GAIT ANALYSIS OF THE HIND LIMB IN LABRADOR RETRIEVERS WITH AND WITHOUT CRANIAL CRUCIATE LIGAMENT DISEASE

BY

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DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in VMS - Veterinary Clinical Medicine in the Graduate College of the University of Illinois at Urbana-Champaign, 2011

Urbana, Illinois

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ABSTRACT

Cranial cruciate ligament (CCL) deficiency is the leading cause of lameness affecting the stifle joints of large breed dogs, especially Labrador Retrievers. Although CCL disease has been studied extensively, its exact pathogenesis and the primary cause leading to CCL rupture remain controversial. However, weakening secondary to repetitive microtrauma is currently believed to cause the majority of CCL instabilities diagnosed in dogs.

Techniques of gait analysis have become the most productive tools to investigate normal and pathological gait in human and veterinary subjects. The inverse dynamics analysis approach models the limb as a series of connected linkages and integrates morphometric data to yield information about the net joint moment, patterns of muscle power and joint reaction forces. The results of these studies have greatly advanced our understanding of the pathogenesis of joint diseases in humans. A muscular imbalance between the hamstring and quadriceps muscles has been suggested as a cause for anterior cruciate ligament rupture in female athletes. Based on these findings, neuromuscular training programs leading to a relative risk reduction of up to 80% has been designed. In spite of the cost and morbidity associated with CCL disease and its management, very few studies have focused on the inverse dynamics gait analysis of this condition in dogs. The general goals of this research were (1) to further define gait mechanism in Labrador Retrievers with and without CCL-deficiency, (2) to identify individual dogs that are susceptible to CCL disease, and (3) to characterize their gait.

The mass, location of the center of mass (COM), and mass moment of inertia of hind limb segments were calculated using a noninvasive method based on computerized tomography of normal and CCL-deficient Labrador Retrievers. Regression models were developed to
determine predictive equations to estimate body segment parameters on the basis of simple morphometric measurements, providing a basis for nonterminal studies of inverse dynamics of the hind limbs in Labrador Retrievers.

Kinematic, ground reaction forces (GRF) and morphometric data were combined in an inverse dynamics approach to compute hock, stifle and hip net moments, powers and joint reaction forces (JRF) while trotting in normal, CCL-deficient or sound contralateral limbs. Reductions in joint moment, power, and loads observed in CCL-deficient limbs were interpreted as modifications adopted to reduce or avoid painful mobilization of the injured stifle joint. Lameness resulting from CCL disease affected predominantly reaction forces during the braking phase and the extension during push-off. Kinetics also identified a greater joint moment and power of the contralateral limbs compared with normal, particularly of the stifle extensor muscles group, which may correlate with the lameness observed, but also with the predisposition of contralateral limbs to CCL deficiency in dogs.

For the first time, surface EMG patterns of major hind limb muscles during trotting gait of healthy Labrador Retrievers were characterized and compared with kinetic and kinematic data of the stifle joint. The use of surface EMG highlighted the co-contraction patterns of the muscles around the stifle joint, which were documented during transition periods between flexion and extension of the joint, but also during the flexion observed in the weight bearing phase. Identification of possible differences in EMG activation characteristics between healthy patients and dogs with or predisposed to orthopedic and neurological disease may help understanding the neuromuscular abnormality and gait mechanics of such disorders in the future.
Conformation parameters, obtained from femoral and tibial radiographs, hind limb CT images, and dual-energy X-ray absorptiometry, of hind limbs predisposed to CCL deficiency were compared with the conformation parameters from hind limbs at low risk. A combination of tibial plateau angle and femoral anteversion angle measured on radiographs was determined optimal for discriminating predisposed and non-predisposed limbs for CCL disease in Labrador Retrievers using a receiver operating characteristic curve analysis method. In the future, the tibial plateau angle (TPA) and femoral anteversion angle (FAA) may be used to screen dogs suspected of being susceptible to CCL disease.

Last, kinematics and kinetics across the hock, stifle and hip joints in Labrador Retrievers presumed to be at low risk based on their radiographic TPA and FAA were compared to gait data from dogs presumed to be predisposed to CCL disease for overground and treadmill trotting gait. For overground trials, extensor moment at the hock and energy generated around the hock and stifle joints were increased in predisposed limbs compared to non predisposed limbs. For treadmill trials, dogs qualified as predisposed to CCL disease held their stifle at a greater degree of flexion, extended their hock less, and generated more energy around the stifle joints while trotting on a treadmill compared with dogs at low risk. This characterization of the gait mechanics of Labrador Retrievers at low risk or predisposed to CCL disease may help developing and monitoring preventive exercise programs to decrease gastrocnemius dominance and strengthened the hamstring muscle group.
To Guillaume and my parents,
I owe you so much
This project would not have been possible without the support of many people. I would like to thank my advisors, Dr Dominique Griffon and Dr Elizabeth Hsiao-Wecksler, for their continuous help and supervision of the project. Thanks to Dr Gerald Pijanowski for his great cooperation with three-dimensional modeling. Also thanks to Dr Wanda Gordon Evans, Dr Santiago Gutierrez Nibeyro, Dr Robert O’Bien, and Dr Michael Thomas for their kind support. Many thanks to Dr David Schaeffer and Dr Richard Evans for their amazing help with the statistical analysis and mathematical modeling part of my project. Many thanks to Dr Ayman Mostafa, (Mei Kuen) Iris Hsu, Michaela Klump, Louis DiBerardino, and Jason Thomas for their tremendous involvement in this project. Thanks to the technicians from the Imaging Section of the Veterinary Teaching Hospital of the University of Illinois, Carrie Bubb, Janet Sinn-Hanlon, John Jang, Sarah Ashton-Szabo, Hsin-Yi Weng, Rosario Vallefuoco, Hae Beom Lee, Chris Knowlton, and Philip Kwon for their assistance with data collection and processing. Thanks to the American Veterinary Medicine Association and for the American Kennel Club for their support in funding the project. And finally thanks to my parents, my brother and sister, and particularly to Guillaume for their kindness, always offering support and love. Thanks!
TABLE OF CONTENTS

LIST OF FIGURES .................................................................................................................... ix

LIST OF TABLES .................................................................................................................. xi

LIST OF ABBREVIATIONS ....................................................................................................... xiii

CHAPTER 1: INTRODUCTION ................................................................................................1

CHAPTER 2: LITERATURE REVIEW ....................................................................................4
  2.1. Techniques of gait analysis .........................................................................................4
  2.2. Clinical applications for the canine hind limb ............................................................48

CHAPTER 3: METHODOLOGY AND RESULTS ....................................................................78
  3.1. Noninvasive determination of body segment parameters of the hind limb .........79
  3.2. Inverse dynamics analysis of the pelvic limbs in Labrador Retrievers
      with and without cranial cruciate ligament disease ......................................................94
  3.3. Association between surface electromyography, kinetics and kinematics
      of the hind limb in healthy trotting Labrador Retrievers ........................................109
  3.4. Multivariate analysis of morphometric characteristics to evaluate risk
      factors for cranial cruciate ligament deficiency in Labrador Retrievers......119
3.5. Kinetic and kinematic analysis of the pelvic limbs in Labrador Retrievers predisposed or at a low risk for cranial cruciate ligament disease ...............128

CHAPTER 4: DISCUSSION...........................................................................................148

4.1. Body segment parameters measurement and estimation by predictive equations .............................................................................................................148

4.2. Inverse dynamic analysis of the pelvic limbs in Labrador Retrievers with and without cranial cruciate ligament disease .................................................154

4.3. Association between surface electromyography, kinetics and kinematics of the hind limb in healthy trotting Labrador Retrievers ............................................160

4.4. Multivariate analysis of morphometric characteristics to evaluate risk factors for cranial cruciate ligament deficiency in Labrador Retrievers............164

4.5. Kinetic and kinematic analysis of the pelvic limbs in Labrador Retrievers predisposed or at a low risk for cranial cruciate ligament disease ...............169

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS ...................................180

REFERENCES ................................................................................................................183
LIST OF FIGURES

Figure 1: Areas of reflective markers placed on the subject’s skin over specific anatomic landmarks .................................................................................................................. 22

Figure 2: Free body diagram of a segment ................................................................... 40

Figure 3: Mean of flexion and extension movements for one gait cycle of the hind limb of trotting Labrador Retrievers .................................................................................. 58

Figure 4: Three-dimensional computed tomography illustrating the outline of fat, muscle and bone tissues based on their respective pixel values using Amira® .......... 84

Figure 5: Assembly of fat, muscle and bone created by Pro/ENGINEER® to determine the body segment parameters of the left crus, such as the location of the center of mass .................................................................................................................. 84

Figure 6: Computer assisted acquisition of ground reaction forces and kinematic data in a trotting subject.................................................................................................................. 97

Figure 7: Mean of hock angular position, angular velocity, net joint moment, power, and joint reaction forces in the vertical and cranio-caudal direction for normal, CCL-deficient and contralateral limbs during one gait cycle......................... 106

Figure 8: Mean of stifle angular position, angular velocity, net joint moment, power, and joint reaction forces in the vertical and cranio-caudal direction for normal, CCL-deficient and contralateral limbs during one gait cycle......................... 107

Figure 9: Mean of hip angular position, angular velocity, net joint moment, power, and joint reaction forces in the vertical and cranio-caudal direction for normal, CCL-deficient and contralateral limbs during one gait cycle......................... 108
Figure 10: Mean vertical ground reaction forces during the stance and the swing phases...114

Figure 11: Patterns of muscular activation for the quadriceps, hamstring and gastrocnemius muscle groups. Mean of angular position, net joint muscle moment, and net joint muscle power at the stifle joint phase.................................118

Figure 12: Femoral anteversion angle...........................................................................124

Figure 13: Scatter plot of tibial plateau angle against femoral anteversion angle measured on radiographs .................................................................127

Figure 14: Scatter plot of femoral anteversion angle measured on radiographs against whole body fat percentage measured on DEXA ..................127

Figure 15: Mean of angular position, angular velocity, net joint muscle moment, and net joint muscle power at the hock joint for limbs at low-risk and limbs predisposed to CCL disease during the stance phase for overground trials ............139

Figure 16: Mean of angular position, angular velocity, net joint muscle moment, and net joint muscle power at the stifle joint for limbs at low-risk or predisposed to CCL disease during the stance phase for overground trials.........................140

Figure 17: Mean of angular position, angular velocity, net joint muscle moment, and net joint muscle power at the hip joint for limbs at low-risk or predisposed to CCL disease during the stance phase for overground trials..............................141

Figure 18: Mean vertical and craniocaudal ground reaction forces for limbs at low-risk or predisposed to CCL disease during the stance phase for overground trials ....142

Figure 19: Mean of angular position, net joint muscle moment, and net joint muscle power at the stifle joint for limbs at low-risk and limbs predisposed to CCL disease during the stance phase for treadmill trials............................147
LIST OF TABLES

Table 1: Criteria for proper collection of force plate data ............................................ 12
Table 2: Body segment parameters of the front and hind limb segments obtained from canine cadavers .................................................................................................... 37
Table 3: Patterns of muscular activation of selected hind limb muscles in trotting dogs via the use of indwelling EMG electrodes ................................................................. 64
Table 4: Mean of the body segment parameters of the foot, crus and thigh of normal, CCL-deficient and contralateral hind limbs of Labrador Retrievers with or without unilateral CCL disease ................................................................................. 89
Table 5: Abbreviations and descriptive statistics for all independent variables included in the regression analysis ..................................................................................... 91
Table 6: Regression equations generated from morphometric measurements and body mass to predict the mass, the location of the center of mass from the proximal joint and the mass moment of inertia of the thigh ................................................. 92
Table 7: Regression equations generated from morphometric measurements and body mass to predict the mass, the location of the center of mass from the proximal joint and the mass moment of inertia of the crus ....................................................... 93
Table 8: Regression equations generated from morphometric measurements and body mass to predict the mass, the location of the center of mass from the proximal joint and the mass moment of inertia of the foot ........................................................ 93
Table 9: Mean animal velocity and acceleration, stance time, stride length, stride
time, peak vertical force, peak braking and propulsive forces, vertical impulse,
braking and propulsive impulses for CCL-deficient, contralateral and normal limbs

Table 10: Peak of mean kinematic and kinetic values for the hock, stifle, and hip
joints for CCL-deficient, contralateral and normal limbs

Table 11: Mean, standard deviation and range of tibial plateau angle and femoral
anteversion angle values presented for predisposed and non-predisposed limbs

Table 12: Mean of gait velocity, stance time, as well as ground reaction forces
for subjects at low-risk or predisposed to CCL disease for overground trials

Table 13: Mean of peak kinematic and kinetic values for the hock, stifle and
hip joints in dogs at low-risk or predisposed to CCL disease for overground trials

Table 14: Mean of gait velocity, stance time, as well as ground reaction forces for
subjects at low-risk or predisposed to CCL disease for treadmill trials

Table 15: Mean of peak kinematic and kinetic values for the hock, stifle and hip
joints in dogs at low-risk or predisposed to CCL disease for treadmill trials
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<td>BSP</td>
<td>Body segment parameters</td>
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<tr>
<td>CCL</td>
<td>Cranial cruciate ligament</td>
</tr>
<tr>
<td>COM</td>
<td>Center of mass</td>
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<td>CT</td>
<td>Computerized tomography</td>
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<td>DEXA</td>
<td>Dual-energy X-ray absorptiometry</td>
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<td>EMG</td>
<td>Electromyography</td>
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<tr>
<td>FAA</td>
<td>Femoral anteversion angle</td>
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<tr>
<td>GRF</td>
<td>Ground reaction forces</td>
</tr>
<tr>
<td>HSD</td>
<td>Honestly significant difference</td>
</tr>
<tr>
<td>JRF</td>
<td>Joint reaction forces</td>
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<td>PVF</td>
<td>Peak vertical forces</td>
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<tr>
<td>ROC</td>
<td>Receiver operating characteristic</td>
</tr>
<tr>
<td>ROM</td>
<td>Range of motion</td>
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<tr>
<td>PVF</td>
<td>Peak vertical forces</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>SEE</td>
<td>Standard error of the estimate</td>
</tr>
<tr>
<td>TPA</td>
<td>Tibial plateau angle</td>
</tr>
<tr>
<td>VI</td>
<td>Vertical impulse</td>
</tr>
<tr>
<td>% BM</td>
<td>Percentage of body mass</td>
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<tr>
<td>% L</td>
<td>Percentage of length of the segment</td>
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CHAPTER 1: INTRODUCTION

Techniques of gait analysis, mostly force plate and computer-assisted kinematic data, have become the most productive tools to investigate normal and pathological gait in human and veterinary subjects. The inverse dynamics analysis approach models the limb as a series of connected linkages and integrates morphometric data. This method yields information about the net moment around the limb joints, patterns of muscle power and joint reaction forces at each joint of interest. The results of these studies, combined with electromyography (EMG) analysis, have greatly advanced our understanding of the pathogenesis of joint diseases in humans. Differences in relative strength of the extensor and flexor muscles of the knee have been found to be factors predisposing female athletes to anterior cruciate ligament rupture (Decker et al 2003; Sigward and Powers 2006; Malizak et al 2001). These findings provided the basis for designing and monitoring neuromuscular training programs leading to a relative risk reduction of cruciate ligament rupture up to 80% (Mandelbaum et al 2005; Hewett et al 1999).

The application of inverse dynamics analysis to compute joint forces, moment and power in veterinary medicine has been limited due to a number of factors, including a lack of morphometric measures for dogs. Regression equations allow approximation of body segment parameters (BSP) from easily obtainable morphometric measurements but are only available for horses (Buchner et al 1997). As a result, no non invasive method has been proposed to calculate BSP in dogs in a representative sample. In dogs, cranial cruciate ligament (CCL) deficiency is the leading cause of lameness affecting the stifle joints of large breeds, especially Labrador Retrievers (Johnson, Austin and Breur
In dogs with CCL rupture, a cyclical pattern of cranial tibial subluxation during the stance phase and cranial tibial reduction during the swing phase occurs (Korvick, Pijanowski and Schaeffer 1994; Tashman et al 2004). The kinematic changes observed in CCL-deficient dogs support the concept of a dynamic imbalance between the extensor and flexor muscles providing support to the stifle joint during the weight bearing phase, similar to that described in humans. However, in spite of the cost and morbidity associated with CCL disease and its management, no studies have focused on the inverse dynamics gait analysis of this condition in dogs. This gap in knowledge limits our understanding about CCL-deficient gait mechanism and neuromuscular abnormality that might lead to higher risk of CCL disease in dogs, which prevents the development of preventive measures.

The general goals of this research were (1) to further define gait mechanism in Labrador Retrievers with and without CCL-deficiency, (2) to identify individual dogs that are susceptible to CCL disease, and (3) to characterize their gait. For the first general goal of the study, our initial objective focused on non-invasive determination of BSP for the hind limb segments in living Labrador Retrievers with or without CCL deficiency and to develop regression equations to estimate those BSP based on easily obtainable morphometric measurements (Sections 3.1 and 4.1). A second objective was to quantify net joint moments, powers and joint reaction forces across the hock, stifle and hip joints in Labrador Retrievers with or without CCL disease, and to investigate differences in joint mechanics between limbs of clinically normal dogs, limbs affected by CCL disease and contralateral limbs of CCL-deficient dogs (Sections 3.2 and 4.2). A third objective was to evaluate the EMG activity of major hind limb muscle groups by means of surface EMG.
and evaluate concurrently kinetics and kinematics of the stifle joint in dogs trotting over a treadmill (Sections 3.3 and 4.3). We hypothesized that BSP would differ between normal, CCL-deficient and contralateral limbs. Consequently, we hypothesized that regression equations would be different for BSP between these three groups of limbs. We hypothesized that net joint moments, powers and joint reaction forces would be different between normal, CCL-deficient and contralateral limbs, with affected limbs revealing decreased values for kinetics compensated by an increase in kinetics of the contralateral side. We also hypothesized that hamstring, quadriceps and gastrocnemius muscles activation patterns would highlight a correlation between EMG patterns, kinetics and kinematics around the stifle joint.

For the second general goal of the study, the objective was to identify an equation combining conformation factors to predict the risk of a limb to become CCL-deficient (Sections 3.4 and 4.4). We hypothesized that specific weighted combinations of hind limb musculoskeletal conformational factors would enable discrimination between limbs at low risk to develop CCL disease and predisposed limbs.

Last, for the third goal of the study, the CCL risk predictive equation was used to assign sound Labrador Retrievers to one of two groups, based on their predictive score for CCL disease. The objective was to examine the pelvic limb mechanics of healthy Labrador Retrievers predisposed to CCL disease and those at low risk to develop the disease. These gait mechanics were examined while trotting overground and on a treadmill (Sections 3.5 and 4.5). We hypothesized that the kinetic and kinematic data around the stifle joint would differ between sound Labrador Retrievers predisposed to CCL disease and sound Labrador Retrievers at low risk for CCL disease.
CHAPTER 2: LITERATURE REVIEW

2.1. Techniques of gait analysis

2.1.1. Definition of gait and gait analysis

Gait is a manner of limb movement, characterized by distinctive, coordinated, and repetitive movements of the feet and limbs. Two main groups of gait exist: symmetric (walk, trot, and pace for example) and asymmetric gaits (gallop for example). The stance phase is defined as the period in which the foot strikes and remains on the ground; it may be further delineated into (i) initial paw strike, (ii) braking or yield, (iii) propulsion and (iv) toe off. The swing phase is defined as the period in which the foot is in the air. Together, the stance and swing phases of one foot equal one stride. The gait cycle of the quadruped may be defined as the series of events to include one stride for each of all four limbs (DeCamp 1997).

