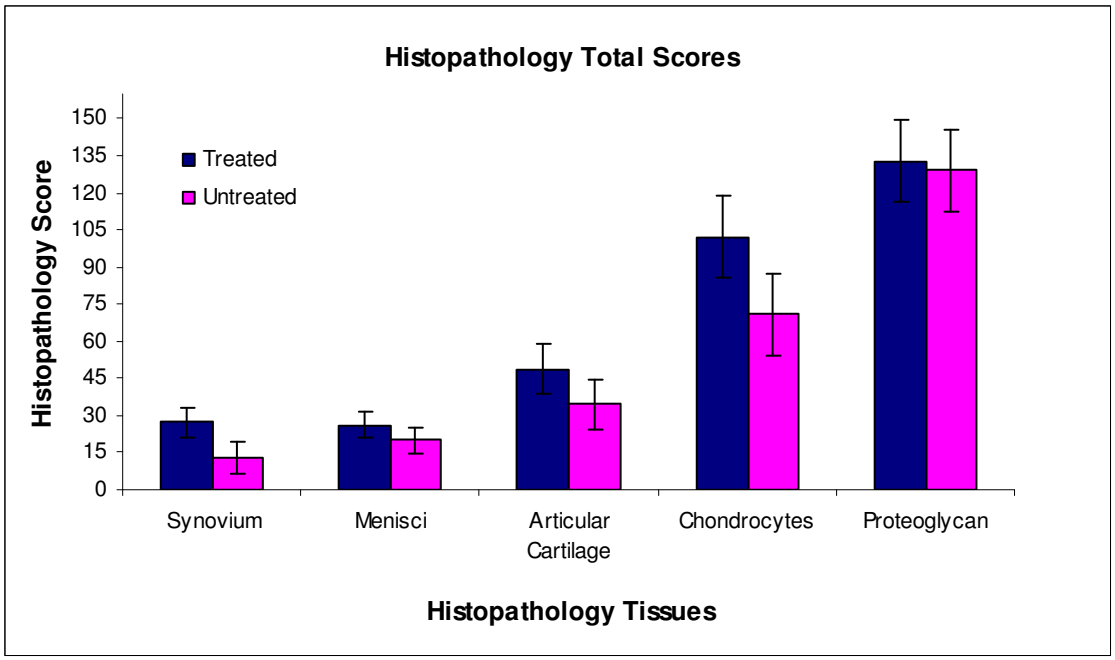

Figure 12 – Mean stifle histopathology in treated and untreated stifles analyzed by cumulative scores for each region at 16 weeks post cranial cruciate ligament rupture (N = 6). Histopathologic outcome parameters did not differ between the treated and untreated stifles.

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CHAPTER 5

SUMMARY OF DISSERTATION FINDINGS

GENERAL CONCLUSIONS

Kinetics

- No kinetic compensations were detected between the ipsilateral or contralateral thoracic limbs during subclinical degeneration of the CCL or post CCL rupture.

- No significant kinetic differences in the treated pelvic limb, compared to the untreated limb, were found post MRFE-induced CCL injury, prior to CCLR.

- Significant kinetic differences in the treated limb, compared to the untreated limb, were found in the majority of outcomes post CCLR.

Kinematics

- No kinematic compensations were detected between the ipsilateral or contralateral thoracic limbs during subclinical degeneration of the CCL or post CCLR.

- Post MRFE-induced CCL injury, hip joint range of motion significantly decreased, limiting motion in the untreated hip during stance phase of gait.

- MRFE-induced CCLR produced alterations in kinematics during stance in both pelvic limbs. The most notable difference was an increase in stifle and tarsal joint flexion angles in the contralateral, untreated limb.

EMG

- No statistically significant differences were found for EMG outcomes, though visually apparent trends are noted.

- Muscle onset times tended to show a delay in muscle activation of all muscles post MRFE-induced CCL injury; while muscle activation tended to occur earlier in all muscles bilaterally post CCLR.

- Activation durations tend to correspond with differences in muscle onset times as durations tended to decrease post MRFE-induced CCL injury and increase post CCLR.

- MRFE-induced CCL injury appears to produce muscle weakness in the vastus lateralis and biceps femoris muscles as evidenced by a decrease in peak EMG amplitude during the stride; however, no significant differences were found.

Clinical Outcomes

- Pelvic limb muscle atrophy, as measured by thigh circumference, begins immediately following CCL rupture and continues to decrease for 16 weeks post CCLR.
- Joint effusion was consistently detected upon subjective evaluation at 4, 8 and 16 weeks post CCLR.
- It is not expected that increased IAP had any effect on muscle activity.
- Additional time post CCLR may be needed to detect signs of OA.