Gait analysis is the study of limb movement. Kinesiology, the science of motion, includes kinetics (the forces that affect motion) and kinematics (the temporal and geometric characteristics of motion) (McLaughlin 2001). The variables that are used in the description and analysis of any movement can be categorized as follows: kinetic and kinematic gait analysis, morphometry, and electromyography. Kinetic, kinematic, and morphometric data may be combined using an inverse dynamics method in order to provide more information about the mechanical events occurring around a specific joint during each phase of the gait.
2.1.2. Kinetic gait analysis

2.1.2.1. Definition

Kinetic represents the internal and external forces that cause motion and provide insight into the cause of the movement and movement strategies. Internal forces are produced by muscle activity, ligaments, or from friction in muscles and joints. External forces are provided by the ground or external loads, from active bodies or from passive sources. A wide variety of kinetic analyses can be done: ground reaction forces, inter-segmental forces at each joint of interest, moments of force (produced by muscles crossing a joint), mechanical power (flowing to or from those same muscles), and energy changes resulting from this power flow (Winter 2005).

Kinetic gait analysis can be performed using a force plate to obtain non-invasive, objective, and quantitative assessment of the forces occurring between the foot and the surface of the plate during the stance phase of the stride. The forces mainly recorded are called ground reaction forces (GRF), which act on the foot. Ground reaction forces have been used extensively in human and animal gait studies in order to characterize how much force a subject applies on each limb during the stance phase, which, for example, is very useful to assess and characterize the degree of lameness.

However, successive strides during locomotion cannot be measured by a single force plate. Using a single force plate to analyze locomotion requires the assumption that all strides, and all of the composite steps of each stride, are identical (Bertram et al 2000). Consecutive strides may only be evaluated with a series of two or four force plates (Bertram et al 2000; Kennedy et al 2003; Lee et al 2004; Lee, Bertram and Todhunter 1999), with force plate(s) built into a treadmill (Brebner, Moens and...
Runciman 2006; Fanchon et al 2006; Mlacnik et al 2006; Bockstahler et al 2007b), or with a pressure walkway measurement system (Lascelles et al 2006; Besancon et al 2003).

2.1.2.2. Force analysis

The system may be set to collect data beginning with the initial forelimb contact or triggered by the operator. The sampling frequency (in hertz) and the sampling time (in seconds) can be adjusted to determine the amount of data collected. A force-versus-time curve is generated. The force-versus-time curve is a graphic representation of the GRF generated during the stance phase of the stride, and impulse represents the area under the curve. Force is generally reported in newton (N), newton normalized by body mass (N/kg) or as a percentage of body weight (%BW).

Ground reaction forces measure limb function but are not joint-specific (DeCamp 1997; Gillette and Angle 2008). As the subject ambulates on a force plate, three orthogonal GRF are calculated: medio-lateral, cranio-caudal and vertical forces. The vertical force is the largest orthogonal and most reproducible force and therefore the most commonly analyzed in animals (McLaughlin 2001). The cranio-caudal (antero-posterior in human) reaction force represents forces exerted in the horizontal plane in the axis of the motion. The braking phase corresponds to the deceleration of the limb in the early stance phase. The propulsion phase corresponds to the acceleration of the limb in the late stance phase, when the limb is pushing off with the foot. Depending on the convention and coordinate system adopted, braking values are assigned either a positive or a negative value, and inversely for propulsive values.
Medio-lateral GRF have the smallest magnitude of all orthogonal forces. The large variations in medio-lateral forces and low initial amplitudes observed in dogs have resulted in few significant results.

2.1.2.3. Equipment for basic force plate analysis

A force plate consists of sensing elements covered by a top plate that should be mounted firmly to a rigid level surface recessed into a floor or a walkway (Winter 2005; McLaughlin 2001). More recently, force plates have been integrated into or under the belt of treadmills to study consecutive strides (Brebner, Moens and Runciman 2006; Fanchon et al 2006; Mlacnik et al 2006; Bockstahler et al 2007b). The magnitude of the force is measured by deflection of the sensing elements within the plate when the subject steps on it. The displacement of the sensing elements is proportional to the force applied on the plate and creates an electric signal, which is amplified and recorded by computer. Sensing elements may consist of strain gauges or quartz crystals for piezoelectric and piezoresistive force-plate systems.

During each trial, the subject’s forward velocity may be measured using a millisecond timer and two photoelectric switches. The photoelectric cells project a beam of light across the force plate runway and are set-up at a known distance apart from another. When the subject crosses the first beam of light, the system is triggered. The system stops when the subject crosses the second beam of light. The subject’s velocity is then calculated using the known distance between the photoelectric switches. To calculate acceleration, three photocells are necessary. Alternatively, subject velocity may be determined via cinematography and video processing software.
2.1.2.4. Collecting force plate data

During over-ground data collection, dogs are led across the force plate by a handler. Each pass across the plate is evaluated by an observer to confirm foot strikes and gait, and to ensure that there is no interference with the stride. A valid trial at the trot generally consists of distinct ipsilateral forefoot and hind foot strikes (McLaughlin 2001). Gait video recording of each pass allows reevaluation and documentation of valid trials. Repeated passes across the plate are usually required to obtain sufficient number of valid trials. Orthogonal ground reaction forces in the front and hind limbs are measured and recorded for each trial, and an average of these measurements is calculated by groups.

Concern has been expressed that variation may be introduced into force plate studies by several parameters, which compromises comparison of results between different experiments. Criteria for proper data collection should be followed (Table 1). Variations in force plate analysis attributable to dog handler, trial repetition, and individual dog have been quantified. The percentage of total variance attributable to handlers varied between 0% and 7% for all ground reaction forces evaluated (Jevens et al 1993). Handler variation was trivial compared with variation introduced into the study by trial repetition (29%-85% of the total variation) and individual dog variation (14%-69% of the total variation). It should be noted that total variation for GRF is quite small, with coefficients of variation for the vertical forces ranging from 5.8% to 8.5% (Jevens et al 1993). The numbers of dogs and trial repetitions must therefore be properly controlled in experimental design, whereas the use of multiple handlers should be of little concern. Moreover,
while the subject is in contact with the plate, he must cross the plate in a consistent manner and the handler must not interfere in anyway. Minor changes in the dog’s body position (such as lowering or raising the head) or pulling on the leash affect the GRF measurements (McLaughlin 2001).

Acceleration and deceleration affect the generation of ground-reaction forces during gait analysis. As velocity increases in dogs at walk and trot, peak vertical forelimb and hind limb forces increase, vertical impulses decrease, and stance time decreases (McLaughlin and Roush 1995; Riggs et al 1993; McLaughlin and Roush 1994). Variations of 0.3 m/s in dog velocity have been shown to significantly alter ground reaction forces (Riggs et al 1993). So it is very important to control the subject’s speed during gait analysis. Only trials collected within a specific velocity range should be considered valid. Unfortunately dogs often mix accelerating and decelerating steps within a stride, and this has a substantial influence on the distribution of vertical forces between the forelimbs and the hind limbs too (Lee, Bertram and Todhunter 1999). Other researchers have suggested that stance phase duration should be used to help standardize gait trials rather than dog velocity (McLaughlin and Roush 1994; McLaughlin et al 1991; Renberg et al 1999). Because stance phase duration will vary with lameness, caution must be employed for its use in gait analysis as a standardization technique, except in gait studies involving normal dogs.

Other factors known to cause variance in force-plate data include interday variation, force-plate sensitivity, limb symmetry (Budsberg et al 1993), selection and habituation of the subject (Rumph, Steiss and Montgomery 1997), exercise (Beraud,
Moreau and Lussier 2010), weight, and morphometric differences between subjects (McLaughlin 2001; Bertram et al 2000; Jevens et al 1993; Budsberg, Verstraete and Soutas-Little 1987; Molsa, Hielm-Bjorkman and Laitinen-Vapaavuori 2010; Voss et al 2010; Moreau et al 2010). Braking, propulsion and vertical impulses correlate with the size of the subject; conversely peak vertical forces inversely correlate with physical size. Thus large dogs have a lower peak force (expressed as a percentage of their body weight) on each limb but have a higher total impulse applied during stance phase compared to small dogs trotting at similar velocity (Budsberg, Verstraete and Soutas-Little 1987). The influence of surface materials with different coefficients of friction on GRF remains controversial. In one study, the GRF of normal dogs did not differ whether they were tested on linoleum or carpet surfaces (Kapatkin et al 2007). However, peak GRF of Thoroughbred racehorses on synthetic surface was 83% and 71% of those on dirt and turf surfaces, respectively (Setterbo et al 2009).

When factors of variation are controlled by consistent data collection methods, the variance in force plate data is low, providing reliable comparisons between dogs and comparisons of clinical cases with known research data.

The advantages of recording gait data from a treadmill with built-in force plate(s) are reliability and consistency of velocity compared to recordings from a force plate embedded in a walkway. In addition, since measurements can be repeated whenever necessary with exactly the same velocity, the treadmill method offers the possibility to develop a standardization of canine gait. Reliability of GRF measured on a treadmill with four built-in forces plates (Type 9011 A, Kistler Instruments AG, Ostfildern, Germany) was assessed by Bockstahler et al. This device enabled
measurement of GRF for each limb separately and provided suitable and consistent results (Bockstahler et al 2007b). Fanchon et al. showed that consistent data can be obtained after a single 10 to 15-minute training session with Malinois Belgian Shepherd dogs using a ADAL 3D-Run treadmill (TecMachine, Andrezieux Boutheon, France) (Fanchon et al 2006). This device enabled recording of the sum of forces for a pair of diagonal limbs.

The most important potential limitation of treadmill kinetic analysis may be whether trotting or walking on a treadmill is comparable to over-ground locomotion. Brebner et al. tried to answer this fundamental question. Sound dogs and dogs with front limb lameness were evaluated using a third model of instrumented treadmill, and these results were compared to measurements generated by a regular force plate. This device, called Gaitway®, is composed of two integrated force plates, one at the front and one at the rear (Gaitway® Instrumented Treadmill Type 9810AS10, Kistler Instrument Corp., Amherst, NY). At the trot, simultaneous contact of the contralateral forelimb and hind limb results in one forelimb foot strike for the front force plate and one hind limb foot strike for the rear force plate on the treadmill. The Gaitway® instrumented treadmill provided rapid peak vertical force measurements, good concordance for the hind limbs (sound $R^2 = 0.90$, lame $R^2 = 0.89$), and substantial concordance for the forelimbs (sound $R^2 = 0.79$, lame $R^2 = 0.73$) in trotting dogs compared to force plate (Brebner, Moens and Runciman 2006).
<table>
<thead>
<tr>
<th>Subjects of similar breed, morphology, and size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant velocity ($v_0, v_1 + 0.3$ m/s)</td>
</tr>
<tr>
<td>Experienced handler(s)</td>
</tr>
<tr>
<td>No interference handler – subject</td>
</tr>
<tr>
<td>Similar habituation to recording device between subjects</td>
</tr>
</tbody>
</table>

Table 1: Criteria for proper collection of force plate data.
2.1.2.5. Pressure walkway system

Pressure sensitive walkways or gait mats have been recently used in dogs (Lascelles et al 2006; Besancon et al 2003). The Industrial Sensing pressure measurement system (Tekscan Inc., South Boston, MA) uses a sensor pad consisting of an ultra thin (0.1 mm) flexible printed circuit. Thousands of pressures sensing elements are contained within the circuit, arranged in rows and columns along a two-meter by 0.75-meter walkway. The sensing elements act as variable resistors in an electrical circuit resulting in the representation of different pressure levels with different colors (Besancon et al 2003). The GAITRite analysis system (GAITRite, CIR Systems Inc., Havertown, PA) is another computerized walkway system for the quantification of temporal and spatial gait parameters. The standard system is a portable, carpeted electronic walkway embedded with pressure sensors that detect a series of footfalls (Light et al 2010).

Pressure walkway systems offer some advantages over traditional force plates for canine gait analysis. The design of these systems eliminates several of the variables that plague the use of a single force plate. First, the length of the system allows multiple readings and simultaneous, consecutive and contralateral foot-strikes recordings with a single pass over the walkway. Second, the design of the pressure walkway systems allows for animals of extreme size to be evaluated. The system makes the objective evaluation of feline orthopedic and neurological conditions possible. Last, these systems are more portable than a force plate, and certain variables such as stride length are easier to obtain than with a force plate system. Moreover, the forces created by
separate limbs, in contact with the mat at one time, can be recorded (Lascelles et al 2006; Besancon et al 2003).

2.1.2.6. Other kinetic recording devices

A large variety of weight bearing measuring techniques have been developed for use in man (Hurkmans et al 2003; Baker 2007). For example, shoe insole inserts with an array of pressure sensors enable the representation of different foot pressure levels with different colors, which is valuable in identifying high pressure points in various foot deformities and in diabetic feet. Transducers have been developed that can be implanted surgically to measure the force exerted by a muscle at the tendon (Winter 2005). Last, Kai et al. used, in Thoroughbred horses, an instrument sandwiched between the hoof and the shoe. The recording instrument, composed of four load cells, could measure in real time the four components of the GRF or their resultant force along with three-dimensional acceleration at the walk, trot, and canter. Patterns of force-time curves recorded for consecutive strides were similar to those previously reported in horses using a force plate (Kai et al 2000).

2.1.2.7. Parameters measured and analysis of forces

Numerous gait parameters may be measured with a force plate and analyzed to characterize the gait of an individual. The mean values of several trials are used for statistical comparisons between dogs and between evaluation times. Data are often normalized with respect to body weight, to allow comparisons between dogs. Peak forces are the most frequently evaluated parameters. Peaks represent the maximal
forces applied during stance in either vertical (PVF), cranio-caudal or medio-lateral
directions. Peak is expressed either in newton (N), in newton per kilogram (N/kg) if
normalized by the body mass or as a percentage of body weight [(value*100)/(body
mass*9.8); %BW]. Cranio-caudal forces may be divided in braking and propulsion,
which are the deceleration and acceleration phases respectively. The weight distribution
among limbs is calculated by dividing the PVF of the limb of interest by the total forces
of the four limbs time 100 (Lascelles et al 2006; Budsberg, Verstraete and Soutas-Little
1987) and is expressed as a percentage (%). Voss et al used a symmetry index (SI)
based on recorded PVF (SI=200[(PVF_1–PVF_2)/(PVF_1+PVF_2)]) to assess the symmetry
between two hind limbs (limbs 1 and 2) of an individual. A symmetry index of zero
indicated perfect symmetry with this calculation (Voss et al 2007).

The vertical, cranio-caudal and medio-lateral forces may also be integrated over
time and described as vertical (VI), cranio-caudal and medio-lateral impulses. Cranio-
caudal impulse is divided in braking (deceleration phase) and propulsive (acceleration
phase) impulses. Impulses measure the forces applied throughout the stance phase, and
are expressed in newton seconds (N.s), newton seconds per kilogram (N.s/kg) or as a
percentage of body weight time second (%BW.s).

Other frequently evaluated parameters include average forces, rate of loading
and rate of unloading in the vertical, cranio-caudal and medio-lateral directions. The
average force is the mean force over a period of time, most often during the whole
stance and can be expressed in N, N/kg or in %BW. The rate of loading, or rising slope,
is the slope of a straight line that connects the start of the stance (ground contact) to the
point of maximum force. The rate of unloading, or falling slope, is the slope of a
straight line that connects the point of maximum force to the end of the stance phase (Evans, Horstman and Conzemius 2005). Rising and falling slopes have been expressed as a percentage of body weight per second squared (%BW/s²). Loading and unloading phases may also be reported and expressed in second or as a percentage of total stance time. The stance time and time to peak can also be measured and expressed in seconds, from which the ratio of time to PVF to stance time may be calculated (Evans, Horstman and Conzemius 2005).

Although GRF data are often presented graphically as force-versus-time curves (Winter 2005; McLaughlin 2001; DeCamp 1997; Budsberg, Verstraete and Soutas-Little 1987), they can be represented numerically too. Mathematical transformation such as Fourier transformation can be made to analyze the curves and identify subtle gait changes indicative of subclinical lameness (McLaughlin 2001; Lee et al 2004; Katic et al 2009).

2.1.3. Kinematic gait analysis

2.1.3.1. Definition

Kinematic variables are involved in the description of the movement, independent of forces that cause that motion. Kinematic data provide information relative to the stance and the swing phases of the dog’s stride, including linear and angular displacements, velocities, and accelerations for each joint analyzed. The trajectory of the center of mass of the whole body may be computed as the location of the weighted average of the center of mass of each segment of the body (Winter 2005). Acquisition of kinematic data provides objective information to further define normal
and pathological gaits of animals, and track changes across time, in response to a specific treatment for example.

Collection of kinematic data allows accurate description of the complex movement of gait but requires specialized equipment. Kinematic gait analysis is mostly performed using a series of cameras and reflective targets placed on the dog’s skin over specific anatomic landmarks: center of gravity of body segments, center of rotation of joints, extremities of limb segments, or key anatomical prominences. Computer programs can be used to calculate the flexion and extension movement of joints and the temporal and distance variables of a subject passing through a three-dimensional testing area, either over ground or on a treadmill (McLaughlin 2001; Gillette and Angle 2008). The spatial reference system can be either relative or absolute. The former requires that all coordinates be reported relative to an anatomical coordinate system, the latter means that the coordinates are referred to an external spatial reference system. The same applies to angular data. Relative angles mean joint angle; absolute angles are referred to the external spatial reference and typically represent segment angles (Winter 2005).

2.1.3.2. Techniques

2.1.3.2.1. History

The first motion picture cameras recorded locomotion pattern of both humans and animals more than a century ago. In 1878, Muybridge triggered 12 stereoscopic cameras sequentially to cover the twenty feet taken by one horse stride. This famous series of photos is called “The horse in motion”. Marey, a French physiologist, used a
photographic “gun” in 1885 to record displacements in human gait and chronophotographic equipment to get a stick diagram of a runner (Baker 2007).

2.1.3.2.2. Direct measurement techniques

a) Goniometry

Goniometry is the measurement of angles, particularly those formed by joints. These angles may be measured in a standing position, in flexion and in extension. Manual goniometry is performed with a measuring device, typically a transparent plastic goniometer. An electrogoniometer may be used too, which is an electrical potentiometer that can be attached to measure a joint angle. As such, one arm of the goniometer is attached to one limb segment, the other to the adjacent limb segment, and the axis of the goniometer is aligned to the joint axis (Jaegger, Marcellin-Little and Levine 2002; Thomas et al 2006; Sato et al 2005).

Goniometry is used by orthopedic surgeons and physical therapists to quantify baseline limits of joint motion, to aid decisions on appropriate therapeutic interventions, and to document the effectiveness of these interventions (Jaegger, Marcellin-Little and Levine 2002; Sato et al 2005; Mann, Wagner-Mann and Tangner 1988). Jaegger et al. showed in Labrador Retrievers (n = 16) that results of goniometry for the front and hind limbs were not significantly different from joint angles measured on radiography (Jaegger, Marcellin-Little and Levine 2002). Goniometry appears to have substantial advantages over radiography, including simplicity, rapidity, lower cost, and no need for sedation or exposure to radiation. On the contrary, in Thomas et al’s study on German Shepherd dogs, goniometric measures differed significantly from
radiographic data for six of twelve joint positions (Thomas et al 2006). This difference may have been influenced by the fact that German Shepherd dogs were anesthetized compared to sedated Labrador Retrievers in the former study (Jaegger, Marcellin-Little and Levine 2002). Moreover, goniometric measures were performed by only one investigator, German Shepherd dogs had less morphologic variability than Labradors, and slightly different anatomic landmarks were used for the two methods (goniometry versus radiography), particularly for stifle joint flexion. Last, standard goniometric measurements are different for a given joint among breeds (Jaegger, Marcellin-Little and Levine 2002; Thomas et al 2006; Mann, Wagner-Mann and Tangner 1988).

When determining the range of motion of a joint, performing goniometric measurements in triplicate, and selecting the median value of these measurements is recommend to improve the accuracy of the process (Jaegger, Marcellin-Little and Levine 2002). The mean variability in measurements of several proximal joints (shoulder, hip and stifle joints) is larger than the mean variability in measurements of several distal joints (elbow and tarsal joints). This variability may be because a larger amount of soft tissue is present around proximal joints, compared with distal joints, potentially interfering with the palpation of body landmarks. Moreover, the larger muscle mass around proximal joints appears to complicate the goniometric measurements (Jaegger, Marcellin-Little and Levine 2002).

Experience with goniometry, either acquired during years of professional experience or during the course of a study, did not appear to affect the reliability of goniometric measurement. Sedation did not appear to influence range of motion despite the various anxiety levels in the dogs (Jaegger, Marcellin-Little and Levine 2002).
However, goniometric measurements made in awake patients is generally considered as indicators of pain-free range of motion and the measurements made in sedated patients as maximal range of motion. While manipulating a painful joint, the protective response of the patient may limit the range of motion of the joint of interest. With presence of pain, range of motion is sensory-limited and not mechanically limited. This sensory limitation may not be present under sedation. In Jaegger et al’s study, the dogs were healthy and pain free, so they assume that they compared the maximal ranges of motion, both awake and under sedation, which appeared to be identical (Jaegger, Marcellin-Little and Levine 2002). But this may not be true for joints with degenerative joint disease or other diseases in which range of motion may be voluntary restricted in response to a painful sensation.

Thomas et al. showed that an electrogoniometer had higher variability than a universal plastic goniometer (Thomas et al 2006). However, the electrogoniometer variability appeared to result from the technique: sensors were manually held in place during measurements. Therefore, it would be interesting to evaluate again the use of electrogoniometer in canine patients. Strapping the sensors to clipped areas could probably decrease sensor motion.

b) Accelerometer

An accelerometer is a device that measures acceleration by a force transducer, usually a strain gauge or piezoresistive type (Winter 2005). The use of an accelerometers does not require a gait laboratory environment and can be used for field studies with a completely body-mounted recording system (Brown, Boston and Farrar 2010; Brown et al 2010).
2.1.3.2.3. Imaging measurement techniques

Because of the complexity of most movements, the only system that can possibly capture all the kinematic data is an imaging system. We used in our study an optical motion capture system. Typically, two to six video cameras linked to a computer are positioned to simultaneously record the subject’s motion as it passes through the testing space. Reflective markers are placed on the subject’s skin over specific anatomic landmarks, most commonly over key anatomical prominences (Figure 1). The optical imaging system is responsible for converting the light from the markers into digital images. The computer collects the data from all the cameras and renders the three-dimensional position of each marker. Active diode markers that emit signals recorded by the cameras are used less frequently in veterinary gait studies. Kinematic data may be generated while the subject is doing sit-to-stand exercises, walks or trots over ground or over a treadmill (Feeney et al 2007; Alton et al 1998; Tashman and Anderst 2003; Owen et al 2004; Clements et al 2005).

An effort should be made to standardize the kinematic method to provide uniform data for analysis. Important sources of variability in the joint angle data include true differences between individual subjects, variations in trial repetition attributable to inconsistency of gait within an individual dog, and error attributable to measurement techniques (Hottinger et al 1996). Body morphology is likely to be a major determinant of baseline kinematics in dogs, and yet defining the
Figure 1: Areas of reflective markers placed on the subject’s skin over specific anatomic landmarks: cranial dorsal iliac spine, sacro-coccygeal articulation, greater trochanter, most distal point of the lateral femoral epicondyle, lateral malleolus of the fibula, and the 5th metatarsophalangeal joint. Note the presence of 2 markers on the front feet to distinguish the forelimbs during gait analysis.
role of morphology as a determinant of gait represents a monumental task. Until
morphology determinants have been defined, gait studies must include careful
restrictions in the sample populations used to limit variance attributable to this factor.

DeCamp et al. minimized the variation of their data by including a single
breed (Greyhound) selected for its uniformity in body conformation and
temperament. They studied a single gait (trot) with a velocity maintained within a
narrow range. Error was further reduced by using a single dog handler and a
single investigator to position the reflective markers on all dogs (DeCamp et al
1993). Inconsistencies in target placement, computer tracking of the target, soft-
tissue movement artefact and mathematic modelling may contribute to
experimental error. Superficial targeting introduces the possibility that kinematic
description of target movement may not fully describe true musculoskeletal
movement. This disparity, called “soft-tissue movement artefact”, is due to skin
motion (DeCamp et al 1993; Riemer, Hsiao-Wecksler and Zhang 2008). Muscular
contraction, ligament, tendon and fabellar movement could also affect the position
of surface markers.

To palliate this limitation, invasive kinematic analysis involves placement of
instrumented spatial linkages rigidly connected to the skeleton with bone plates
(Korvick, Pijanowski and Schaeffer 1994) or intra-cortical Steinmann traction pins
(Lafortune et al 1992). However, the risk and discomfort associated with these
techniques limit the number of willing volunteers, and may make serial studies
impossible. The validity of data obtained under these conditions is further limited by
the potential effects of implants (and their surgical placement) on the gait. Such techniques consequently had limited applications in human or animal experiments.

Dynamic Magnetic Resonance Imaging (MRI) and Computerized Tomography (CT) methods show promise but are limited by low frame rates and environments too restrictive for most dynamic, weight-bearing activities. Conventional fluoroscopy permits direct visualization of bone motion, but is limited to two-dimensional assessment and is prone to errors due to parallax and motion blur (Tashman and Anderst 2003). Biplane or stereo radiographic imaging enables accurate quantitative three-dimensional motion assessment as well as direct evaluation of bone motion. Radiopaque tantalum bone markers are implanted percutaneously with a cannulated drill (Tashman and Anderst 2003). This approach is particularly well suited for the development, validation and implementation of dynamic musculoskeletal models aimed at estimating in vivo behavior of internal joint structures. It is also well suited for studies involving rapid acceleration or deceleration, as skin movement would be especially prone to affect measurements based on surface markers.

The support for locomotion may be another source of variation. Kinematic data may be recorded as the subject walks or trots through the testing area (overground) or he may ambulate over a treadmill. Although the treadmill offers a convenient and controlled environment for studying gait, Alton et al, who compared over-ground and treadmill walking in human, demonstrated that the kinematic data differ between the two conditions. The significant differences between hip range of motion, maximum hip flexion angle and stance time may be attributable to subjects not being habituated to treadmill walking (Alton et al 1998).
Habituation is defined as the decline of a conditioned response after repeated exposure to the conditioned stimulus. For movement on a treadmill, habituation is the point at which a steady, repeatable gait is achieved. In Greyhounds, the mean elbow and stifle joint angles and their respective velocities at thirty seconds of trotting did not differ from that at two minutes. This suggests that after walking for two minutes over the treadmill, consistent elbow and stifle joint kinematics at a trot can be obtained for treadmill-naïve Greyhound within only 30 seconds (Owen et al 2004). In Labradors Retrievers, a consistent trotting gait was not obtained after five two-minute trials over a treadmill (Clements et al 2005). Thus, further studies are needed to establish how long Labrador Retrievers need to become habituated to walking or trotting on a treadmill before this technique can be applied to the analysis of the gait in this breed. However, the patterns of movement of the elbow and stifle joint recorded on the treadmill were very similar to the patterns observed in dogs trotting overground, and the average range of motion of both joints were similar to those observed in dogs walking and trotting overground (Clements et al 2005).

2.1.3.3. Parameters measured and analysis

A great deal of information can be gathered with kinematic gait analysis from the accumulation of data points from reflective tracking of the limb targets. Kinematics provide input about the stance and the swing phases of the gait cycle, including subject velocity, joint angles, linear and angular velocities and accelerations of each segment of the body or of the center of mass of the whole body (McLaughlin 2001). Stride length and frequency, temporal data and limb symmetry may also be
documented (Schaefer et al 1998; Gillette and Zebas 1999). Two-dimensional kinematic analyses are reported most commonly in the literature because of ease in data collection and processing compared with three-dimension analyses. Moreover, the amplitude of movement predominates in the sagittal plane compared with transverse and longitudinal planes (Fu, Torres and Budsberg 2010).

Each joint has a characteristic pattern of flexion and extension movement (sagittal plane) for a defined gait. When a joint flexes, the numeric value of the joint angle decreases. On the contrary, when a joint is extending, the numeric value of joint angle increases. Peaks of maximum flexion or extension and range of motion (ROM = peak of extension – peak of flexion) during the stance, the swing or the whole gait cycle may be reported (in degrees) and compared between groups. Mean joint angle during the stance and the swing phases and during the whole gait cycle may also be documented. In the transverse and longitudinal planes, adduction-abduction angles and internal-external rotation angles may be documented respectively (Fu, Torres and Budsberg 2010; Bulgheroni et al 1997; Tapper et al 2008; Chailleux et al 2007).

Angular velocities and accelerations for each joint of interest may also be reported, in degrees per second (°/s) and degrees per second squared (°/s²) respectively, and compared between groups. Joint angular velocities can be important determinants in detecting joint abnormalities. Subtle changes in the control of joint motions during gait may be detected only by changes in angular velocities and not by changes in joint angles (Marghitu, Kincaid and Rumph 1996).
Alternatives include the modelling of the kinematic curves with polynomial equations and Fourier transformation (Hottinger et al 1996; Schaefer et al 1998; DeCamp et al 1996; Torres et al 2010).

Aside from dynamic waveforms, kinematic analysis provides discrete variables to describe movement. Discrete temporal and spatial events are described for the canine trot and walk, including stride length, frequency and velocity, maximum foot velocity, and duration of the stance and swing phases (Hottinger et al 1996; DeCamp et al 1993). Linear velocities and accelerations, for example foot velocity and acceleration in the forward direction, are documented in meters per second (m/s) and meters per second squared (m/s²) respectively. The stride length, in meter (m), represents the distance between two consecutive foot strikes for a limb of interest and the stride time the duration of a stride in second (s). The stride frequency, cadence, is the number of strides per second (in s⁻¹). The stance phase duration represents the amount of time that the foot of the limb of interest is in contact with the ground (weight bearing phase, in s). The stance phase may also be expressed as a percentage of the duration of the whole gait cycle (i.e. duration of the stride). Values of stride length and time and stance time may be absolute or relative, if normalized by a characteristic length (tibial length or functional limb length for example). The duty factor represents the fraction of the total stride period that the foot was in contact with the ground and is calculated by dividing the stance time by the stride time (Evans, Horstman and Conzemius 2005). Analyses of discrete variables require no mathematical modelling and may proceed with a more straightforward approach using standard analysis of variance.
The last type of kinematic analysis relies on phase plane portraits to characterize joint motion (Marghitu, Kincaid and Rumph 1996). With this technique, locomotion is represented as a nonlinear periodic system. Sagittal phase plane portraits are obtained by plotting the joint rotations against the corresponding velocities. The events of paw strike and paw off are identified on the phase plane portraits. Phase plane trajectory of a joint may be used to highlight variations in locomotion compared to normal subjects, thereby enabling detection of subtle lameness (Marghitu, Kincaid and Rumph 1996).

Kinematic and kinetic analyses provide quantitative data to describe the gait of a subject. A better insight into subject locomotion is therefore gained compared with subjective methods to evaluate lameness. However, combined analyses, using data of both kinematic and kinetic studies, in an inverse dynamics analysis approach, would provide further information about gait, such as internal forces at each joint of interest or net moments around particular joints. To be able to gather that further information, morphometric data of the individual segments are needed in addition to kinematic data and GRF for inclusion of gravitational and inertial forces in the model.

2.1.4. Morphometry

2.1.4.1. Definition

Morphometry is the study of physical measurements of the animal body to determine differences between individuals and groups. Anthropometry is similar but applies to humans only (Winter 2005). Body segment parameters of the individual
segments are needed to account for inclusion of gravitational and inertial forces data in gait analysis.

2.1.4.2. Segment length

The most basic body dimension consists of the length of segments between each joint. Historically, measurements have been performed on cadavers of Japanese and Caucasian males and females (Winter 2005; Dempster 1955; Fujikawa 1963; Liu, Laborde and Van Buskirk 1971; Mori and Yamamoto 1959; Clauser, McConville and Young 1969). These data were the basis to estimate the length of a segment as a percentage of the body height. These segment proportions serve as a good approximation in the absence of measurements obtained directly on the individual.

2.1.4.3. Segment density

Each body segment contains a unique combination of bone, muscle, fat and other tissues, resulting in heterogeneity within each segment. Cortical bone has an approximate density of 1.8 g/cm$^3$, which was determined using computerized tomography scanning. Cancellous bone has a density of 1.1 g/cm$^3$, muscle tissue of 1.06 to 1.08 g/cm$^3$, visceral organs and body fluids such as blood of 1.06 g/cm$^3$ and fat of 0.94 to 0.96 g/cm$^3$ (Henson, Ackland and Fox 1987; Zatsiorsky 2002). The density of distal segments is generally greater than that of proximal segments because of the higher proportion of bone. Moreover, the density of individual segments correlates with the average body density (Zatsiorsky 2002).
2.1.4.4. Segment mass and location of the center of mass

The mass of individual segments correlates with the total body mass and may therefore be expressed as a percentage of the total body mass. The body weight represents the body mass (in kg) time the gravity (in m.s\(^{-2}\)) and is expressed in Newtons. Using cadavers, each segment mass can be weighed on a scale after disarticulation.

The terms center of mass and center of gravity are often used interchangeably. However, the more general term is center of mass. The center of gravity refers to the center of mass in one axis only, defined by the direction of gravity. The location of the center of mass is expressed as a percentage of the segment length, in relation to the distal or proximal end of the segment (Dempster 1955; Clauser, McConville and Young 1969; Pearsall and Costigan 1999). With the balance board technique, the center of mass is located by determining the center of balance of each individual segment in each direction. The balance board system can also be used in living subjects to establish a relation between the mass and the displacement of the center of mass of a body segment. The relation is given by:

\[ W = D \times (\Delta R)/d_w, \]

where \( W \) is the weight of the body segment, \( D \) is the distance between the two supporting knife edges of the balance board, \( d_w \) is the displacement of the center of mass of the body segment and \( \Delta R \) is the change in pressure exerted on the scale due to this displacement (Bernstein 1967; Drillis, Contini and Bluestein 1964). Using advanced imaging, the profile of cross-sectional area and the length of the segment can provide the location of the center of mass. The segment is broken into \( n \)
sections. The mass of each section is determined according to its volume and the density of the different materials within this section (Winter 2005).

2.1.4.5. Mass moment of inertia

Whereas the location of the center of mass of each segment is needed to analyze translational movement through space, the mass moment of inertia \( I \) is needed to evaluate rotation.

\[
M = I \times \alpha, \\
\]

with \( M \) (N.m) the moment of force causing the angular acceleration \( \alpha \) (rad.s\(^{-2}\) or ms\(^{-2}\)). Thus, \( I \) (kg.m\(^2\)) is the constant of proportionality that measures the ability of the segment to resist changes in angular velocity. The value of \( I \) depends on the point about which the rotation is taking place, and is minimum when the rotation takes place about its center of mass. Torque and moment are both equivalent forces with the same units (force x distance). A torque is a purely rotational force, whereas a moment is a force applied at a specified distance from an axis of rotation.

The constant \( I \) can be directly calculated using a method called the quick release experiment, which is based on Newton’s law for rotation. This law states that the moment acting on a body \( M \) is proportional to its angular acceleration \( \alpha \), the proportionality constant being the mass moment of inertia \( I \). Thus if the body segment can be made to move at a known acceleration by a moment which can be evaluated by applying a known force at a given distance, its moment of inertia could be determined (Drillis, Contini and Bluestein 1964).
A pendulum technique can also be used to compute the mass moment of inertia. The segment is allowed to oscillate about a fixed suspension point and its period, or time for a complete cycle, is measured. The moment of inertia of the system is found from the measurement of the period (Drillis, Contini and Bluestein 1964).

2.1.4.6. Determination of body segment parameters

Historically, body segment data have been collected by direct measurements, which is time-consuming and requires cadavers (Buchner et al 1997; Fujikawa 1963; Liu, Laborde and Van Buskirk 1971; Mori and Yamamoto 1959; Clauser, McConville and Young 1969; Dempster, Gabel and Felts 1959; Nielsen et al 2003; Colborne et al 2005; Dogan et al 1991). Moreover, only a few such studies in humans have been performed, with a total of less than 50 cadavers measured, predominantly old adult male Caucasians or Asians. Pearsall et al. showed that substantial variation in segment parameters estimates were produced when using various predictive formulae from the literature. In some cases, the values of mass and mass moment of inertia of a segment differed more than 40% from one study to another (Pearsall and Costigan 1999). However, these published data have frequently been applied in development of mathematic models of the human body and in analysis of human motion. Segment parameters variations were found to significantly affect most of the kinetic estimates produced, particularly those taken during the swing phase (Riemer, Hsiao-Wecksler and Zhang 2008; Pearsall and Costigan 1999). Although the magnitude of these effects was generally less than one percent of body weight, accuracy of segment parameter prediction should be of concern in biomechanical
research particularly for open chain and high acceleration activities, and for distal
segments (Pearsall and Costigan 1999; Colborne, Shellard and Morris 2007; Lanovaz
and Clayton 2001). Moreover, Dowling et al. showed that the pendulum method to
determine the mass moment of inertia of an object is quite sensitive to errors in the
period of oscillation. Although the uncertainty of this method could be drastically
reduced to less than three percent by suspending the object with the axis located at the
radius of gyration, most studies have adopted a proximal suspension, including the
often cited work by Dempster (Dempster 1955; Dowling, Durkin and Andrews 2006).

Another method is to use geometrical models, so the shape of the segments can
be approximated. From this, the inertial properties can be calculated thanks to volume
and density. However, while easy and useful in large population groups for
epidemiological studies, Buckley showed that anthropometry using geometrical models
in lower extremity in human overestimated total area and total volume by five to ten
percent. Moreover, it overestimated muscle plus bone area and muscle plus bone
volume by as much as 40 % (Buckley et al 1987).