GENERAL DISCUSSION

The focus of this dissertation was evaluation of kinetic, kinematic, electromyographic, and selected clinical and physiologic parameters during subacute, acute and chronic phases of cranial cruciate ligament (CCL) injury and subsequent rupture. CCL transection is the most common model of CCL rupture, however, we chose the MRFE model of CCL injury that allowed for novel insights into subclinical evaluation of these outcome parameters as the CCL fibers degenerated, and eventually ruptured. This study is the first to suggest that a gradual adjustment to stifle instability produces a compensation pattern different than what is reported in kinematic studies that have used the acute CCL transection model. A key difference in MRFE-induced CCL injury is the gradual destabilization and degeneration of the CCL, allowing a window into subclinical descriptions of movement of the pelvic limb.

Kinematics

When immediate instability is induced through complete transection of the CCL, an increase in stifle flexion occurred in the CCL-deficient limb[1-4], but with increased variability in gait adaptations post CCL transection, showing that each dog compensates differently for the loss of stifle stability.[3,4] A major kinematic finding in our study showed altered kinematics in the untreated limb, as the stifle is held in significantly more flexion during the stance phase of gait post CCLR. We acknowledge that limitations exist when using 3D kinematic analysis systems, such as difficulty in locating centers of joint rotation, skin movement and conformational variability between and within breeds.[5] To minimize error, placement of reflective markers was performed by one researcher. This study is the first to suggest that a gradual adjustment to stifle instability produces a compensation pattern different than what is reported in kinematic articles that use the acute CCL transection model.

EMG

This project was novel since we investigated neuromuscular contributions to stifle stability via EMG post MRFE-induced CCL injury and rupture. A limitation in our study was that significance was not determined for any EMG results, potentially due to a small sample size of 5 dogs. Power analysis was recalculated using results from this study and a sample size of 20 dogs is needed to detect a 10% difference in muscle onset times between treated and untreated limbs.

Measures of muscle activity appear to demonstrate a possible delay in muscle activation of all muscles in the treated and untreated limbs post MRFE-induced CCL injury, with the exception of the gastrocnemius in the untreated limb. Earlier

activation of the gastrocnemius in the untreated limb may have occurred in preparation to bear more load, unload the affected limb sooner during stance, and/or as a response to co-contraction at the tarsal joint; however, the cranial tibial muscle was not monitored. Post CCLR, muscle activation appeared to occur earlier in all muscles bilaterally, possibly in an effort to stabilize the affected limb and prepare the untreated limb to accept more load during stance. The exception is the biceps femoris in the untreated limb where a delay was noted. This delay could possibly be due to a reduced need for activation of stifle flexors, as the stifle has a significant increase in flexion during stance.

Interestingly, peak EMG amplitude generally showed a decrease in the vastus lateralis and biceps femoris post MRFE-induced CCL injury and rupture and an increase in the gastrocnemius. Post CCLR, peak EMG amplitude normalized to baseline showed a general tendency to increase in the vastus lateralis and decrease in the biceps femoris in bilateral pelvic limbs. This finding may support the need for increased vastus lateralis activity in the untreated limb to prevent collapse as the stifle is held in increased flexion. In the treated limb, increased vastus lateralis EMG amplitudes may be explained by the need to resist further stifle flexion, maintaining a stiffer limb with less total excursion, to avoid cranial tibial translation.

As muscle activation patterns influenced the magnitude and loading rate of the stifle, uncoordinated muscle activity may contributed to destabilization of the stifle, increasing joint load and contributing to increased CCL strain, which ultimately leads to CCLR.[6] Though, due to a small sample size, a large number of insignificant findings, and limitations in the ability to normalize EMG across time when an MVC

is not an option, caution must be exercised when drawing any conclusions related to muscle timing, duration and amplitude of muscles providing stifle stabilization.

Clinical and Physiologic Outcomes

Traumatic rupture of the CCL occurs in 20% of dogs[7], the majority of CCL disruption occurs from progressive degeneration leading to elongation and weakening, rather than instantaneous discontinuity, with eventual complete rupture of the ligament.[8] Animal models have shown that by applying the cranial drawer test to the point of mechanical failure, intact CCL fibers remain even after femorotibial displacement, despite elongation and progressive macro- and microfailure.[9] In contrast to transection models that report complete disruption of the CCL fibers[1-4], our study showed 20-40% of the caudal fibers of the CCL that remained intact in 4 out of 6 (67%) dogs at 16 weeks post CCLR. Lopez and Markel reported that 3 out of 18 (17%) dogs had any sign of remaining ligament fibers at 28 weeks post CCLR, demonstrating the progressive nature of CCL degeneration.[10] Their additional 12 weeks to necropsy appears to allow for continued degradation of the CCL, to a point where no CCL fibers remain, though no fibers existed as early as 8 weeks post CCLR.[10] Dogs in their study were also described as having an extremely high level of activity post MRFE (M. Lopez, personal communication), which may also have contributed to complete CCL degeneration at 8 and 28 weeks post CCLR.[10,11]

Based on decreases in thigh circumferences, muscle atrophy showed a trend toward decrease following CCL injury, though a significant decrease was not shown until post CCLR. Since muscle weakness has been shown to be associated with a loss in muscle cross-sectional area (muscle atrophy) and/or a decrease in the ability to

activate muscle fibers,[12] we had confidence in the selection of thigh circumference to represent muscle atrophy. These subclinical findings correspond to EMG data that show a decrease in peak EMG amplitude in the vastus lateralis and biceps femoris at 2 and 4 weeks post MRFE. Future correlative studies could investigate use of thigh circumference (or other observable gait changes such as limb unweighting or asymmetry) as a clinical indicator of CCL injury, prior to actual rupture.

Clinical and physiologic indicators of inflammation were measured in this study to investigate the contribution of inflammation within the stifle joint to CCL injury and rupture. Increased IAP in effused joints has been shown to cause quadriceps muscle weakness in humans.[13] Joint effusion was consistently detected upon subjective evaluation at 4, 8 and 16 weeks post CCLR. Our results also showed an increase in IAP in the treated limb (3.5-10.9 mm Hg) post CCLR, indicative of an increase in joint effusion. However, pressures in the canine stifle of 10-13 mm Hg are needed before arthrogenic mechanoreceptor signals are activated, indicating distention or joint damage and potentially resulting in neural inhibition.[14] Thus, it is not expected that increased IAP had any effect on muscle activity in our study, with the possible exception of the IAP reading of 10.9 mm Hg found at 4 weeks post CCLR. It appeared that the percentage of peak EMG amplitude increased in the vastus lateralis post CCLR, but no significant difference. Therefore, it is not expected that the increased IAP resulted in muscle inhibition throughout the course of the study.

PGE2 concentrations are a major indicator of inflammatory disease and were found to be significantly increased at all time points post CCLR. These values were

higher than a previously reported study who showed baseline PGE2 values in a CCL transection model to be approximately 40-50 pg/ml[15]; whereas, our results indicate baseline levels of 500-600 pg/ml. Higher values are most likely explained by the fact that thermal MRFE treatment incites inflammatory responses that are proposed to contribute to the degradation of the cruciate ligament.[10,16]

This study reports the first comprehensive OA severity scores at post mortem examination of stifles at that had undergone MRFE-induced CCL injury and rupture. The original paper describing the MRFE-induced CCL injury technique stated that pathologic changes were similar to changes seen post CCL transection, without further elaboration.[10] Transection models typically report a single area for analysis, not allowing for global assessment of all tissues, such as cartilage, subchondral bone, synovium and menisci.[17] In addition, a large variation in the grades and severity of OA lesions post CCL transection is reported.[17] Our study included a comprehensive grading scheme for global assessment of tissues that may be reproduced at specific sites within the joint to determine repeatability of OA lesions in an MRFE-induced model of CCL injury and rupture. Altered kinematics have been implicated as a causative factor in the development of OA; however, most studies have found this association to occur in CCL-deficient stifles.[18-20] Studies on altered kinematics in normal stifles are warranted to determine the influence of kinematics on normal cartilage and its possible relationship to contralateral CCLR. Future investigations may lead to insights related to kinematic and EMG differences in a gradual model of CCLR over longer study durations contributing knowledge related to slowing the degenerative process and contralateral CCLR.

FUTURE DIRECTIONS

This study stimulates a variety of future directions that are relevant to neuromuscular alterations in gait and may provide a basis for surgical and rehabilitative treatment of CCL disease. If muscle weakness is significant post CCL injury, as evidenced by a decrease in peak EMG amplitudes, why does weakness occur in first place? Does fatigue play a role in muscle weakness and delays in muscle activation? What types of physical activity (e.g. athletic trials, repetitive motion) trigger muscle fatigue and/or microtrauma within the CCL that are theorized to contribute to CCL degeneration and OA development? How might outcome parameters be utilized in the clinic to detect and possibly avoid early microtrauma in the stifle and possibly prevent CCL disease? What types of preventative surgical and/or rehabilitation interventions may be established to decrease morbidity associated with CCL disease? What types of therapeutic interventions are necessary to reduce the risk of contralateral CCL rupture? Investigating the in depth relationship of the 3 variables (kinetics, kinematics and electromyography), which is required for a biomechanical analysis, may provide the scientific rationale to interpret their meaning related to analysis of movement (primarily for non-clinicians, as well as interested clinicians) and application to patient care (primarily clinicians). One example of a clinical intervention that might change based on the findings of this study may be that if the goal was to increase co-contraction, or joint stability and stiffening of the stifle, one might consider targeting strengthening the hip extensors and plantarflexors. Inclusion of observational gait analysis is important to compare biomechanical variables to movement analyses performed in the clinic.

Consequences of CCL injury and rupture and gait compensations as seen only from the sagittal plane may become clearer when observed from other planes. Ground reaction forces and kinematics combined with inverse dynamics, or calculating the net muscular moments acting about the stifle, are an important next step in providing net flexor and extensor muscle contributions to gait mechanics and stifle stability post MRFE-induced CCL injury and rupture. This information may contribute to the development of neuromuscular training programs to target specific affected muscles, whether designed for perturbation or strength training, and aimed at reducing the risk for CCL disease.[21] Further investigation of the neuromuscular contribution to stifle stability is warranted. Results from this study indicate that a sample size of 20 dogs is needed to detect EMG differences between the treated and untreated limbs. Despite variability in CCL time to rupture, the MRFE-induced CCL injury and rupture model may be considered for future studies, as gradual degeneration of the CCL produced kinematic results different than those reported in the literature with CCL transection models of CCL rupture.