More recently, modern techniques have been developed using scanning systems
that produce the cross-sectional image at many intervals across the segment. Individual
body segment parameters may now be determined from living subjects by use of
noninvasive techniques such as computerized tomography, magnetic resonance
imaging, gamma scanning and dual energy X-ray absorptiometry (Winter
2005; Pearsall, Reid and Livingston 1996; Zatsiorsky and Seluyanov 1983; Durkin and
Dowling 2003; Cheng et al 2000; Huang and Suarez 1983; Martin et al 1989; Durkin,
2.1.4.7. Direct experimental measures

For more exact kinematic and kinetic calculations, it is preferable to have directly measured morphometric values. But the equipment and techniques that have been developed have limited capability and sometimes are not much of an improvement over the values for humans obtained from tables. Unfortunately, few morphometric studies have been performed with veterinary patients, and very few body segment parameter data or regression models have been published. Tables available in horses and dogs are based on studies with a low number of subjects (Buchner et al 1997; Nielsen et al 2003; Colborne et al 2005). As a result, most of the studies on animals require direct measurement for each individual, particularly for dogs.

Dogan et al. published a study about cemented total hip replacement in dogs, in which he used five adult mixed-breed dogs, with body masses of 31.8 – 34.0 kg. At the end of the study, after euthanizing each dog, hind limb segments were isolated by disarticulating the hip, stifle, and talo-crural joints. The center of mass was determined for each segment using mass balances. Limb segment dimensions, masses, and mass moments of inertia were also determined. A pendulum technique and transformation calculations were used to compute the mass moment of inertia. An electronic sensing device accurately measured the pendulum period of each segment (Dogan et al 1991). Unfortunately these morphometric data are not available in the literature.

To compare power distribution across the hind limb joints between Labrador Retriever and Greyhound dogs, Colborne et al. collected morphometric data on hind limb segments from cadavers of client-owned animals (Colborne et al 2005). Three Labradors weighing 30.3 ± 3.6 kg and four Greyhounds weighing 32.3 ± 3.5 kg were
used. Cadavers’ specimens were disarticulated through the hip, femoro-tibial, tibio-
tarsal, and metatarsophalangeal joints on a plane perpendicular to the long axis of the
proximal bone segment. Segments were weight on a digital scale, and weight of each
segment was recorded as a percentage of total body weight (Table 2). The location of
the center of gravity along the long axis of each segment was determined in accordance
with the balance board technique. Segment inertial quantities were unfortunately
neglected and therefore mass moment of inertia was not documented for the segments
(Colborne et al 2005). This study found the location of the segmental center of gravity
to be similar between the two breeds, but proximal segments were heavier in
Greyhounds when their weight were expressed as a percentage of body weight
(Colborne et al 2005).

Nielsen et al. published the mass, the location of the center of mass and the
mass moment of inertia for forelimb segments (Table 2) of six adult mixed-breed dogs
(mean body mass 25.3 ± 2.5 kg) (Nielsen et al 2003). After euthanasia, the left forelimb
was removed from each cadaver and forelimb segments were isolated by disarticulation
of the shoulder, elbow, antebrachiocarpal and metacarpophalangeal joints. The location
of the center of mass was determined by use of a balance board technique. Limb
segment masses and lengths were measured directly. Mass moments of inertia were
determined by use of a pendulum technique and the parallel-axis theorem.

Most equine gait analysis studies refer to the morphometric data published by
Buchner et al. (Buchner et al 1997), who determined a complete set of three-
dimensional inertial properties of Dutch Warmblood horses, including segment mass,
location of the center of mass, mass moment of inertia and density of all body
segments. However, this study has several limitations: (i) data are only from 6 horses (body mass range from 470 to 620 kg, mean 538 kg), (ii) mass moments of inertia around the longitudinal axis in the smallest segments were considerably underestimated, (iii) the regression model did not perform well for the small horse, which lied outside the range of the material. This stresses the importance of using regression models only for horses within the range of the material. Furthermore, it precludes direct extrapolation of models between breeds, and implies that inertial properties and segment masses have to be determined in each individual horse breed (Buchner et al 1997).

Available BSP, breed-specific tables from a numerous population of domestic animals or a non-invasive and easy way to determine the BSP on living subjects represent future progress that should be accomplished.

Ground reaction forces, kinematic, and morphometric data may now be combined in an inverse dynamics analysis method using a segmental model of the limb, which would yield information in two- or three-dimensions about the internal forces, the net moments around the joints, and patterns of muscle work or joint power.
Table 2: Body segment parameters of the front and hind limb segments obtained from canine cadavers. Front limbs data were documented from 6 large breed dogs with a mean (±SD) body mass of 25.3 ± 2.5 kg. 4 Greyhounds (G) and 3 Labrador Retrievers (L) provided support to determine hind limb data. # represents the percentage of total body mass. Location of the center of mass as a percentage of the distance form the proximal (§) or distal (*) end of the segment. Modified from Nielsen et al., 2003 and Colborne et al., 2005.
2.1.5. Dynamic gait analysis

2.1.5.1. Inverse dynamics analysis

Inverse dynamics analysis is a standard tool for biomechanical studies of locomotion. The traditional approach is to use kinematics of the body segments, combined with measurements of external (ground reaction) forces and morphometric data in a segmental model of the limb. The kinematic data are known and they are used to solve the kinetics in order to obtain resultant intersegmental force and net moment at each joint of interest. The moment provides information about muscle function and can be used to estimate joint power and work.

The first step in the process is to calculate joint reaction force and net joint moment for each joint of interest using rigid body link-segment models in order to draw a free-body diagram (Winter 2005). The free body diagram is a graphical representation of a body and all of the external forces acting on it (Figure 2). Three-dimensional calculations are used less frequently, most likely because of the complexity of the 3-dimensional system and because most of the motion takes place in the sagittal plane. The force and moment at a joint of the model can be calculated in 2-dimensions with the following equations:

\[ \Sigma F_x = m*a_x \quad \Rightarrow R_{xp} - R_{xd} = m*a_x \]
\[ \Sigma F_y = m*a_y \quad \Rightarrow R_{yp} - R_{yd} - m*g = m*a_y \]
\[ \Sigma M = I_0*a = M_p + M_d + R_{yd}*d_j*cos\theta - R_{xd}*d_j*sin\theta - R_{xp}*d_2*cos\theta + R_{yp}*d_2*sin\theta \]

(M calculated about the segment center of mass, COM).

Known are \( m \) (mass of the segment), \( a_x \) and \( a_y \) (acceleration of the segment center of mass in the \( x \) and \( y \) directions), \( \theta \) (angle of the segment in plane of movement), \( a \)
(angular acceleration of the segment in plane of movement), \( R_{xd} \) and \( R_{yd} \) (reaction forces acting at distal end of the segment, determined from \textit{a priori} analysis of the proximal forces acting on the distal segment), \( d_1 \) (distance from the distal joint center to the center of mass), \( d_2 \) (distance from the proximal joint center to the center of mass), and \( M_d \) (net muscle moment acting at the distal joint, determined from an analysis of the proximal muscle acting on the distal segment). Unknown are \( R_{xp} \) and \( R_{yp} \) (reaction forces acting at the proximal joint) and \( M_p \) (net muscle moment acting on the segment at the proximal joint) (Figure 2). By definition, flexor moments are negative and extensor moments are positive. Forces are expressed in newton (N) and moments in newton meters (N.m).

Moreover, joint power and work can be determined. To calculate joint power for the ankle, knee and hip joints, the same equation is used:

\[
P = M \times \omega,
\]

with \( M \) the moment applied at the joint and \( \omega \) the joint’s angular velocity. Power is timed rate of work done by a system and is expressed in watts (W). The power is positive (power generation) for concentric contractions (muscle moment and joint movement in the same direction), and negative (power absorption) for eccentric contractions (muscle moment and joint movement in opposite direction) (Winter 1987).

The work done (energy) is by definition the integral of power over time. Therefore the net joint work done between an interval of time is given by calculating the area under a plot of joint power against time and is expressed in joule (J) or watt seconds. A positive value of joint work indicates that energy has been generated by the joint, while a negative value indicates that energy has been absorbed in the joint.
Figure 2: Free body diagram of a segment. $m$ is the mass of the segment, $\alpha_x$ and $\alpha_y$ are accelerations of the segment center of mass in the $x$ and $y$ directions, $\theta$ is the angle of the segment in plane of movement, $\alpha$ is the angular acceleration of the segment in plane of movement, $R_{\text{ad}}$ and $R_{\text{vd}}$ are the reaction forces acting at distal end of the segment, $M_d$ and $M_p$ are the net muscle moment acting at the distal and the proximal joint respectively, $R_{xp}$ and $R_{yp}$ are the reaction forces acting at the proximal joint, $d_1$ is the distance from the distal joint center to the center of mass (COM), and $d_2$ is the distance from the proximal joint to the COM.
2.1.5.2. Forward dynamics analysis

Most biomechanical modeling involves inverse dynamics analyses to predict variables such as reaction force, moment of force, mechanical energy and power, none of which are directly measurable. The reverse of this analysis is called forward dynamics analysis or synthesis of movement. The kinematic data are predicted from assumed moments of force (or muscle forces) and a complete set of inertial properties including mass, location of the center of mass, and mass moment of inertia. The ultimate goal, once a valid model has been developed, is to investigate response of the model to various modifications. However, the validity of the predictions are limited by a lack of correct morphometric data (Winter 2005).

2.1.5.3. Parameters measured

Inverse dynamics analysis has been applied to gait of human, and recently in horses and dogs (Winter 2005;Nielsen et al 2003;Colborne et al 2005;Dogan et al 1991;Clayton et al 2002;Khumsap et al 2003;Colborne et al 2006). Published inverse dynamics data from animal subject are restricted to two-dimensional analyses (sagittal plane only), however rare human studies describe the use of inverse dynamics method in three-dimension. For example, Bulgheroni et al. reported the value of the flexion-extension (sagittal plane), adduction-abduction (transverse plane) and internal-external rotation (longitudinal plane) moments at the hip, knee and ankle joints (Bulgheroni et al 1997).

Parameters measured in equine studies are normalized by body mass; therefore net joint moments are expressed in newton meters per kilogram (N.m/kg), joint power
in watts per kilogram (W/kg), work in joules per kilogram (J/kg) and internal joint reaction forces in newton per kilogram (N/kg). In horses, inverse dynamics solutions have been applied to the coffin (distal interphalangeal), fetlock, carpal, elbow, shoulder, tarsal, stifle and hip joints (Clayton et al 2002; Khumsap et al 2003; Clayton et al 2000).

In one of the first canine inverse dynamics study, Wentik et al. documented the vertical and horizontal total forces applied to the centers of gravity of the hind limb segments (foot, leg and thigh) in newtons (Wentink 1977). However, this study didn’t describe the internal reaction force at each joint of interest. Other canine inverse dynamics studies reported the peak of vertical and horizontal inter-segmental forces (as a percentage of body weight [%BW] or in newtons per kilogram [N/kg]), net joint moments (in N.m and N.m/kg) and joint powers (in W and W/kg). No studies describe the use of integrating joint reaction forces over time (i.e. impulse) to characterize the canine gait. Inverse dynamics solutions have been applied to the metacarpophalangeal, carpal, elbow, shoulder, metatarsophalangeal, hock, stifle and hip joints of walking or trotting Labrador Retrievers, Greyhounds or large mixed breed dogs (Nielsen et al 2003; Colborne et al 2005; Dogan et al 1991; Colborne et al 2006). Colborne et al. showed that increases in trotting velocity in Greyhound do not alter the basic patterns of work and power for various joints of the hind limbs in the sagittal plane, but local burst amplitudes during the stance phase increase incrementally (Colborne et al 2006).

2.1.5.4. Limitations

Inverse dynamics studies are based on assumptions inherent to the model. It is assumed that each body segment has a constant mass, constant length, fixed center of
mass location, and a constant mass moment of inertia about its center of mass throughout the movement, and that all joints act as simple hinge joints with air friction playing a limited role (Winter 2005). However, most joints cannot be considered as a standard hinge joint. Moreover, segment parameters variations and soft-tissue movement artefact were found to affect the kinetic estimates produced, especially during the swing phase (Riemer, Hsiao-Wecksler and Zhang 2008). Therefore inverse dynamics solutions don’t completely represent real joint mechanics.

Inverse dynamics analyses provide information about joint power among other things, which power depends on the net joint moment and angular velocity of the joint excursion. When co-contraction of antagonistic muscle groups stabilizes a joint against motion, then angular velocity and calculated power will be low. This may be well the case at the stifle joint for example, where intuitively the stifle extensors must be coactive with the flexors, given the angular position of the joint at the time of ground contact (Colborne et al 2005). So it would be helpful to combine joint kinetic and kinematic patterns with electromyographic analysis for a complete assessment of the manner by which muscle coactivation affects joint motion.

2.1.6. Electromyography

2.1.6.1. Techniques

The electrical signal associated with the contraction of a muscle is called an electromyogram. Electromyography (EMG) is mainly used to identify the muscle(s) responsible for a muscle moment and detect any antagonistic activity. However, EMG activity is not necessarily directly related to muscular force or contraction. The
electrical signal generated in the muscle fibers that results from the recruitment of a motor unit is called motor unit action potential. Electrodes placed inside the muscle tissue (indwelling electrodes) or on the surface of a muscle will record the sum of all motor unit action potentials being transmitted along the muscle fibers at that point in time. However, there are many variables that can influence the signal at any given time: velocity of shortening or lengthening of the muscle, rate of tension build-up, fatigue and reflex activity (Winter 2005).

The two broad classification of EMG electrodes are surface versus indwelling (intramuscular) electrodes. Surface electrodes typically consist of disks of metal, usually silver/silver chloride. They may also consist of two parallel in relief metal lines embedded in a plastic device. Surface electrodes detect the average activity of superficial muscles. Indwelling electrodes are required, however, for the assessment of deep muscles or fine movements. Surface electrodes register electrical activities nonselectively from a wider region, covering the recording radius of some 20 mm compared to selective pickup from a 500 µm radius by a needle electrode (Winter 2005). Both surface and indwelling electrodes are influenced by waves that actually pass by their conductive surfaces, but also by waves passing within a few millimeters. Surface electrodes have typically a finite surface area, so the potential on the surface represent the average of all point source potentials. Surface electrodes can be placed over the muscle belly along an axis parallel to the muscle fibers or perpendicular to the muscle fibers.

The signal represents the summation of motor unit action potentials and should be undistorted and free of noise or artifacts before it can be evaluated. The EMG signal
must first be amplified; then, calibration must be done. Electromyograms in humans are typically calibrated against resting activity and isometric maximum voluntary contraction of the muscle of interest (Williams et al 2005). Alternatively, calibration may be adjusted against maximum involuntary contraction (Branch, Hunter and Donath 1989; Besier, Lloyd and Ackland 2003; Neptune, Wright and van den Bogert 1999; Rand and Ohtsuki 2000; Peham et al 2001) or a mean value of muscle activation (Kaya, Leonard and Herzog 2003). Reproducibility was improved when the normalization method was based on the maximum voluntary isometric contraction compared with the use of maximum involuntary contraction or a mean dynamic EMG value. (Knutson et al 1994) However, precision was improved when using the maximum involuntary contraction and mean dynamic EMG methods. Calculation in animals can only be based on maximum involuntary contraction, mean or resting activity because maximum voluntary contraction cannot be determined.

The premise behind EMG studies is based on the relationship between EMG and muscle function. This relationship was evaluated by Komi and Buskirk, in a study where a subject was asked to generate maximum tension while the muscle lengthened (eccentric contraction) or shortened (concentric contraction) at controlled velocities. Decreased tension during shortening and increased tension during lengthening were observed. However, the EMG amplitude remained fairly constant. Such results support the theory that the EMG amplitude indicates the state of activation of the contractile element, which is quite different from the tension recorded at the tendon (Komi and Buskirk 1972; Winter 2005).
2.1.6.2. Parameters measured and analysis

The following variables can be calculated for each muscle from the processed electromyographic data: duration of muscle burst, muscle onset, off time (termination of the activity), time of peak muscle amplitude, peak, mean, minimum and maximum values of muscle amplitude, co-contraction index, and the integral of muscle activity. Times are often expressed in milliseconds (ms). EMG amplitude is measured in millivolts (mV) and reflects the spatial and temporal summation of motor unit action potentials in proximity of the recording electrodes. Peak muscle amplitude is often a normalized peak amplitude as a percentage of maximum voluntary or involuntary contraction of the corresponding muscle (Wentink 1977; Branch, Hunter and Donath 1989; Besier, Lloyd and Ackland 2003; Neptune, Wright and van den Bogert 1999; Rand and Ohtsuki 2000; Cowling, Steele and McNair 2003; Hurd, Chmielewski and Snyder-Mackler 2006; Chappell et al 2007; Buford and Smith 1990). Neptune et al. defined the criteria for the onset and offset values based on a minimum threshold of three standard deviations of the resting baseline and a minimum burst duration of 50 ms (Neptune, Kautz and Hull 1997). Zaneb et al. reported the use of mean, maximum, and minimum muscle activities of the pelvic limb and maximum-to-mean and minimum-to-mean activity ratios in horses (Zaneb et al 2009). Recently, Lauer et al. reported their results of surface EMG as a mean activity per muscle during the first and second halves of the stance phase and during the swing phase. Ratio of activity of distal quadriceps to hamstring, proximal quadriceps to hamstring and distal quadriceps to gluteal muscles were documented as well during the first and second halves of the stance phase (Lauer et al 2009).
Many factors can affect EMG amplitude such as muscle mechanics (muscle architecture, muscle length, type of muscle contraction, and velocity of contraction), and skin impedance (hair, oils, dead skin cells, electrode electrolyte interface). The amplitude of surface EMG may be affected by crosstalk from nearby muscles or part of muscles. In addition, there are non-neural activity related factors that affect EMG amplitude such as electrode and lead motion artifact, electromagnetic energy, and various types of equipment such as electrocardiograms (Gillette and Angle 2008; Bolton et al 2000).

Thus, various techniques of gait analysis exist from kinetic to kinematic, morphometry to electromyography, that may be used alone or combined in inverse or forward dynamics analysis. All these methods have been used extensively in human medicine for decades, providing objective information about gait, limb movement, degree of weight-bearing or pattern of muscle activation that are useful for clinical purposes. More recently, the application of these techniques to veterinary gait analysis has improved the characterization and understanding of animal normal and abnormal gaits, especially in horses and dogs. It is now well admitted that gait analysis techniques are objective tools that must be incorporated in the design of veterinary studies to overcome bias due to subjective gait assessment.
2.2. Clinical applications for the canine hind limb

2.2.1. Relevance of gait analysis

In spite of the fast evolution of orthopedics, the sophistication of surgical techniques developed for fracture and joint repair in small animal, the outcome of these techniques has largely been evaluated based on subjective clinical observations until recently. The outcome may be evaluated by physical examination and subjective grading assessment of different variables, including joint effusion or signs of pain elicited on palpation. Owners may fill questionnaires to assess their animal’s condition. Radiographic data may be used too, but they have their limits, as it has been previously reported that there is no relationship between limb function and radiographic osteoarthritis score in dogs with stifle osteoarthritis (Gordon et al 2003).