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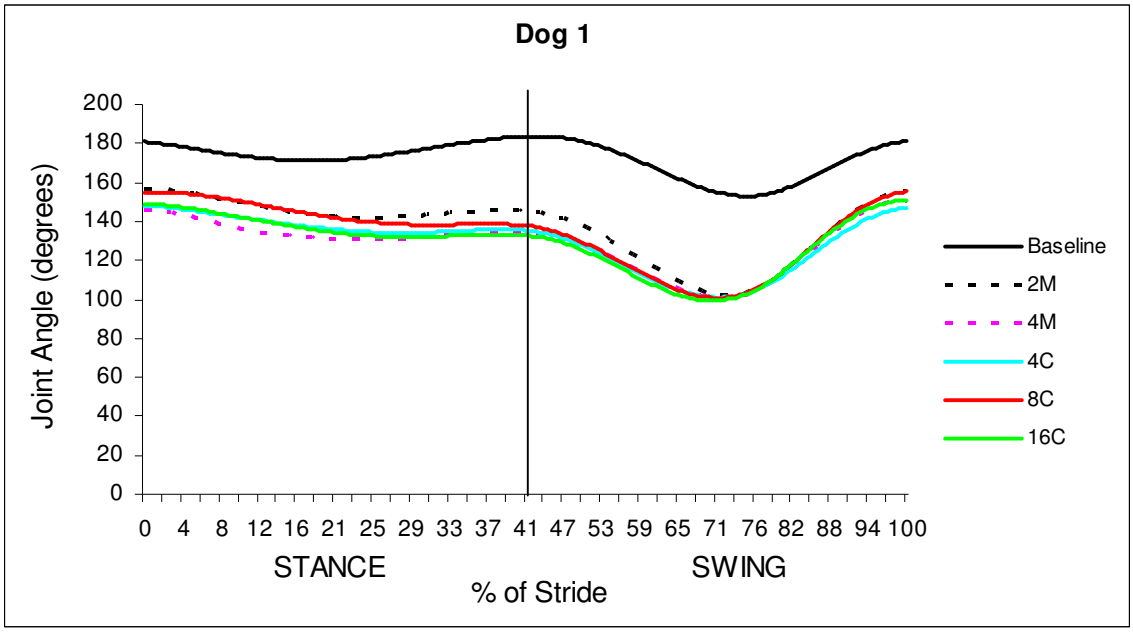
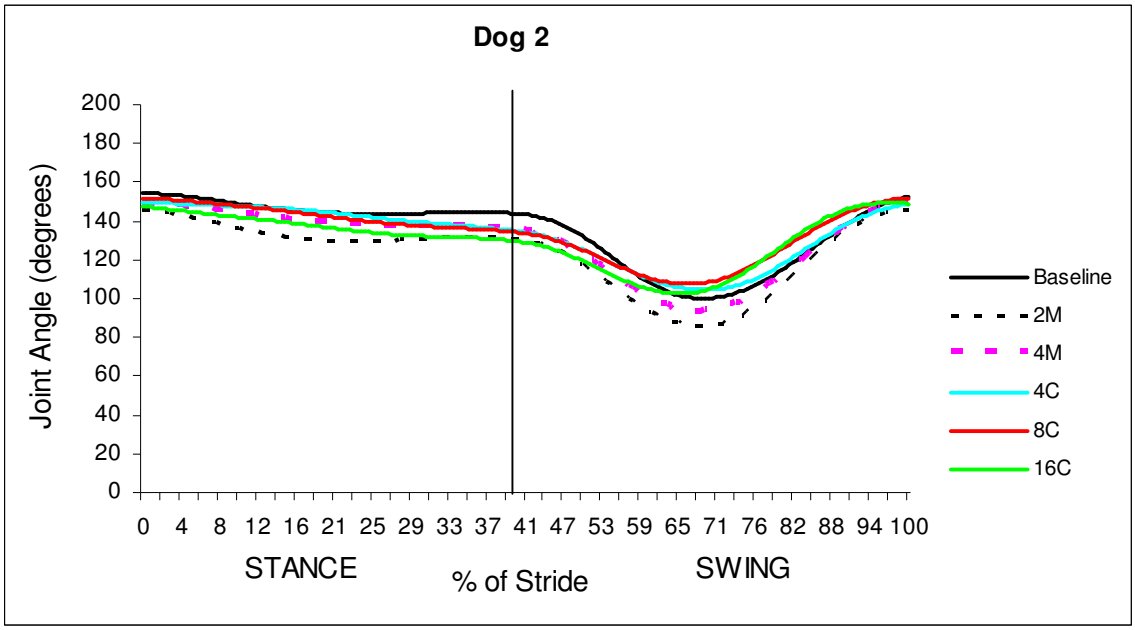
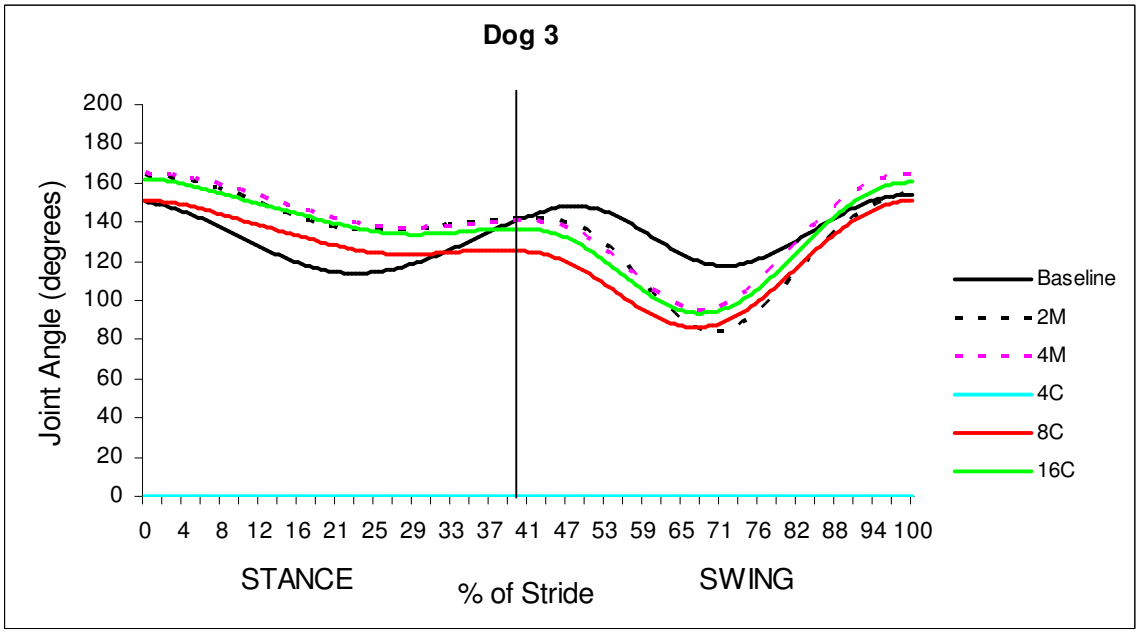
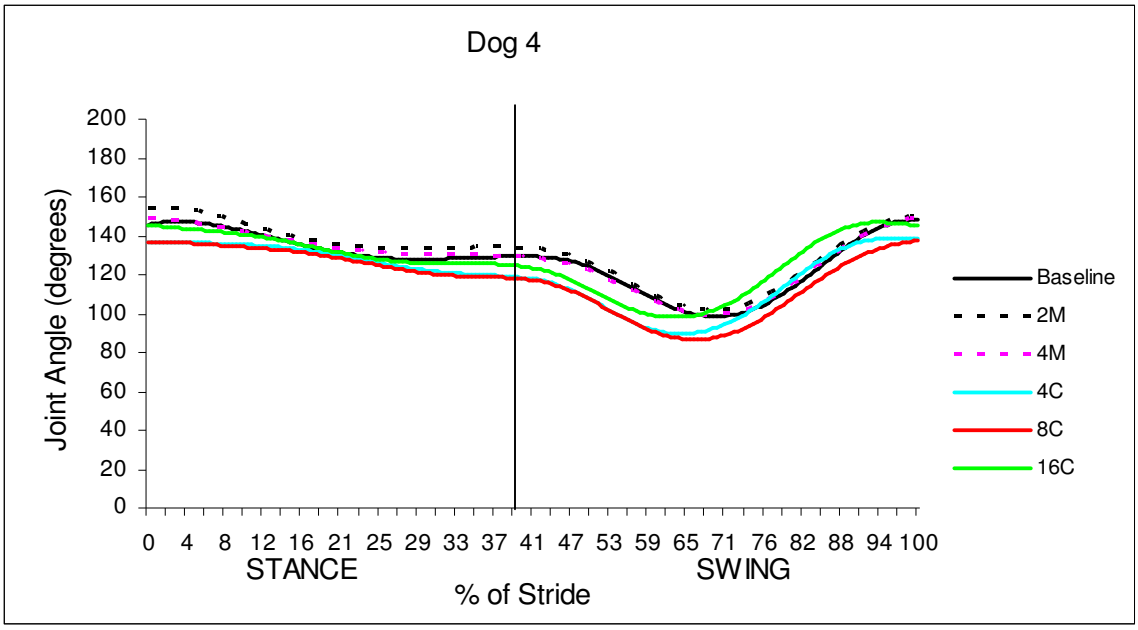
Appendix A