Scale of lameness was often used to quantify the degree of gait abnormality. Shires et al described two grading system, one to evaluate the type of lameness and another one to evaluate the frequency of lameness (Shires, Hulse and Lui 1984), but one of the most used lameness scale is graded from 1 to 4 (no to severe lameness). Lameness score may also be assigned by use of a visual analog scale, graduated from 0 to 10 (none to non weight-bearing lameness) (Hudson et al 2004). The degree of weight bearing when the limb is at rest may be graded too (Trumble et al 2005). However, human perceptive skills are clearly subjective by nature and relate to the clinical knowledge and acumen of the operator.

Currently, force plate and computer-assisted kinematic analysis have become the most used mean of objective gait assessment in the veterinary and medical professions. The proper use of these tools provides three distinct advantages over
subjective clinical evaluations alone: less human observer bias, improved perception and accuracy of measurement of gait dysfunction, and a tremendous capacity for data collection (DeCamp 1997; Burton et al. 2009; Waxman et al. 2008; Quinn et al. 2007). Specialized gait analysis techniques may eventually enable veterinarians to accurately diagnose subtle lameness, better evaluate dogs with resolving lameness and accurately select the appropriate time to return an athletic dog to exercise after recovering from an injury (Winter 2005).

Using a cranial cruciate ligament deficiency model in Labrador Retrievers, Evans et al. showed that, given two randomly chosen dogs (one sound, one lame), there is a 98% chance that the force plate analysis will correctly discriminate the lame from the normal dog. They also found that all dogs with observable gait abnormalities had a very low probability of being normal, i.e. having GRF consistent with the sound Labrador Retrievers. In contrast, 75% of the dogs that had no observable gait abnormalities had a probability of less than 50% of being sound; and the abnormality was easily discriminated by their GRF (Evans, Horstman and Conzemius 2005). Thus, this creates a clinical dilemma. If visual observation of gait cannot reliably discriminate abnormal from normal gait, nor radiographs can assess limb function, how does one simply tell an owner that their dog’s limb function has or has not returned to normal?

As we saw it previously, accuracy of kinetic and kinematic analysis is dependent of many factors, recorded data may therefore vary with different breeds and different type of lameness. A thorough canine gait database should be created for most of the breeds and orthopedic or neurological diseases.
2.2.2. Creation of a normal gait database

The major aim of treating patients with orthopedic diseases is to correct or improve joint and limb diseases and to restore functional normalcy. To accomplish this goal, it is necessary to develop the ability to quantify the structural and functional changes in limb use resulting from pathology in terms of well defined and meaningful parameters. The first step in this process is to establish a normative database from a sample population to define these parameters and their associated variability.

2.2.2.1. Definition of canine gaits

The walk and trot are the two gaits studied in the majority of canine gait analysis publications. The walk includes two-, three- and four-limb support phases, with no single limb support phase. Walking is a slow, symmetrical gait in which the limbs on one side of the animal perform the same movements as on the other side but a half stride later. Examination of this gait on a reflecting walkway has shown that the main pad takes the weight first and then the weight is put on the toe pads (Summer-Smith 1993). The trot is composed of a succession of two-limb support phases, with diagonal pairs of limbs involved, separated by an aerial phase during which none of the feet touch the ground (DeCamp 1997). The forelimbs are free of the ground longer than the hind limbs, permitting those limbs to clear the ground in advance of placement of the hind limbs. The animal forward velocity reported in the literature at a walking gait ranges from 0.95 to 1.3 m/s (Besancon et al 2003; Evans, Horstman and Conzemius 2005; Hottinger et al 1996), and from 1.91 to 2.86 m/s at a trotting gait (Lascelles et al 2006; Rumph, Steiss and Montgomery 1997; DeCamp et al 1993; Gillette and Zebas...
1999; Madore et al 2007). However, absolute gait velocities vary with the size of the subject, with small animals ambulating slower than large animals. Relative velocities normalized by subject’s height or a characteristic length are recommended to compare groups with different size.

2.2.2.2. Ground reaction force data

Numerous studies based on force plate and GRF have been performed in healthy dogs to document normal gait. Budsberg et al.’s work is considered as a founding study in this field despite rare previous less thorough reports (Budsberg, Verstraete and Soutas-Little 1987; Wentink 1977). Force-plates have been used in the evaluation of GRF associated with dogs at a walk (Besancon et al 2003; Budsberg, Verstraete and Soutas-Little 1987; Griffon, McLaughlin and Roush 1994), trot (Bertram et al 2000; Lee et al 2004; Lee, Bertram and Todhunter 1999; Lascelles et al 2006; Riggs et al 1993; McLaughlin and Roush 1994; DeCamp et al 1993; Rumph et al 1994) and while jumping (Yanoff, Hulse and Hogan 1992). These studies have improved our knowledge about normal canine gaits.

For the hind limbs at walk, the vertical force has a very characteristic “M-shape”, with a rapid rise at foot contact, followed by a drop as the stifle flexes during mid-stance, partially unloading the force plate. This phase represents the rollover of the foot between paw strike and toe off. At push-off, a second peak is recorded. Finally, the vertical force drops to zero as the opposite limb takes up the body weight (Winter 2005; DeCamp 1997; Budsberg, Verstraete and Soutas-Little 1987). In trotting, the vertical GRF of dogs are graphed as single, sharper peaks for both forelimbs and hind
limbs (DeCamp 1997). Immediately after ground contact, the cranio-caudal force is negative, indicating a backward horizontal friction force between the floor and the foot (braking phase). Near mid-stance, the cranio-caudal force becomes positive as muscle forces cause the foot to push back against the plate (propulsive phase).

Body weight is distributed at 28.0 ± 1.3 % to 29.6 ± 1.7 % to each forelimb and 20.4 ± 1.6 % to 22.7 ± 3.0 % to each hind limb at a walk (Budsberg, Verstraete and Soutas-Little 1987; Griffon, McLaughlin and Roush 1994). Approximately 50% of each stance phase is spent in each of the braking and propulsive phases in the forelimbs of a walking dog. In the hind limbs, the braking and propulsive phases represent respectively 35% and 65% of the stance (DeCamp 1997). In addition to general support, the forelimbs mainly function to decelerate the dog and the hind limbs serve to propel the animal forward (Budsberg, Verstraete and Soutas-Little 1987).

In normal large breed dogs at a walking gait, the peak vertical forces (PVF) in the hind limbs range from 33.9 to 49.0 percent of body weight (%BW) (mean 42.3 %BW) (Voss et al 2007). In walking Greyhounds, the PVF in the forelimbs average 61.6 ± 3.4 %BW and 42.3 ± 1.4 %BW in the hind limbs. In the same population, vertical impulses (VI) average 28.0 ± 1.9 %BW.s in the front limbs and 18.8 ± 1.0 %BW.s in the hind limbs (Besancon et al 2003).

In normal large breed dogs during trotting, the values of PVF in the front limbs range from 107 ± 9 to 115 ± 9 %BW and the values of VI range from 16.6 ± 1.1 to 17 ± 2 %BW.s. In the hind limbs, PVF equals 65 ± 7 to 72 ± 9 %BW and the VI equals 9 ± 1 %BW.s (Lascelles et al 2006; Rumph et al 1994). The forelimb braking and propulsive peaks are equal to -17 ± 3 and +7 ± 2 %BW respectively. In
the hind limbs, these peaks equal -4 ± 2 and +8 ± 2 %BW respectively. The usual medio-lateral force pattern found in forelimb is directed laterally, with a peak magnitude of 6 ± 4 %BW, whereas the hind limb patterns are variable (Rumph et al 1994). Peak vertical forces in the hind limbs are lower in trotting Labrador Retrievers compared with Greyhounds trotting at the same velocity (76 ± 8 %BW versus 107 ± 13 %BW) (Bertram et al 2000). In Rottweilers, PVF in front limbs are lower (117 ± 6 %BW versus 125 ± 11 %BW) and VI in both pelvic and front limbs are higher than in Labrador Retrievers (17.1 ± 1.3 %BW.s versus 15.7 ± 0.8 %BW.s for the front limbs; 9.3 ± 0.6 %BW.s versus 8.3 ± 0.6 %BW.s for the hind limbs) (Molsa, Hielm-Bjorkman and Laitinen-Vapaavuori 2010).

The variations in kinetic characteristics reported for the canine gait is not surprising. Although methods used to collect force plate data are known to affect objective results (Table 1), the canine gait varies also with the morphology of dogs. The effects of morphology on gait analysis measurements have been incompletely studied. It has been described that PVF, normalized as a percentage of body weight, correlates inversely with physical size of the dog, with larger dogs having lower maximum forces (Budsberg, Verstraete and Soutas-Little 1987; Molsa, Hielm-Bjorkman and Laitinen-Vapaavuori 2010). Bertram et al reported lower values for mean and maximum vertical forces in the hind limbs in Labradors (mean ± SD mass, 24.2 ± 3.0 kg) compared with Greyhounds (mean ± SD mass, 17.6 ± 1.8 kg). Labrador Retrievers put less force on their hind limbs than Greyhounds (Bertram et al 2000). On the contrary, the duration of the stance phase as well as braking and propulsion impulses increase with increasing physical size. Vertical, propulsive and
braking impulses also are significantly correlated with body weight (Budsberg, Verstraete and Soutas-Little 1987; Molsa, Hielm-Bjorkman and Laitinen-Vapaavuori 2010).

Because the effects of morphology on gait variables are not fully described, a population with a homogeneous morphology should be selected to reduce this inherent source of variance in kinetics. Small-breed dogs should not be compared with large-breed dogs in gait analysis. Chondrodystrophic breeds, giant breeds, or other dogs with unusual morphology should not be introduced into study samples of "normal" populations until the effects of morphology are better described. The effect of dog size on dispersion of force plate data is reduced in most studies of gait by normalization of GRF and impulses to body weight of the animal (Jevens et al 1993; McLaughlin et al 1991; Budsberg, Verstraete and Soutas-Little 1987; Molsa, Hielm-Bjorkman and Laitinen-Vapaavuori 2010; Voss et al 2010; Budsberg et al 1996; Jevens et al 1996). Unfortunately, this simple normalization is of very little practical value unless the temporal aspects of the gait and the size of the subjects are stringently controlled. This normalization method may yield misleading results if comparisons are not made at functionally comparable velocities.
2.2.2.3. Goniometric and accelerometer data

Orthopedic surgeons and physical therapists use common goniometric measures in human to quantify the baseline limits of joint motion, in order to choose appropriate therapeutic interventions and to document the outcome of these interventions (Sato et al 2005).

Reference ranges for joint range of motion in adult Labrador Retrievers free of orthopedic disease have been established for the carpal joint in flexion, extension, valgus and varus positions, and for elbow, shoulder, tarsus, stifle and hip joints in flexion and extension (Jaegger, Marcellin-Little and Levine 2002). The 95 % confidence interval of the means for these fourteen joint positions is within a narrow two- to four-degree range. This suggests excellent precision and repeatability of goniometry in the population of Labrador Retrievers included in this study (n = 16). These results could potentially be extrapolated to other canine breeds with similar conformation but may not be valid in breeds with substantially different conformations (Jaegger, Marcellin-Little and Levine 2002).

Goniometric measurements for German Shepherd dogs are lower than those for Labrador Retrievers during flexion and extension of the elbow, shoulder, hock, stifle and hip joints. Values for range of motion are significantly lower in the hock of German Shepherd dogs than in the hock of Labrador Retrievers. However, the range of motion of other joints is similar between the breeds. The combination of lower values for flexion and extension with a similar range of motion suggests that differences exist in bone shape but not in joint shape between these two breeds (Thomas et al 2006).
Although the use of goniometry has been validated in healthy dogs of certain breeds, additional research is needed to provide objective information regarding joint angles measured at rest or during locomotion in healthy dogs and with orthopedic diseases. Information regarding these joint angles is important to assess the efficacy of medical and surgical treatments, to plan joint arthrodesis and correction of limb deformities, or to detect early signs of osteoarthritis and decreased range of motion, which may enable early intervention (Thomas et al 2006).

Recently, a watch-sized, omnidirectional, accelerometer-based activity monitor has been reported to continuously measure the intensity, frequency, and duration of movement for extended periods, as an objective assessment of locomotor activity in dogs (Brown, Boston and Farrar 2010; Brown et al 2010). The baseline activity count in dogs with osteoarthritis is about 1.4 million over a week period (Brown, Boston and Farrar 2010).

2.2.2.4. Kinematic data

Dynamic flexion and extension movement patterns have been characterized for Greyhounds, Labrador Retrievers and mixed large-breed dogs doing sit-to-stand, swimming, stair ascent and descent exercises, and trotting and walking over ground or on a treadmill (Feeney et al 2007; Owen et al 2004; Clements et al 2005; Hottinger et al 1996; DeCamp et al 1993; Schaefer et al 1998; Gillette and Zebas 1999; Colborne et al 2005; Colborne et al 2006; Richards et al 2010). Recently, the sagittal (flexion-extension), transverse (internal and external rotation), and frontal (abduction-adduction) plane kinematics measurements were acquired during walking and
trotting trials for hip, stifle, and tarsal joints in six healthy mixed-breed dogs (Fu, Torres and Budsberg 2010). If kinematic gait analysis has shown great potential for development in orthopedic research, it is because studies have been carefully designed and controlled, as many factors can impact the kinematic results.

Kinematic patterns of movements are similar between dogs of similar morphology. They are characteristic for each joint and each gait studied. The waveform seen in Figure 3 illustrates the flexion and extension angles of the joints of the hind limb in one gait cycle of trotting Labrador Retrievers. While trotting, the hip joint slowly extends throughout the stance phase. The swing phase follows with rapid hip flexion. The stifle joint begins the stance phase in extension and flexes, corresponding to braking. While trotting, the stifle joint extends at the end of the stance phase, which corresponds to propulsion. The early swing phase is followed by larger excursions of flexion followed by extension, preparing for the next stride. While trotting, the hock joint follows a pattern similar to that of the stifle joint, except for a greater magnitude of extension in the late stance in the tarsus. Trotting and walking gaits display similar joint excursions, with a greater range of motion for all joints while trotting (Hottinger et al 1996; DeCamp et al 1993).

Establishing regular waveforms of joints flexion and extension from healthy subjects allows kinematic data to be compared with other subjects, and may be helpful in detecting subtle gait differences or abnormalities. The effects of morphology on gait analysis measurements have been incompletely documented; however evidence of difference in kinematics between subjects with different morphology has already been published. The stride period and length are shorter in
Figure 3: Mean of flexion and extension movements for one gait cycle of the hind limb of trotting Labrador Retrievers. Kinematic data are illustrated for the hip (A), stifle (B), and hock joint (C).
Labradors compared to Greyhounds (0.43 ± 0.003 s versus 0.46 ± 0.006 s and 1.05 ± 0.008 m versus 1.13 ± 0.013 m respectively); whereas the stride frequency is higher in Labradors compared to Greyhounds (2.32 ± 0.016 s\(^{-1}\) versus 2.17 ± 0.028 s\(^{-1}\)). However, after normalization of stride period and length for functional limb length, no significant differences are observed between the two breeds. The limb length is calculated as the distance from the ground to the elbow joint plus one third of the distance from the elbow joint to the highest point of the back at mid-stance. Therefore, the trotting gait of Labrador Retrievers differs from that of Greyhounds, mainly as a result of their difference in size (Bertram et al 2000). The duty factor, fraction of the total stride period that the foot is in contact with the ground, is higher for the front limbs in Labradors compared with Greyhounds (0.505 ± 0.023 versus 0.426 ± 0.027) (Bertram et al 2000). Therefore, Labrador Retrievers spend more time on their forelimbs compared with Greyhounds.

Because the effects of morphology on gait variables are not fully described, a population with a homogeneous morphology should be selected to reduce this inherent source of variation in kinematics studies.

### 2.2.2.5. Inverse dynamics analysis data

Colborne et al. combined kinematics, force plate and morphometric data in an inverse dynamics analysis. The authors studied the mechanical function of the hip, stifle, tarsal and metatarsophalangeal joints in clinically normal Greyhounds (n = 6, 30.3 ± 3.6 kg, velocity 3.13 ± 0.12 m/s) and Labrador Retrievers (n = 6, 32.3 ± 3.5 kg, velocity 1.98 ± 0.14 m/s) (Colborne et al 2005). After adjusting for body weight,
Greyhounds exhibited greater vertical and propulsive joint reaction forces at all hind limb joints. However, horizontal joint reaction forces at the stifle during braking were larger in Labrador Retrievers. Ground reaction forces and stature results are unfortunately unavailable. Comparing these findings with previous results from Budsberg et al. concerning GRF would have been really interesting (Budsberg, Verstraete and Soutas-Little 1987). Peaks of vertical joint reaction force may also inversely correlate with dog size as ground PVF do.

The stifle joint of Labrador Retrievers flexed through the early portion of the stance phase and midstance, and it remained flexed for the rest of the stance phase. Conversely, at the end of the stance phase, the stifle joint in Greyhounds extended for a small but active contribution to push-off. At the stifle and tarsal joints, moment and power patterns were similar in shape, with larger amplitudes in Greyhounds. In both breeds, the moment across the stifle joint was flexor for the first half of the stance phase. This indicates a net activity by the stifle flexors muscles (semi-membranosus, semi-tendinosus, biceps femoris, and gastrocnemius muscles). Then, the stifle joint moment reversed from flexor to extensor and caused extension of the stifle (Colborne et al 2005).

In limbs, muscles do negative work as they lengthen to absorb energy during weight acceptance in the early stance phase. Then they typically do positive work in the late stance to provide an active push-off, when the foot is caudal to the more proximal joints. The metatarsophalangeal joint was a net absorber of energy, and this effect was greater in Greyhounds. The more vertical attitude of the limb in Greyhounds, with extended metatarsophalangeal and tarsal joints, gives it potential to absorb more energy.
in the early stance. Much of this energy is then returned during the late stance phase to contribute to forward propulsion. The horizontal position of the foot of Labrador Retrievers at the beginning of the stance phase does not allow dorsiflexion of the metatarsophalangeal joint until the metatarsus begins to rotate forward over the paw in mid-stance. The phase of negative work at this joint through mid-stance is not followed by a positive burst of any amplitude. This implies that the flexors of the metatarsophalangeal joint contribute little to propulsion in Labrador Retrievers (Colborne et al 2005).

Moreover, co-contraction of antagonistic muscle groups at any joint yields a cloudy picture of joint mechanics. The power calculated at a joint depends on the net joint moment and angular velocity of the joint excursion. When co-contraction of muscles stabilizes a joint against motion, then angular velocity and calculated power will be low. This may well be the case at the stifle joint, where the stifle extensors must be coactive with the flexors, given the angular position of the joint at the time of ground contact.

The difference in velocity between the two breeds (3.13 ± 0.12 m/s versus 1.98 ± 0.14 m/s) was a potential limitation of the study, as the higher velocity of Greyhounds may affect results. Local burst of power amplitudes during the early portion of the stance phase increased incrementally in response to increased velocity in Greyhounds (Colborne et al 2006). Therefore, differences in joint moment and power reported previously between Labradors and Greyhounds may not only reflect differences between breeds, but also differences between subjects’ velocities (higher for Greyhounds), particularly because major differences between breeds were observed
in the early portion of the stance phase. Changes in timing are observed with increased velocity too. The peak of power amplitude during the stance occurs relatively later in the stride. During the swing phase, no difference in kinetics is noticed with increased velocity despite variations in angular excursion and velocity (Colborne et al 2006).