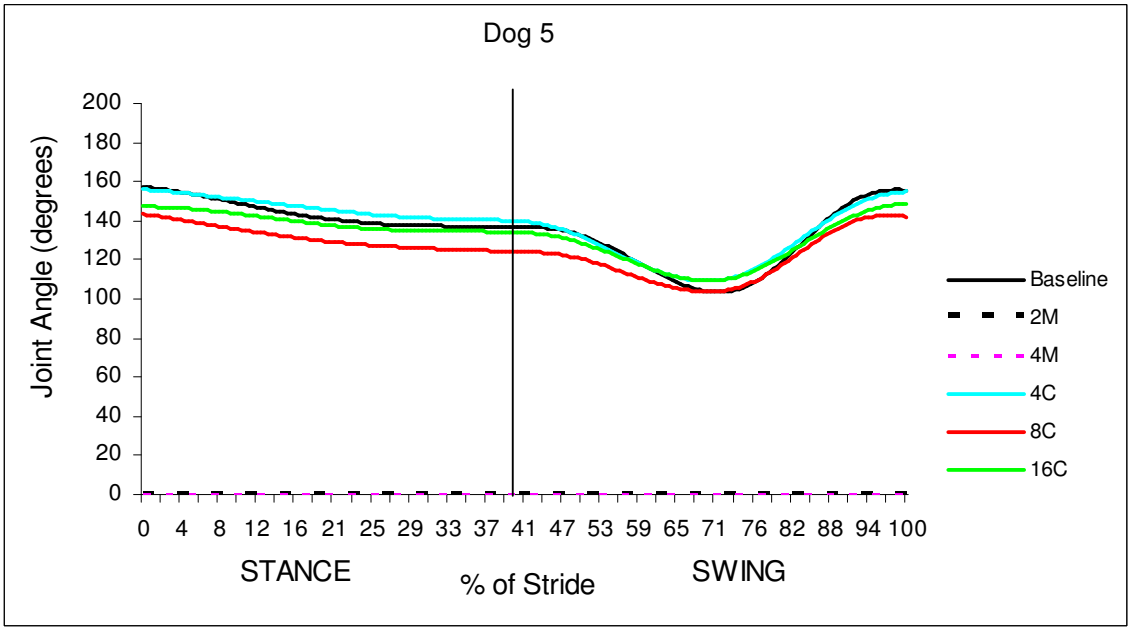
Individual dog graphs showing variability in kinematic trial averaged data for femorotibial joint angles of the treated limb during stance phase as a percent of stride. The solid black vertical line indicates the transition between stance and swing phase of the stride. 'M' refers to the number of weeks post MRFE surgery, prior to CCL rupture. 'C' refers to the number of weeks post CCL rupture.