Inverse dynamics method of gait analysis provides useful insight in animal locomotion as it allows assessment of muscles moment and power pattern around various joints. These patterns may enable more accurate description of gait which could be used to make comparison between breeds or to assess the outcome of a medical or a surgical treatment. But, there is a need to develop a database of breed-specific morphometric tables for inverse dynamic method to be applied accurately. Errors caused by gross differences in conformation and body condition among and within breeds may overshadow subtle changes in the moment, power and joint reaction force patterns caused by clinical conditions when an accurate morphometric model is not used.

**2.2.2.6. Electromyographic data**

Electromyography (EMG) measures electrical signal transmission along muscle fibers at rest, in reflex contraction, and during voluntary contraction. Such systems can reveal the activity of specific muscles during locomotion. Electromyography is used to identify which muscles are active during particular movements; it does not reveal whether the muscle contributes to the movement, opposes it or is merely adjusting its length to the altered positions of its attachments (Wentink 1976). Until recently, there has been few studies using EMG for locomotor analysis in dogs. This could be because

The activation pattern of the hind limb muscles in trotting dogs has been sparsely described (Table 3). Historically, the EMG data were gathered using indwelling EMG electrodes. According to Gregersen et al., the semi-membranous muscle of all three studied trotting dogs was activated from the end of swing to the first fourth of the stance phase (Gregersen, Silverton and Carrier 1998). Individual variations in muscular activity were observed. In one dog, EMG activity was also observed during early swing (Gregersen, Silverton and Carrier 1998). The vastus lateralis muscle of trotting dogs was activated in all four studied dogs from ground contact through two-thirds of the stance phase, but also in two of the dogs during the second half of the swing phase (Carrier, Gregersen and Silverton 1998). Goslow et al. studied the activation patterns of multiple muscles of the front and hind limbs in two
<table>
<thead>
<tr>
<th>Muscles</th>
<th># dogs</th>
<th>Activation pattern</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>vastus lateralis</td>
<td>4</td>
<td>ground contact to 2/3 stance (4/4 dogs) and during second 1/2 swing (2/4 dogs)</td>
<td>Carrier et al. 1998</td>
</tr>
<tr>
<td>vastus lateralis</td>
<td>2</td>
<td>just before ground contact to 2/3 stance</td>
<td>Goslow et al. 1981</td>
</tr>
<tr>
<td>semi-membranosus</td>
<td>3</td>
<td>end of swing to first 1/4 of stance (3/3 dogs) and early swing (1/3 dogs)</td>
<td>Gregersen et al. 1998</td>
</tr>
<tr>
<td>biceps femoris</td>
<td>2</td>
<td>end of swing to mid stance</td>
<td>Goslow et al. 1981</td>
</tr>
<tr>
<td>gastrocnemius (medial head)</td>
<td>2</td>
<td>after ground contact to 3/4 stance</td>
<td>Goslow et al. 1981</td>
</tr>
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Table 3: Patterns of muscular activation of selected hind limb muscles in trotting dogs using indwelling EMG electrodes.
mixed-breed dogs at the walk, trot and gallop using indwelling EMG electrodes (Goslow et al 1981). The biceps femoris muscle (cranial head) became active prior to foot-down (25% of the swing phase) and remained active for about 46% of the stance phase (34% of the stride period). The vastus lateralis muscle became active just before the hind foot touched the ground and remained active for 70% of the stance phase (32% of the stride period). The medial head of the gastrocnemius became active after foot-down and remained active for 73% of the stance phase (33% of the stride) at the trot (Goslow et al 1981). For the walking gait, Wentink et al, described the muscular activity being largely concentrated during transition periods between joint flexion and extension (Wentink 1976). The vastus lateralis was active from the end of the swing phase to most of the stance phase. The rectus femoris had two peaks of activation: during the second half of stance phase and during mid swing. The semitendinosus and the caudal part of the biceps femoris and the semi-membranosus also had two peaks of activation: during the first half of the stance and at the transition between stance and swing. The cranial part of the semi-membranosus was active from the end of the swing phase to most of the stance. Last, the medial and lateral head of the gastrocnemius were activated from the end of swing to most of the stance phase (Wentink 1976).

Very little information has been published regarding the activation pattern of the gastrocnemius in small animals. Kaya et al. determined that the medial gastrocnemius muscle in cats was primarily responsible for generating the moment required at the hock during most of the stance phase, but also helped to satisfy the moments at the stifle joint in the initial phase of stance. In the second half of stance, the medial gastrocnemius muscle transferred mechanical energy from the stifle to the hock.
Based on these results, the medial gastrocnemius muscle primary function consists mostly in extending the hock in cats. However, the medial gastrocnemius muscle satisfies two joint moments in early stance with the combination of a net extensor moment at the hock joint with a net flexor moment at the stifle joint. Moreover, the medial gastrocnemius muscle also transfers mechanical energy from the stifle to the hock joint in late stance based on the net extensor moment observed at the stifle joint in the final phase of stance (Kaya et al 2005).

Major limitations of canine EMG studies stem from the use of invasive indwelling EMG electrodes, the small number of subjects recruited (two to four), inconsistency in the EMG activation pattern between dogs, and the inclusion of multiple breeds. Using a surface EMG method, Bockstahler et al. reported the muscle activity pattern of the vastus lateralis muscle in eleven Malinois walking on a treadmill. The vastus lateralis was activated from the end of the swing phase through most of the stance phase with two peaks of activation (Bockstahler et al 2009b). The first maximum occurred at the beginning of early stance phase (11.7 ± 6.2 % stride duration). After this first peak, the muscle activity decreased to a minimum in the middle of the stance phase. The second peak was observed in the middle of the second half of the stance (42.1 ± 4.3 % stride duration). A positive correlation was reported between the vastus lateralis muscle activity on one side and GRF, and the stifle joint angle during the swing phase on the other side (Bockstahler et al 2009b). The vastus lateralis contributes to stifle extension and prevents flexion, leading to stabilizing effect during the stance phase. Moreover, this muscle acts as a stifle extensor during the swing phase.
In another study, activities of the hamstring, gluteal and quadriceps muscle groups were measured with surface EMG electrodes in hounds walking on a treadmill at inclines 5%, 0% and -5% (Lauer et al 2009). At the beginning of the stance phase, activity of the hamstring muscle group was significantly increased when walking at a 5% incline versus a 5% decline. At the end of the stance phase, the hamstring muscle was more active when walking at a 5% incline versus walking on a flat surface or at a decline. Activity of the gluteal and quadriceps muscle groups was not affected when treadmill inclination changed. Therefore, walking on an inclined treadmill triggered more hamstring muscle activation than walking on a treadmill with a flat or declined surface (Lauer et al 2009).

Despite an increased use of EMG in canine gait analysis, more practice and research is warranted to standardize EMG in dogs and apply this method in the clinical setting. If data collection can be improved, EMG may become an asset to biomechanical and neuromuscular research by revealing specific muscular activity during locomotion (Gillette and Angle 2008).

2.2.3. Lameness assessment

Force plate and kinematic analysis have been used extensively in dogs to compare function before and after surgery of the hind limbs such as: stabilization of the ruptured cranial cruciate ligament (Jevens et al 1996; Dupuis et al 1994; Evans, Gordon and Conzemius 2003; Lopez et al 2003; Marsolais et al 2003; Conzemius et al 2005; Robinson et al 2006), triple pelvic osteotomy (McLaughlin et al 1991), total hip arthroplasty (Dogan et al 1991; Budsberg et al 1996), juvenile pubic symphysiodesis

These studies have traditionally relied on a single ground reaction force (often PVF or VI) to describe limb function in a population of dogs or to discriminate gait among groups of dogs (Robinson et al 2006; Lopez et al 2003a; Ballagas et al 2004). Changes expected with lameness include the following: reduced vertical forces relative to normal, reduced cranio-caudal forces if the lameness is moderate-to-severe and asymmetry. In addition to PVF, VI should also be assessed in dogs, because important data about total force applied during a stance phase may be missed by simply measuring the peak magnitude (Mlacnik et al 2006; Budsberg 2001). Evans et al. recently proposed that a multivariate approach would improve the ability to discriminate sound from lame dogs compared to a univariate approach (Evans,
Horstman and Conzemius 2005). The gait of walking Labrador Retrievers was studied (17 normal dogs, 100 with unilateral cruciate disease and 131 six months after surgery for unilateral cruciate disease) and rising slope, falling slope, ratio of time to PVF to stance time, PVF and VI were documented. Normal and pre-operative groups were the “gold standard” and used to determine the GRF that best discriminated lame and sound Labradors with stifle injury. The combination of PVF and falling slope had the best sensitivity (93%, probability of classifying a normal dog as normal) and specificity (94%, probability of classifying a lame dog as lame). Normal dogs had both large PVF and shallow (small negative) falling slope compared with lame dogs. Few injured Labradors had large PVF. However, these injured dogs distinguished themselves from normal dogs because injured dogs had a large negative falling slope value, which indicates that these dogs unloaded their limb quickly. This could be explained as pain response once the peak force reaches a normal threshold (Evans, Horstman and Conzemius 2005). According to Fanchon and Grandjean, a multivariate approach combining asymmetry assessments of PVF and maximal rising slope yielded the optimum combination to distinguish healthy dogs from those affected by either CCL deficiency or hip dysplasia. This combination was calculated as: 

$$3(I_{PVF}) + I_{maxRS}$$

where $$I_{PVF}$$ and $$I_{maxRS}$$ represent the asymmetry indices of the PVF and maximal rising slope, respectively. The asymmetry index for each gait variable for each dog was calculated by use of the following equation: 

$$\left( \frac{|X_R - X_L|}{|X_R + X_L| \times 0.5} \right) \times 100$$

where $$X_R$$ is the mean of a given gait variable for right footfalls during a 10-second recording and $$X_L$$ is the mean of a given gait variable for left footfalls during a 10-second recording. A cutoff value of 15.7% yielded sensitivity of
92% and a high probability that the dog was sound, whereas a cutoff value of 19.5% yielded a specificity of 95% and a high probability that the dog was lame (Fanchon and Grandjean 2007). The discrepancy between Evans et al. and Fanchon and Grandjean’s studies regarding the most appropriate combination of parameters to discriminate between normal and lame dogs, may be due to differences in gait (walk versus trot, overground versus treadmill trials), differences in population (Labradors only versus multiple different breeds, 100 versus only 13 lame dogs) and differences in source of lameness (cranial cruciate ligament disease only versus a combination of cranial cruciate ligament disease (n=4) and canine hip dysplasia (n=9)).

However, GRF are neither joint specific nor truly limb specific in the description of various pathological gaits. In addition to force-plate data, changes in kinematic data such as range of motion of joints, degree of flexion or extension, and angular velocity and acceleration, may underline subtle gait abnormalities and provide information about limb function.

Moreover, it is essential to evaluate the status of all limbs in subjects undergoing gait analysis as lameness in any limb will affect the gait characteristics of all limbs. Redistribution of forces to other limbs occurs during single limb lameness, particularly in the acute phase of injury (Griffon, McLaughlin and Roush 1994; Jevens et al 1996; Rumph et al 1995; Rumph et al 1993; Berchuck et al 1990; Bockstahler et al 2009a). Redistribution of vertical force between forelimbs and hind limbs develops in association with severe acute hind limb lameness (Rumph et al 1993), but this redistribution is noticeable in hind limbs only during chronic affection. Force redistribution does not seem to be limited to the vertical axis. A study of chronic
CCL insufficiency has documented significant decreases in ipsilateral forelimb braking impulses too (Jevens et al 1996). Redistribution of limb forces attributable to lameness may be affected by many factors, including degree of lameness, joints affected and duration of lameness. Therefore, the contralateral limb must not be used as a control in any study with significant lameness present (Griffon, McLaughlin and Roush 1994; Rumph et al 1993). Kinematic studies of limb movement compensation attributable to lameness of other limbs have not been performed. Nevertheless, we suggest that the "normal" contralateral limb should not be used as a control for kinematic studies of lameness.

Investigators should also be cautious that they do not introduce a bias when selecting a gait (walk versus trot) for their study. Some lame dogs will use their affected limb while walking, but not while trotting. Dogs may fatigue quickly at a faster velocity, leading to incomplete data collection on the affected limb and an increase in trial failures. Incomplete data acquisition and increase in trial failure would also increase trial variation. Thirty eight percent of dogs with a cranial cruciate ligament deficiency not surgically corrected failed to provide acceptable trotting trials (Evans, Gordon and Conzemius 2003). This is a large population and it indicates a potential impact of subject selection for clinical studies. The mean ground reaction force at a walk is significantly different among dogs that could and could not successfully trot, essentially because the most severely lame dogs fail to trot. Mean walking PVF and VI for dogs that can successfully trot is significantly higher than from dogs that failed to trot. The very lame dogs would be eliminated from a clinical study with a trotting gait, so the study would be biased toward dogs that are less lame. In that situation,
differences between interventions may be less pronounced, because they would be
evaluated on dogs that are less affected. Dogs with the poorest outcomes would also be
eliminated, possibly making a procedure or medication appear better than had the
evaluation been performed for dogs at a walk. Moreover, walking has less inherent
variation than trotting. Thus, smaller differences among interventions may be detected,
and smaller sample sizes are required to detect such difference (Evans, Gordon and
Conzemius 2003).

Other sources of bias recently studied include exercise before data collection
and changes in body weight. A moderate amount of exercise (1.2 km trot) exacerbated
hindlimb lameness in dogs clinically affected by osteoarthritis (Beraud, Moreau and
Lussier 2010). Changes in body weight in osteoarthritic dogs interfered significantly
with PVF values already normalized as a percentage of body weight (%BW). An
increase in body weight in dogs with osteoarthritis could exacerbate a preexisting
lameness (Moreau et al 2010).

2.2.4. Cranial cruciate ligament disease

The cranial cruciate ligament (CCL) is a critical stabilizer of the stifle joint and
controls cranial drawer motion, internal tibial rotation, and stifle hyperextension
cruciate ligament deficiency is the leading cause of lameness affecting the stifle joints of
large breed dogs (Johnson, Austin and Breur 1994;Innes et al 2000). The prevalence of
CCL disease is between 1.55 and 4.87 cases per 100 patients according to the Veterinary
Medical Data Base and has gradually increase over the last 40 years (Johnson, Austin and
Breur 1994; Whitehair, Vasseur and Willits 1993; Slauterbeck et al 2004; Witsberger et al 2008). The annual economic impact of medical and surgical treatment of CCL disease in veterinary patients has been estimated to be 1.32 billion dollars in the United States (Wilke et al 2005). Large breed dogs, especially Labrador Retrievers have been identified as a predisposed breed to this disease (Whitehair, Vasseur and Willits 1993; Duval et al 1999; Lampman, Lund and Lipowitz 2003). The prevalence of CCL disease for Labrador Retriever is 3.81 to 5.79 % (Whitehair, Vasseur and Willits 1993; Witsberger et al 2008; Lampman, Lund and Lipowitz 2003). Cranial cruciate ligament deficiency is bilateral in 31 to 69% of affected dogs, with a contralateral rupture occurring in a mean of 13 to 17 months after initial diagnosis of unilateral rupture (Duval et al 1999; Cabrera et al 2008; Duerr et al 2007; Moore and Read 1995; Doverspike, Vasseur and Harb 1993). Therefore, these contralateral limbs can be considered as predisposed to CCL deficiency based on the high incidence of bilateral and contralateral CCL disease in dogs. According to a recent meta-analysis of CCL deficiency repair, there is not a single surgical procedure that can consistently allow return to normal function (Aragon, Hofmeister and Budsberg 2007). Unfortunately, despite surgical treatment to address stifle instability, degenerative joint disease will progress, which lead to decreased range of motion, pain, and decreased function (Innes et al 2000; Lazar et al 2005; Lineberger et al 2005; Jandi and Schulman 2007).

Although CCL disease has been studied extensively, its exact pathogenesis and the primary cause leading to CCL rupture remain controversial. A CCL rupture can occur after trauma; however, in most dogs, mid-substance rupture occurs under conditions of normal loading because of pre-existing progressive fatigue (Vasseur et al 1985; Johnson
and Johnson 1993; Lampman, Lund and Lipowitz 2003). As the disease progresses, the collagen fibrils become hyalinized, and the tensile strength of the cruciate ligament is reduced (Arnoczky and Marshall 1981; Lampman, Lund and Lipowitz 2003; Hayashi et al 2003). Ligament degeneration, immune-mediated disease, conformational abnormalities, obesity, bacterial DNA, and trauma to the stifle joint have been incriminated (Duval et al 1999; Lampman, Lund and Lipowitz 2003; Duerr et al 2007; Barrett et al 2005; Muir et al 2005; Read and Robins 1982; Mostafa et al 2009; Mostafa et al 2010; Clements et al 2008; Doom et al 2008; Doom et al 2008; Guerrero et al 2007; Zeltzman et al 2005; Morris and Lipowitz 2001; Reif and Probst 2003; Wilke et al 2002; Comerford et al 2006; Comerford et al 2005; Hayashi, Manley and Muir 2004; Muir et al 2007). In Newfoundlands, a genetic basis for CCL disease has been identified (Wilke et al 2006). Obese animals have a two-fold greater risk to be affected and more neutered animals are diagnosed with CCL disease compared to sexually intact dogs (Whitehair, Vasseur and Willits 1993; Slauterbeck et al 2004; Witsberger et al 2008; Duval et al 1999; Lampman, Lund and Lipowitz 2003). Last, it is unclear whether female have a higher prevalence of CCL rupture than male (Whitehair, Vasseur and Willits 1993; Duval et al 1999). These etiologies are not mutually exclusive, and the pathogenesis may be multifactorial as what has been described in human (Griffin et al 2000; Griffin et al 2006; Bahr and Krosshaug 2005; Hewett, Myer and Ford 2006; de Rooster, de Bruin and van Bree 2006; Jerram and Walker 2003). However, weakening secondary to repetitive microtrauma is currently believed to cause the majority of CCL instabilities diagnosed in dogs, especially in large breeds such as Labrador Retrievers (Arnoczky and Marshall 1981; Hayashi et al 2003; Slocum and Devine 1983). A great deal of interest has recently focused on the
importance of hind limb conformation as an underlying cause for repetitive microtrauma to the CCL (Griffon 2010). A greater tibial plateau slope is believed to increase the cranial tibial thrust, thereby increasing the risk of weakening of the CCL secondary to repetitive microtrauma (Arnoczky and Marshall 1981; Morris and Lipowitz 2001; Slocum and Devine 1983). The intercondylar notch in predisposed dogs may be naturally narrower than dogs at low risk for CCL disease (Greyhounds) (Comerford et al 2006; Aiken, Kass and Toombs 1995; Lewis et al 2008). Moreover, internal torsion of the femur distal to the lesser trochanter observed in predisposed and affected limb may lead to further impingement of the CCL in the intercondylar notch, causing it to weaken over time (Mostafa et al 2009). Increased DTA/PTA (diaphyseal tibial axis/proximal tibial axis) angle or a less developed tibial tuberosity were other risk factors previously identified (Mostafa et al 2009; Guerrero et al 2007; Osmond et al 2006; Inauen et al 2009), which highlight the complexity of CCL disease in dogs.