Dog 2
Joint Angle (degrees)
200
180
160
140
120
100
80
60
40
20
0
0 4 8 12 16 21 25 29 33 37 41 47 53 59 65 71 76 82 88 94 100
STANCE
% of Stride
SWING
Baseline
2M
4M
4C
8C
16C


Dog 3
Joint Angle (degrees)
200
180
160
140
120
100
80
60
40
20
0
0 4 8 12 16 21 25 29 33 37 41 47 53 59 65 71 76 82 88 94 100
STANCE
% of Stride
SWING
Baseline
2M
4M
4C
8C
16C


Dog 4
Joint Angle (degrees)
200
180
160
140
120
100
80
60
40
20
0
0 4 8 12 16 21 25 29 33 37 41 47 53 59 65 71 76 82 88 94 100
STANCE
SWING
% of Stride
Baseline
2M
4M
4C
8C
16C


Dog 5
Joint Angle (degrees)
200
180
160
140
120
100
80
60
40
20
0
0 4 8 12 16 21 25 29 33 37 41 47 53 59 65 71 76 82 88 94 100
STANCE
% of Stride
SWING
Baseline
2M
4M
4C
8C
16C

Appendix B

Mean percent of stride for the minimal joint angles at 3 joints for the treated and untreated pelvic limbs during swing phase (N = 5).

	Coxofemoral Joint		Femorotibial Joint		Tarsal Joint	
Time Points	**Untreated**	**Treated**	**Untreated**	**Treated**	**Untreated**	**Treated**
Baseline	92.8 ± 2.6	89.1 ± 2.6	69.7 ± 0.9	69.6 ± 0.9	74.7 ± 1.0	75.1 ± 1.0
Post MRFE						
Week 2	88.2 ± 2.9	88.8 ± 2.9	68.8 ± 1.0	69.7 ± 1.0	74.1 ± 1.1	75.0 ± 1.1
Week 4	88.4 ± 2.9	90.5 ± 2.9	68.7 ± 1.0	69.3 ± 1.0	74.3 ± 1.1	74.7 ± 1.1
Post CCL Rupture						
Week 4	91.6 ± 3.3	95.8 ± 2.9	71.3 ± 1.2	68.4 ± 1.2†	73.7 ± 1.2	75.4 ± 1.1
Week 8	94.5 ± 2.9	94.0 ± 2.6	72.9 ± 1.0‡	68.7 ± 1.0†	77.0 ± 1.1	75.4 ± 1.0
Week 16	91.9 ± 2.6	89.2 ± 2.6	72.0 ± 0.9	67.6 ± 0.9†	76.6 ± 1.0	74.1 ± 1.0

† Significantly ($P < 0.05$) different from contralateral pelvic limb. ‡ Significantly ($P < 0.05$) different compared to baseline, presurgical values.