Ground reaction forces and kinematics combined in an inverse dynamics analysis method have helped improve the understanding of the pathophysiology of anterior cruciate ligament diseases in humans (Decker et al 2003; Krosshaug et al 2007). In human, the risk for females to sustain an anterior cruciate ligament deficiency is two to eight times higher than in males (Bahr and Krosshaug 2005; Hewett, Myer and Ford 2006; Arendt and Dick 1995). Gender differences in joint mechanics have been evaluated to identify intrinsic factors predisposing female athletes to anterior cruciate ligament rupture compared with males. Based on these studies, increased quadriceps activation, decreased hamstring recruitment and strength (quadriceps to hamstring dominance), increased knee laxity, along with knee valgus are now considered as
factors predisposing female athletes to knee injuries (Decker et al 2003; Myer et al 2008; Sigward and Powers 2006; Hanson et al 2008; Malinzak et al 2001). Moreover, female athletes demonstrated reduced hamstrings stiffness compared to males, indicating a compromised ability to resist against joint perturbation, which was attributable in large part to differences in muscle size (Blackburn et al 2009). These findings provided the basis for designing and monitoring neuromuscular training programs for humans that led to a relative risk reduction of 80% among individuals participating in such programs (Mandelbaum et al 2005; Hewett et al 1999).

Ground reaction forces and kinematic data associated with CCL disease have been previously reported in dogs (Korvick, Pijanowski and Schaeffer 1994; DeCamp et al 1996; Jevens et al 1996; Evans, Gordon and Conzemius 2003; Marsolais et al 2003; Vilensky et al 1994; Rumph et al 1995; Vilensky et al 1997; Budsberg 2001; Lopez et al 2003a; Ballagas et al 2004; Sanchez-Bustinduy et al 2010). In dogs with CCL rupture, a cyclical pattern of cranial tibial subluxation during the stance phase and cranial tibial reduction during the swing phase occurs (Korvick, Pijanowski and Schaeffer 1994; Tashman et al 2004). The kinematic changes observed in CCL-deficient dogs support the concept of a dynamic imbalance, similar to that described in humans (Korvick, Pijanowski and Schaeffer 1994; Tashman et al 2004). Colborne et al. reported differences in distribution of muscle moment across the hind limb joints in trotting adult Labrador Retrievers and Greyhounds (a breed at low risk for CCL disease) (Colborne et al 2005). When the moment of the stifle joint of Labrador Retrievers was examined for its contribution to the total limb moment of support, the peak stifle moment during the propulsion phase accounted for 30% of the total moment, compared
with only 12% in the Greyhounds (Colborne et al 2005). Moreover, the amplitude of
the net flexor muscle moment across the stifle joint was more than doubled in
Greyhounds during the early stance phase compared to Labrador Retrievers (Colborne
et al 2005). These results are encouraging as they support the concept of a dynamic
muscular imbalance around the stifle joint predisposing Labradors to CCL disease.
However, they warrant further investigation in order to further characterize the
neuromuscular imbalances affecting the agonist-antagonist relationship between active
stabilizers of the hind limb joints with EMG analysis and to confirm the functional
implications of the observed kinetics differences. Moreover, the morphometric data in
Colborne et al.’s study was derived from a very small number of cadavers (four
Greyhounds and three Labradors) and the difference in gait observed may reflect
differences in morphology between the two breeds rather than predisposition to CCL
disease.

To our knowledge, net joint muscle moment, net joint muscle power and joint
reaction forces have not been compared between dogs with CCL deficiency, those
predisposed to CCL disease and dogs at low risk for the disease. The exact nature of the
previously observed imbalance and its significance in relationship with CCL deficiency
in dogs have not been defined. This gap in knowledge prevents the development of
preventive measures against CCL disease in dogs.
CHAPTER 3: METHODOLOGY AND RESULTS

In this section, the author presents the methods and results for each study successively. The general goals of this research were (1) to further define gait mechanism in Labrador Retrievers with and without CCL-deficiency (Sections 3.1 to 3.3), (2) to identify individual dogs that are susceptible to CCL disease (Section 3.4), and (3) to characterize their gait (Section 3.5). The Institutional Animal Care and Use Committee of the University of Illinois approved all study procedures prior to subject enrollment. Informed consent was obtained from all the owners.

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3.1. Noninvasive determination of body segment parameters of the hind limb

For the first general goal of the study, our initial objective focused on non-invasive determination of BSP for the hind limb segments in living Labrador Retrievers with or without CCL deficiency, and to develop regression equations to estimate those BSP based on easily obtainable morphometric measurements (Ragetly et al 2008).

3.1.1. Materials and methods

3.1.1.1. Subjects

Informed consent was obtained from the owners of 24 adult pure-bred Labrador Retrievers. Group 1 included 14 dogs (28 normal hind limbs) over 6 years of age and without history or current orthopedic disease of the stifle joint based on history, physical examination, radiographs and computerized tomography. Group 2 included 10 adult dogs that were presented to the Veterinary Teaching Hospital (VTH, University of Illinois, Urbana-Champaign, Illinois) with a weight-bearing lameness due to non-traumatic unilateral CCL disease. The diagnosis of CCL disease was confirmed either by arthroscopy or by exploratory arthrotomy at the time of surgical treatment of the CCL disease. Group 2a consisted of ten CCL-deficient limbs, whereas group 2b included the ten sound contralateral limbs of the CCL-deficient dogs. Each limb in this study was analyzed separately and classified as normal (group 1), CCL-deficient (group 2a) or contralateral limb (group 2b).
3.1.1.2. Body segment parameters

Signalment and body mass were recorded prior to complete physical and orthopedic examinations in each dog. Dogs underwent additional procedures as part of other studies (Mostafa et al. 2009; Mostafa et al. 2010). Standing angles and range of motion (flexion and extension) of the hip, stifle and tarsal joints were measured. Radiographs of the pelvis, femur and tibia, and dual energy X-ray absorptiometries (DEXA, QDR 4500® Fan Beam X-ray Bone Densitometer, Hologic Inc., Bedford, MA) of the whole body were obtained prior to gait analysis at the Human Dynamics and Controls Laboratory, College of Engineering, University of Illinois (Urbana-Champaign, IL).

Morphometric measurements were obtained in triplicate and averaged prior to analysis. The length of the thigh (Thigh_L) was measured between the greater trochanter and the most distal point of the lateral femoral epicondyle. The length of the crus (Crus_L) was defined as the distance between the most distal point of the lateral femoral epicondyle and the lateral malleolus of the fibula. The length of the foot (Foot_L) was measured between the lateral malleolus of the fibula and the fifth metatarsophalangeal joint. Lengths were measured between skin markers designed for kinematic studies placed on previously described landmarks using a motion capture software (Vicon Motion Systems, Inc., Lake Forest, CA). Measurements were performed after recording kinematic data from those individuals (Ragetly et al. 2010). Lengths consisted of the average length of the segment over a complete gait cycle. The circumference of the thigh (thigh girth, Thigh_G) was measured at the level of the femoral mid-shaft, midway between the greater trochanter and the lateral femoral
epicondyle. The circumference of the crus (Crus_G) was measured at the level of the tibial crest. Circumferences were measured using a single standard plastic non-stretchable metric tape with the dogs in lateral recumbency. The latero-medial widths of the stifle, hock and metatarsophalangeal joints (Stifle_W, Hock_W and Meta_W) were measured with a caliper at the level of the lateral femoral epicondyle, lateral malleolus of the fibula and fifth metatarsophalangeal joint, respectively.

Length of the foot was measured from the lateral malleolus of the fibula to the distal portion of the toes on DEXA images. Total area, bone mineral content, bone mineral density, lean mass, and total mass of the feet were also all provided by DEXA analysis.

Both hind limbs and pelvis of each anesthetized subject were imaged using a helical computerized tomography (CT) system (General Electric High Speed F/X Helical Scanner, Milwaukee, WI) at the VTH (University of Illinois, Urbana-Champaign, IL). Dogs were positioned in dorsal recumbency with both stifle and hock joints extended. All CT scans were associated with a coordinate system in the x, y and z axes with a common reference origin at the bottom of the field of view. Because the dogs were in dorsal recumbency with the hind limbs extended parallel to the z axis, these axes of the CT images approximately aligned with the transverse, cranio-caudal and longitudinal directions, respectively, of the hind limb segments. Each image was subsequently analyzed using Amira software (Amira 4®, Mercurey Computer System Inc, Chelmsford, MA) to yield data on the cross-sectional area of bone, muscle, and fat tissues. The pixel intensity values of every image were correlated to tissue densities by the software. The operator adjusted these ranges of pixel values per material by
selecting different points for each tissue material and comparing the pixel intensity recorded to the range of pixel provided by the software for the selected material (Figure 4). The range of pixel intensity for each tissue type was held constant throughout the study. Following three dimensional (3-D) reconstruction of CT images, each limb was divided into thigh, crus and foot segments. A transverse plane was defined as parallel to the transverse (x) and cranio-caudal (y) axes and perpendicular to the longitudinal axis (z) of the segment. The proximal boundary of the thigh was defined by the transverse plane that crossed through the proximal end of the greater trochanter. The pelvic bones and pelvic inlet, tail and sheath were excluded. The distal boundary of the thigh was defined by the transverse plane crossing the most distal point of the lateral femoral condyle. The crus segment extended from the transverse plane crossing the most distal point of the lateral femoral condyle, to the transverse plane crossing the distal point of the fibular malleolus. The tarsal elements proximal to the fibular malleolus, with the tarsal joint extended, were included in the crus segment. The foot segment was defined from the transverse plane crossing the distal point of the fibular malleolus to the tip of the most distal phalanx among all digits.

To determine the inertial properties of each segment using CT scans, the tissue densities of muscle, fat, and bone were fixed at 1.06, 0.95, and 1.8 g/cm³ respectively (Zatsiorsky 2002). The density of each tissue type within a segment was assumed uniform. Muscles, tendons, and ligaments were not distinguished in this analysis since they have similar densities. Dermal tissues were treated as the layer in closest contact to them, either muscle or fat. The CT volumes obtained from each tissue section using a solid modeling software (Pro/ENGINEER®, Wildfire 3.0, Parametric Technology
Corp., Needham, MA) were multiplied by the individual tissue density value to
determine the mass of each tissue within each segment. The segment mass (in g) was
then calculated by summing individual tissue masses and expressed as a percentage of
the body mass (% BM). The location of the COM was represented along the
longitudinal axis of each segment and the distance (in cm) of the estimated COM from
the proximal joint of the segment was determined using a solid modeling software. This
position was also expressed as a percentage of the length of the segment (% L). The
mass moment of inertia of each segment about a transverse (medio-lateral) axis through
its estimated COM was calculated (in g.cm²) using a solid modeling software and
normalized by the body mass (Figure 5).
Figure 4: Three-dimensional computed tomography illustrating the outline of fat (yellow outline, yellow arrow), muscle (red outline, red pointer) and bone (dark grey outline, curved white arrow) tissues based on their respective pixel values using Amira 4® (transverse view at the hip joint (A), frontal (B) and para-sagittal (C) views at mid femoral level).

Figure 5: Assembly of fat (yellow), muscle (red) and bone (grey) created by Pro/ENGINEER® to determine the body segment parameters of the left crus, such as the location of the center of mass (arrow).
3.1.1.3. Statistical analyses

3.1.1.3.1. Demographics and body segment parameters

Groups were compared for gender distribution with an exact Chi-square test (StatXact 9®, Cytel Software, Cambridge, MA). Body mass (in kg) was log-transformed to achieve normality. Differences among the groups for age (months) and log-body mass (in kg) were determined by 1-way ANOVA followed by Tukey’s Honestly Significant Difference (HSD) tests.

Body segment parameters (BSP) measurements for the thigh and crus required special methods because the CCL-deficient and contralateral groups were paired within each dog while limbs in the normal group were paired within each dog but were not related to limbs in either the CCL-deficient or contralateral groups. The limbs are not independent but rather are “repeated measurements” on each dog. In order to account for the within-dog pairing of limbs, cluster-correlated analysis of variance (mixed regression), followed by pair wise tests of group differences (Tukey’s HSD tests), were carried out using Systat 12® (Systat Inc., Richmond, CA). The segment mass was expressed as a percentage of whole body mass (% BM). The location of the COM was expressed as a percentage of segment length (% L). The mass moment of inertia (I) was normalized by dividing it by the body mass (I/BM). Because the I/BM data were not normally distributed, inverse transformation was required. Differences among groups for normalized segment mass, COM location, and inverse transformation of normalized moment of inertia were determined by 1-way ANOVA followed by Tukey’s HSD tests. A paired t-test was used when the comparison of BSP involved only right and left sides for crus and thigh in normal dogs.
Due to technical difficulties, the distal extremity of the foot segment was entirely scanned in only 20 limbs (18 normal, one CCL-deficient and one contralateral limb). Morphometric measurements obtained on physical examination and DEXA of the 48 feet included in the study (in normal, CCL-deficient and contralateral limbs) were analyzed using mixed regression and Tukey’s HSD tests (Systat 12®) to validate the extrapolation of BSP of the foot in normal to CCL-deficient Labradors. Because the data of some variables of the feet was not normally distributed, log transformation was required for those variables. Missing values were estimated using procedures developed by Little and Rubin (Systat 12®). Morphometric, radiographic and DEXA measurements of all the limbs included in the study were imported in an expectation-maximization algorithm (Dempster, Laird and Rubin 1977;Little and Rubin 1987). For all analyses, P<0.05 was considered significant.

3.1.1.3.2. Regression models

Estimation equations were constructed from morphometric dimensions to predict mass, location of the COM and mass moment of inertia for thigh, crus and foot of normal, CCL-deficient and contralateral limbs in Labrador Retrievers. These equations were tested to find the more adequate model for each BSP using step-wise forward and backward regression with $\alpha = 0.15$ to enter or be removed from the regression (Systat 12®). Independent variables (predictors) consisted of the morphometric measurements and body mass. The model with the highest $R^2$ (coefficient of determination) was selected for the mass, location of the COM and mass moment of inertia for the foot segment. For the crus and thigh segments, different
models were selected according to the highest $R^2$ per status (normal, diseased and contralateral limbs) for the mass, location of the COM, and mass moment of inertia (Staniar et al 2004; Hinrichs 1985; Schneider and Zernicke 1992). $R^2$ was higher than 0.85. Standard error of the estimate (SEE) helped assessing models as previously described (Buchner et al 1997; Clauser, McConville and Young 1969; Dempster, Sherr and Priest 1964; Staniar et al 2004; Young, Chandler and Snow 1983). In order to ensure that these equations were appropriate, they were placed in context to all-subsets regression from the allsubsrc macro of SAS 9.1® (SAS Institute, Cary, NC). Then, the accuracy of the model was tested using a criterion that the percentage of error ($\% error = \text{absolute value of } [(\text{estimated value} - \text{measured value}) \times 100 / \text{measured value}]$) was <10.5%. $R^2$ and SEE reflect the precision of predictive equations and percentage of error reflects the accuracy (Staniar et al 2004). Finally, residuals and estimates were compared against measurements. When these analyses did not confirm that the estimation requirements of the linear models were met, models from the all-subsets regression were examined both to identify a possibly better model and to help identify ways to improve the estimation, such as incorporation of non-linear terms.

3.1.2. Results

3.1.2.1. Population study

Labrador Retrievers enrolled in the normal group (group 1) were older (100.6 ± 23.0 months) than dogs with CCL disease (group 2, 56.4 ± 20.1 months, $P<0.05$). The average body mass for dogs was 36.9 ± 9.1 Kg in group 1 and 37.5 ± 7.4 Kg for dogs in group 2. Body mass did not differ between groups and the average body mass was 36.2
± 8.1 Kg. Group 1 included 35.7% spayed females, 21.4% intact females, 21.4% castrated males, and 21.4% intact males. Group 2 included 62.5% spayed females and 37.5% castrated males. Gender distribution did not differ statistically between groups, consisting of 58.3% female and 41.7% male dogs. The duration of lameness averaged three months, varying from a week to a year.

3.1.2.2. Body segment parameters

Body segment parameters of the thigh, crus and foot segments are presented for the normal, CCL-deficient and contralateral limbs in Table 4. Due to technical difficulties, the distal extremity of the foot segment was entirely scanned in only 18 normal, one CCL-deficient and one contralateral limb. Hence, the mass, location of the COM and mass moment of inertia values of the feet were only documented for a total of 20 limbs. Morphometric measurements obtained on physical examination and DEXA of the 48 feet included in the study (in normal, CCL-deficient and contralateral limbs) were compared to validate the extrapolation of BSP of the foot in normal to CCL-deficient Labradors. None of the following parameters differed at P<0.05 between normal, CCL-deficient and contralateral limbs, when analyzed with ANOVA and Tukey’s HSD: 1- Length of the foot (Foot\_L), 2- Mediolateral hock width (Hock\_W), 3- Mediolateral metatarsophalangeal width (Meta\_W), 4- Length of the foot, measured on DEXA images, 5- Total area, bone mineral content, bone mineral density, lean mass, and total mass of the feet as all measured on DEXA. Based on these results, the morphometry and composition of the foot were similar for normal, CCL-deficient, and contralateral limbs for this sample of Labrador Retrievers. These findings support the
Table 4: Mean (±SD) of the body segment parameters of the foot (n=20), crus and thigh of normal (n=28), CCL-deficient (n=10) and contralateral hind limbs (n=10) of Labrador Retrievers with or without unilateral cranial cruciate ligament (CCL) disease. A, B: groups with different letters differ statistically (P<0.05).

<table>
<thead>
<tr>
<th>Segment</th>
<th>Group</th>
<th>n</th>
<th>Mass</th>
<th>% BM</th>
<th>Mass moment of inertia</th>
<th>Center of mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>g</td>
<td>% BM</td>
<td>g.cm²</td>
<td>cm</td>
</tr>
<tr>
<td>Thigh</td>
<td>Normal</td>
<td>28</td>
<td>2231 (±381)</td>
<td>6.05 (±0.51)</td>
<td>92.875 (±44,284)</td>
<td>2,423 (±561)</td>
</tr>
<tr>
<td></td>
<td>CCL-deficient</td>
<td>10</td>
<td>1939 (±398)</td>
<td>5.48 (±0.34)</td>
<td>79.074 (±34,566)</td>
<td>2,199 (±276)</td>
</tr>
<tr>
<td></td>
<td>Contralateral</td>
<td>10</td>
<td>2192 (±374)</td>
<td>6.22 (±0.20)</td>
<td>87.981 (±23,735)</td>
<td>2,459 (±210)</td>
</tr>
<tr>
<td>Crus</td>
<td>Normal</td>
<td>28</td>
<td>516 (±113)</td>
<td>1.41 (±0.16)</td>
<td>16.242 (±6,778)</td>
<td>420 (±35)</td>
</tr>
<tr>
<td></td>
<td>CCL-deficient</td>
<td>10</td>
<td>464 (±112)</td>
<td>1.36 (±0.09)</td>
<td>15.174 (±5,699)</td>
<td>421 (±60)</td>
</tr>
<tr>
<td></td>
<td>Contralateral</td>
<td>10</td>
<td>512 (±116)</td>
<td>1.44 (±0.11)</td>
<td>15.941 (±6,216)</td>
<td>441 (±35)</td>
</tr>
<tr>
<td>Foot</td>
<td></td>
<td>20</td>
<td>249 (±25)</td>
<td>0.70 (±0.09)</td>
<td>7,228 (±1,223)</td>
<td>203 (±24)</td>
</tr>
</tbody>
</table>
extrapolation of BSP for the foot segment between healthy, CCL-deficient and contralateral limbs in our population of Labrador Retrievers.