Appendix C

Mean treated and untreated pelvic limb range of motion at 3 joints during stance phase (N = 5).

	Coxofemoral Joint		Femortibial Joint		Tarsal Joint	
Time Points	**Untreated**	**Treated**	**Untreated**	**Treated**	**Untreated**	**Treated**
Baseline	26.7 ± 1.5	24.5 ± 1.5	19.3 ± 3.2	19.0 ± 3.2	38.2 ± 2.9	41.7 ± 2.9
Post MRFE						
Week 2	22.6 ± 1.7‡	25.7 ± 1.7	21.2 ± 3.3	20.3 ± 3.3	40.7 ± 2.9	42.6 ± 2.9
Week 4	22.2 ± 1.7‡	25.1 ± 1.7	21.9 ± 3.3	20.5 ± 3.3	39.2 ± 3.0	38.9 ± 3.0
Post CCL Rupture						
Week 4	24.1 ± 1.7	28.3 ± 1.7†‡	28.9 ± 3.3‡	19.8 ± 3.3	53.5 ± 3.0‡	33.1 ± 3.0†‡
Week 8	23.2 ± 1.5‡	28.0 ± 1.5†‡	25.8 ± 3.2‡	21.4 ± 3.2	51.2 ± 2.9‡	35.5 ± 2.9†‡
Week 16	25.8 ± 1.5	26.4 ± 1.5	24.7 ± 3.2‡	21.1 ± 3.2	46.5 ± 2.9‡	37.7 ± 2.9†

† Significantly ($P < 0.05$) different from contralateral limb. ‡ Significantly ($P < 0.05$) different compared to baseline, presurgical values.

Appendix D
Subjective Radiographic Score Form*

Canine ID_____
Date_____
Time point_____weeks

Reference range:
0 = Normal
1 = Mild OA
2 = Moderate OA
3 = Severe OA

Right stifle

1. Femur
   a. Femoral trochlea _____
   b. Medial femoral condyle _____
   c. Lateral femoral condyle _____
   d. Medial femoral epicondyle _____
   e. Lateral femoral epicondyle _____
2. Patella
   a. Proximal patella _____
   b. Distal patella _____
3. Tibia
   a. Lateral tibial condyle _____
   b. Medial tibial condyle _____

Right total score _____

Left stifle

4. Femur
   a. Femoral trochlea _____
   b. Medial femoral condyle _____
   c. Lateral femoral condyle _____
   d. Medial femoral epicondyle _____
   e. Lateral femoral epicondyle _____
5. Patella
   a. Proximal patella _____
   b. Distal patella _____
6. Tibia
   a. Lateral tibial condyle _____
   b. Medial tibial condyle _____

Left total score _____

Overall Cumulative Score Categories	
0-9	Mild OA
10-18	Moderate OA
19-27	Severe OA

* Roy RG, Wallace LJ, Johnston GR, Wickstrom SL. A retrospective evaluation of stifle osteoarthritis in dogs with bilateral medial patellar luxation and unilateral surgical repair. *Vet Surgery* 1992;21(6): 475-79.

Appendix E
Colorado State University Veterinary Medical Center Acute Pain Scale

Colorado State University

Date _____

Time _____

Colorado State University Veterinary Medical Center
Canine Acute Pain Scale

Rescore when awake
☐ **Animal is sleeping, but can be aroused - Not evaluated for pain**
☐ **Animal can't be aroused, check vital signs, assess therapy**

Pain Score	Example	Psychological & Behavioral	Response to Palpation	Body Tension
0		☐ **Comfortable** when resting ☐ **Happy, content** ☐ Not bothering wound or surgery site ☐ Interested in or curious about surroundings	☐ **Nontender** to palpation of wound or surgery site, or to palpation elsewhere	Minimal
1		☐ **Content to slightly unsettled** or restless ☐ **Distracted easily** by surroundings	☐ **Reacts to palpation** of wound, surgery site, or other body part by **looking around, flinching,** or **whimpering**	Mild
2		☐ Looks **uncomfortable** when resting ☐ May **whimper** or cry and may **lick or rub wound** or surgery site when unattended ☐ Droopy ears, **worried facial expression** (arched eye brows, darting eyes) ☐ **Reluctant to respond** when beckoned ☐ **Not eager to interact** with people or surroundings but will look around to see what is going on	☐ Flinches, whimpers cries, or guards/pulls away	Mild to Moderate **Reassess analgesic plan**
3		☐ **Unsettled, crying, groaning, biting or chewing** wound when unattended ☐ **Guards or protects** wound or surgery site by altering weight distribution (i.e., limping, shifting body position) ☐ **May be unwilling to move** all or part of body	☐ May be **subtle** (shifting eyes or increased respiratory rate) if dog is too painful to move or is stoic ☐ May be **dramatic**, such as a sharp cry, growl, bite or bite threat, and/or pulling away	Moderate **Reassess analgesic plan**
4		☐ **Constantly groaning or screaming** when unattended ☐ May bite or chew at wound, but unlikely to move ☐ **Potentially unresponsive** to surroundings ☐ **Difficult to distract** from pain	☐ **Cries at non-painful palpation** (may be experiencing allodynia, wind-up, or fearful that pain could be made worse) ☐ May react aggressively to palpation	Moderate to Severe **May be rigid to avoid painful movement** **Reassess analgesic plan**

Comments _____

Supported by an Unrestricted Educational Grant from Pfizer Animal Health

Appendix F
Necropsy Procedures*

PROCEDURES FOR NECROPSY
CANINE STIFLE STUDY (IACUC #: 08-323A-01)
ORC Study Number: 28,000.001

1. Dogs will be sedated and radiographs, intra-articular pressures and synovial fluid samples will be performed. Dogs will be euthanized immediately following and moved to necropsy where stifles will be shaved with a #40 blade.

2. Dogs will be marked with a label that includes ORC Study #, Dog ID # and Name, Date and Limb (Right or Left) and pictures of the following will be taken for each dog in sequential order:
    a. Face
    b. Left side
    c. Right side
    d. Left hind limb
    e. Right hind limb

3. Each stifle will have the skinned removed and the joint opened (cut proximally and reflected distally). Again, stifles are labeled according to #2 above, and pictures of the following will be taken for each joint (photos will be kept in sequential order):
    a. Open joint with ID tag
    b. Close-up of open joint (no ID tag)
    c. Opposite side of the joint (close-up with no ID tag)

4. Tissue processing may occur up to 30 days following necropsy and sample collection. Tissues will be collected and graded in the following order:
    a. **Gross Score – Synovial Membrane**
        1. Right and Left Limb
            a) Middle compartment
            b) Axial compartment
            c) Lateral compartment
        2. One sample from each compartment will be harvested for histology (no map available)

    b. The joint will be disarticulated and the cruciate ligaments cut.

c. **Gross Score – Meniscus**
    1. Right and Left Limb
    2. Medial and Lateral meniscus
        a) Anterior Zone
        b) Middle Zone
        c) Posterior Zone

d. Photographs of the medial and lateral menisci will be taken in situ. Menisci will then be removed and cut into an anterior, middle and posterior zone for histology (no map available). Each sample will be labeled appropriately.

e. Close up photographs of the tibial plateau will be taken once the menisci are removed and gross scores of the articular cartilage surface of the femoral condyles and tibial plateaus will be recorded.

f. **Gross Score – Articular Cartilage Surface**
    1. Right and Left Limb
        a) Medial Femoral Condyle
        b) Lateral Femoral Condyle
        c) Medial Tibial Plateau
        d) Lateral Tibial Plateau

g. Four cartilage explants will be harvested from each region according to the maps below and saved for histological evaluation.

h. Using a band saw, the distal femur and proximal tibia will be cut approximately two inches above the joint. These bones will be wrapped in saline-soaked gauze sponges then saran wrap, labeled accordingly, and stored in a freezer for future analysis.

* Cook JL, et.al., Osteoarthritis Cartilage, 2010

Articular Cartilage Sampling Areas

Key:
S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4

Excerpt from Cook JL, et.al., Osteoarthritis Cartilage, 2010, p.575

Appendix G
Histologic Sectioning*

HISTOLOGICAL EVALUATION of the CANINE STIFLE
CANINE STIFLE STUDY (IACUC #: 08-323A-01)
ORC Study Number: 28,000.001

1. **Synovial Membrane Histology (H&E only) – 6 samples/dog**
   i. Right and Left Limb – 1 sample from each region
      1. Medial Joint
      2. Axial Joint
      3. Lateral Joint

2. **Meniscal Histology (H&E and SOFG) – 12 samples/dog; 24 slides/dog**
   j. Right and Left Limb
   k. Medial and Lateral meniscus – 1 sample from each zone
      1. Anterior Zone
      2. Middle Zone
      3. Posterior Zone

3. **Articular Cartilage Histology** – evaluate cartilage **(H&E),** chondrocyte **(H&E)**, and proteoglycan pathology **(SOFG) – 32 samples/dog; 64 slides /dog**
   l. Right and Left Limb
      1. Medial Femoral Condyle - Samples 1-4
      2. Lateral Femoral Condyle - Samples 1-4
      3. Medial Tibial Plateau - Samples 1-4
      4. Lateral Tibial Plateau - Samples 1-4

* Cook JL, et.al., Osteoarthritis Cartilage, 2010