None of the BSP differed between left and right limbs in normal dogs. The normalized mass of the thigh and crus of CCL-deficient limbs (expressed as a % BM) were lighter than their matched contralateral segments (Table 4). The thigh weighed less in CCL-deficient limbs than in normal limbs. The normalized mass moment of inertia of the thigh (I/BM) was smaller in CCL-deficient limbs than in their matched contralateral limbs. The position of the crural COM expressed as a % L was more proximal in CCL-deficient and contralateral limbs compared to normal limbs.

3.1.2.3. Regression equations

Table 5 lists the abbreviations used for the morphometric dimensions in tables 6, 7 and 8. Also included in table 5 are range, average and standard deviation (SD) for each dimension measured in each group.

The regression equations are presented in tables 6, 7 and 8 for the thigh, crus and foot segments respectively with corresponding $R^2$ values, absolute percentage of error (% error) and SEE. The % error varied between 1.0 and 5.7 % for the mass of different segments, between 1.6 and 6.5 % for the location of the COM and between 3.4 and 10.4 % for the mass moment of inertia. The average SEE of all regression equations was 4.7 % for the mass of the segment, 3.8 % for the position of the COM and 7.9 % for the mass moment of inertia. All regression equations accounted for more than 86 % of the variance in the dependent variables.
Table 5: Abbreviations and descriptive statistics for all independent variables included in the regression analysis for normal (n=28), CCL-deficient (n=10) and contralateral hind limbs (n=10) of Labrador Retrievers with or without unilateral cranial cruciate ligament (CCL) disease.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Dimension</th>
<th>Status</th>
<th>Range</th>
<th>Units</th>
<th>Average</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM</td>
<td>Body mass</td>
<td>Normal</td>
<td>23.9 - 35.7</td>
<td>kg</td>
<td>26.9</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCL-deficient</td>
<td>26.8 - 30.0</td>
<td>kg</td>
<td>25.4</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contralateral</td>
<td>26.8 - 30.0</td>
<td>kg</td>
<td>23.4</td>
<td>6.8</td>
</tr>
<tr>
<td>Thig_L</td>
<td>Thigh length</td>
<td>Normal</td>
<td>17.9 - 24.9</td>
<td>cm</td>
<td>21.3</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCL-deficient</td>
<td>18.2 - 23.7</td>
<td>cm</td>
<td>20.4</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contralateral</td>
<td>19.3 - 23.3</td>
<td>cm</td>
<td>20.9</td>
<td>1.4</td>
</tr>
<tr>
<td>Crus_L</td>
<td>Crus length</td>
<td>Normal</td>
<td>17.1 - 24.2</td>
<td>cm</td>
<td>20.2</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCL-deficient</td>
<td>17.2 - 21.0</td>
<td>cm</td>
<td>19.6</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contralateral</td>
<td>16.9 - 21.2</td>
<td>cm</td>
<td>19.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Foot_L</td>
<td>Foot length</td>
<td>Normal</td>
<td>2.4 - 10.8</td>
<td>cm</td>
<td>9.5</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCL-deficient</td>
<td>3.0 - 11.0</td>
<td>cm</td>
<td>9.3</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contralateral</td>
<td>3.0 - 10.1</td>
<td>cm</td>
<td>9.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Thig_D</td>
<td>Thigh girth</td>
<td>Normal</td>
<td>37.5 - 50.0</td>
<td>cm</td>
<td>42.5</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCL-deficient</td>
<td>35.5 - 47.5</td>
<td>cm</td>
<td>40.2</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contralateral</td>
<td>37.5 - 53.0</td>
<td>cm</td>
<td>42.4</td>
<td>4.8</td>
</tr>
<tr>
<td>Crus_D</td>
<td>Crus girth</td>
<td>Normal</td>
<td>22.0 - 31.0</td>
<td>cm</td>
<td>25.1</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCL-deficient</td>
<td>23.0 - 25.8</td>
<td>cm</td>
<td>24.4</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contralateral</td>
<td>23.0 - 27.5</td>
<td>cm</td>
<td>21.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Stifle_W</td>
<td>Stifle width</td>
<td>Normal</td>
<td>46.6 - 67.7</td>
<td>cm</td>
<td>56.6</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCL-deficient</td>
<td>50.6 - 65.0</td>
<td>cm</td>
<td>56.6</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contralateral</td>
<td>48.6 - 65.0</td>
<td>cm</td>
<td>55.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Hock_W</td>
<td>Hock width</td>
<td>Normal</td>
<td>30.0 - 46.6</td>
<td>cm</td>
<td>37.7</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCL-deficient</td>
<td>31.1 - 39.9</td>
<td>cm</td>
<td>35.5</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contralateral</td>
<td>31.1 - 40.0</td>
<td>cm</td>
<td>35.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Meta_W</td>
<td>Metatarsal width</td>
<td>Normal</td>
<td>29.0 - 45.5</td>
<td>cm</td>
<td>33.8</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCL-deficient</td>
<td>31.1 - 41.0</td>
<td>cm</td>
<td>33.8</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contralateral</td>
<td>30.0 - 40.0</td>
<td>cm</td>
<td>35.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Table 6: Regression equations generated from morphometric measurements and body mass (BM, kg) to predict the mass (g), the location of the COM from the proximal joint (cm) and the mass moment of inertia (g.cm$^2$) of the thigh in normal (n=28), CCL-deficient (n=10) and contralateral hind limbs (n=10) of Labrador Retrievers with or without unilateral cranial cruciate ligament (CCL) disease. Segment lengths, widths and girths are expressed in cm. Abbreviations are defined in Table 5. $R^2$ is the coefficient of determination, % error is the percentage of error, and SEE is the standard error of estimate.

<table>
<thead>
<tr>
<th>Segment</th>
<th>Predictive Equations</th>
<th>$R^2$</th>
<th>% Error</th>
<th>SEE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>THIGH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Normal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T_Mass</td>
<td>-2.23 + 38.5 * BM + 51.2 * Thigh_G + 296.4 * Stifle_W</td>
<td>0.95</td>
<td>5.0</td>
<td>127.6</td>
</tr>
<tr>
<td>T_COM</td>
<td>0.30 * Foot_L + 0.30 * Crus_G - 0.26 * Thigh_G + 0.70 * Stifle_W + 13.3 * Hook_W + 11.1 * Meta_V</td>
<td>0.99</td>
<td>6.5</td>
<td>0.55</td>
</tr>
<tr>
<td>T_Inertia</td>
<td>10.15[2^log10(BM)] + 0.94[10^log10(Crus_L)] + 0.70[10^log10(Thigh_L)] - 0.78[10^log10(Hook_W)]</td>
<td>0.83</td>
<td>10.4</td>
<td>12,306</td>
</tr>
<tr>
<td><strong>CCL-deficient</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T_Mass</td>
<td>-27.7 + 65.9 * BM</td>
<td>0.91</td>
<td>5.5</td>
<td>130.1</td>
</tr>
<tr>
<td>T_COM</td>
<td>-0.04 * BM + 0.40 * Crus_L + 0.06 * Crus_G</td>
<td>1.00</td>
<td>5.4</td>
<td>0.21</td>
</tr>
<tr>
<td>T_Inertia</td>
<td>9.506.1 + 3458.7 * BM - 15,153.0 * Meta_V</td>
<td>0.96</td>
<td>6.1</td>
<td>5,356</td>
</tr>
<tr>
<td><strong>Contralateral</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T_Mass</td>
<td>-1,773.2 + 18.7 * BM + 31.2 * Crus_G + 483.3 * Thigh_G - 158.3 * Hook_W + 173.9 * Stifle_W</td>
<td>0.93</td>
<td>1.0</td>
<td>38.5</td>
</tr>
<tr>
<td>T_COM</td>
<td>0.49 * Foot_L + 0.22 * Crus_G</td>
<td>1.00</td>
<td>1.6</td>
<td>0.14</td>
</tr>
<tr>
<td>T_Inertia</td>
<td>-66.72 + 2,494.8 * BM + 1641.8 * Thigh_G</td>
<td>0.97</td>
<td>3.4</td>
<td>4,873</td>
</tr>
</tbody>
</table>
Table 7: Regression equations generated from morphometric measurements and body mass (BM, kg) to predict the mass (g), the location of the COM from the proximal joint (cm) and the mass moment of inertia (g.cm$^2$) of the crus in normal (n=28), CCL-deficient (n=10) and contralateral hind limbs (n=10) of Labrador Retrievers with or without unilateral cranial cruciate ligament (CCL) disease. Segment lengths, widths and girths are expressed in cm. Abbreviations are defined in table 5. $R^2$ is the coefficient of determination, % error is the percentage of error and SEE is the standard error of estimate.

Table 8: Regression equations generated from morphometric measurements and body mass (BM, kg) to predict the mass (g), the location of the COM from the proximal joint (cm) and the mass moment of inertia (g.cm$^2$) of the foot of Labrador Retrievers with or without unilateral cranial cruciate ligament (CCL) disease. Segment lengths, widths and girths are expressed in cm. Abbreviations are defined in table 5. $R^2$ is the coefficient of determination, % error is the percentage of error and SEE is the standard error of estimate.
3.2. Inverse dynamics analysis of the pelvic limbs in Labrador Retrievers with and without cranial cruciate ligament disease

The second objective of the first general goal was to quantify net joint moments, powers and joint reaction forces across the hock, stifle and hip joints in Labrador Retrievers with or without CCL disease, and to investigate differences in joint mechanics between limbs of clinically normal dogs, limbs affected by CCL disease and contralateral limbs of CCL-deficient dogs (Ragetly et al 2010).

3.2.1. Materials and methods

3.2.1.1. Subjects

For this part of the study, the same dogs as in part 3.1. were recruited except one dog with CCL deficiency for which we were unable to collect gait trials. Group 1 included 14 dogs (28 normal rear limbs) over 72 months of age (range, 72 to 144 months). Group 2 included nine adult dogs (range, 36 to 82 months) with a weight-bearing lameness due to non-traumatic unilateral CCL disease. Mean duration of lameness for the dogs with CCL disease was three months (range, one week to one year with seven dogs lame for three months or less). The inertial properties of each segment of the hind limbs were determined (see above 3.1.).

3.2.1.2. Gait data collection

Thirteen spherical 14-millimeter diameter reflective markers were affixed to the clipped skin overlying skeletal landmarks for both hind limbs of each dog. Markers
were placed bilaterally on the skin over the cranial dorsal iliac spine, greater trochanter, most distal point on the lateral epicondyle of the femur, lateral malleolus of the fibula, lateral fifth metatarsophalangeal joint and on the sacro-coccygeal articulation. Two additional markers were placed on the dorsum of the front foot to distinguish the forelimbs during gait analysis (Figure 6). Markers identified the thigh, crus and foot segments in accordance with the aforementioned morphometric model. The landmarks were estimated by palpation of the end points of the bones. Manual flexion and extension of the joints after application of the markers was used to verify marker positions. Following application of the markers, the dogs were allowed to become familiarized with the runway used for gait analysis before data collection.

Kinematic data were collected using a 6-camera optical motion capture system (Vicon Motion Systems, Inc., Lake Forest, CA) synchronized with a force plate (AMTI, model BP600900, Watertown, MA) embedded halfway down a 30-feet walkway. Force and kinematic data were simultaneously collected at 100 Hz using the Vicon workstation software. A volume of space was calibrated to track markers over the walkway portion containing the force plate when dogs trotted in either direction. The same handler led all dogs throughout the study at a comfortable trotting pace with an average velocity of 1.92 ± 0.22 m/s. A minimum of three and maximum of five valid trials (stance and swing phases over one gait cycle) were examined for each hind limb. The selected trials from each limb were used to calculate a mean value per limb. The grand mean value was calculated among all limbs for the normal, CCL-deficient and contralateral groups. Trials were considered valid if (i) one hind limb of a trotting dog was the only limb on the force plate at the point of impact, or minimal overlap of the
hind limb of interest and contralateral front limb at ground contact was observed, and (ii) no other limbs touched the force plate while the investigated limb was in contact with the force plate. Trials confounded by avoidance reaction or other unnatural movement were discarded. Each gait cycle, or stride, started from ground contact of the hind paw onto the force plate and ended with the subsequent ground contact of the same hind paw. Kinematic and kinetic data were filtered using a forward-backward fourth-order Butterworth low-pass filter, 12 Hz cut-off frequency. X-, y- and z-directions were defined as cranio-caudal (forward-backward), medio-lateral and vertical, respectively (Figure 6).

3.2.1.3. Data processing

Kinetics and kinematics were determined for each selected trial. For trials with minimal overlap of the hind limb and contralateral front limb at ground contact, the interpolating method was used to identify the start of the gait cycle and remove additional ground reaction force due to the front limb (DiBerardino et al 2008). Trotting velocity and acceleration during each trial were calculated based on the displacement of the sacro-coccygeal marker during one hind limb stride. Kinematic data were obtained for the hip, stifle and hock joints. These included stance time (in s), stride time (in s), stride length (in m), angular displacement (in deg), velocity (in deg/s) and acceleration (in deg/s²). The duration of the stance phase was expressed as a percentage of duration of the gait cycle. The timing of each trial was expressed as a percentage of duration of the gait cycle. Joint angles were measured on the anatomic flexor side of each joint, with the value of the angle decreasing during flexion and increasing during extension.
Figure 6: Computer assisted acquisition of ground reaction forces and kinematic data in a trotting subject. The coordinate system represents the vertical (z), cranio-caudal (y) and medio-lateral (y) direction for the motion recording system. The z-direction is pointing toward the ground for the force plate data.
The peaks of flexion and extension and the range of motion (ROM) during the stance and the swing phases were documented for each joint of interest. Angular velocity was positive during extension and negative during flexion.

The GRF from each trial were analyzed to determine peak vertical, braking, and propulsion forces (in N/kg). Stance phase was divided into two subphases, early stance (braking) and late stance (propulsion), which were distinguished by examining the cranio-caudal GRF behavior. Impulses were calculated by time integration of the force curves and expressed as vertical, braking, and propulsive impulses (in N.s/kg). Force peaks and impulses were normalized by the mass of each dog. Peaks were also expressed as percentage of body weight (%BW) and impulses as percentage of body weight time seconds (%BW.s).

The GRF and kinematic data were imported into a custom program (MATLAB, MathWorks, Inc., Natick, MA) and combined with dog-specific morphometric data to obtain an inverse dynamics solution for the resultant parameters of interest. Net joint moments, joint powers and joint reaction forces (JRF) were computed in the sagittal plane for the hock, stifle and hip joints during the stance and swing phases of the gait cycle, and were normalized by the mass of each dog. The net joint moment represents the net torque produced by the action of soft tissues around the representative center of rotation of the joint. Extensor joint moments were assigned as positive, and flexor moments were assigned as negative (Clayton et al 2000). A positive joint power indicates power generation by soft tissues around the joint in which the muscle shortens as it generates tension (concentric muscular contraction). A negative joint power indicates power absorption in which the muscle lengthens as it generates tension.
(eccentric muscular contraction). Peak and impulse of JRF were evaluated in the vertical and cranio-caudal directions.

3.2.1.4. Statistical analysis

3.2.1.4.1. Demographics parameters

Groups were compared for gender distribution with an exact Chi-square test (StatXact 9, Cytel Software, Cambridge, MA). Body mass (in kg) was log-transformed to achieve normality. Differences among groups for age (in months) and log-body mass (in kg) were determined by one-way ANOVA followed by Tukey’s Honestly Significant Difference tests.

3.2.1.4.2. Gait analysis parameters

Group mean values for trotting velocity and acceleration; stride time, stride length and stance duration; peak and impulse for vertical and cranio-caudal GRF were calculated. Peak values of joint angular position, angular velocity, net moment, power, and reaction forces for each joint were also documented over a gait cycle. A normal distribution and homogeneity of variance were not assumed for the collected data. The values of right and left limbs for each of these parameters were compared in normal dogs with the use of a Wilcoxon test (SPSS 16.0, SPSS Inc., Chicago, IL) and no statistical difference was observed between the two sides. For each of these parameters, the averaged values of the right and left limbs of normal dogs were then compared to the CCL-deficient and contralateral limbs using a Mann-Whitney test with a Bonferroni
correction (SPSS 16.0). CCL-deficient and contralateral groups were compared using a Wilcoxon test. For all analyses, P<0.05 was considered significant.

3.2.2. Results

3.2.2.1. Population study

Labrador Retrievers enrolled in the normal group (group 1) were older (100.6 ± 23.0 months) than dogs with CCL disease (group 2, 58.2 ± 19.2 months, P<0.05). The difference in age (42.4 months) between normal and CCL-deficient Labradors in our study was expected because of the minimum age requirement for inclusion in our normal group (older than 72 month-old), which was based on data suggesting the decreased risk for CCL disease in older dogs (Reif and Probst 2003). Moreover, the normal dogs were followed for an additional two years to ascertain absence of CCL disease. The average body mass (36.2 ± 8.3 kg) did not differ between groups. Gender distribution did not differ statistically between groups, consisting of 60.9 % female and 39.1 % male dogs.

3.2.2.2. Gait measurements

A minimum of three valid trials were examined for each hind limb, with five valid trials examined for more than 80 % of the limbs. Three limbs (in three different dogs, two normal and one CCL-deficient limb) were excluded from the study as less than three valid trials were available for assessment for those limbs. Therefore, data were analyzed from eight CCL-deficient limbs, nine contralateral limbs, and 26 normal limbs. Trotting velocity of the subject did not differ between groups and the average
velocity was 1.92 ± 0.22 m/s (Table 9). Tables 9 and 10 present all variables analyzed and compared between CCL-deficient, contralateral and normal limbs in this study (mean value ± SD). Curves of mean ± standard error (SE) values are plotted to facilitate appreciation of differences in amplitude for the hock, stifle and hip joints angular positions (Figures 7-9 A), angular velocities (Figures 7-9 B), net joint moments (Figures 7-9 C), joint powers (Figures 7-9 D), and JRF in the vertical and cranio-caudal directions (Figures 7-9 E and F). All curves followed a similar pattern but quantitative differences were found between normal, CCL-deficient and contralateral limbs.

a) Normal limbs

An extensor moment was generated at the hock during the entire stance phase, reaching a peak immediately before mid-stance (MHo, Figure 7 C). The extensor muscles around the hock first absorbed energy, controlling the rate of tarsal flexion in the first half of the stance phase and then contracted concentrically to extend the hock joint for an active push-off in the second half of the stance phase (PHo1 and PHo2, Figure 7 D). A flexor moment was first generated across the stifle joint from ground contact until mid-stance (MS1, Figure 8 C). During mid stance the extensor muscles of the stifle absorbed energy, controlling the rate of stifle joint flexion, and then generated energy to a peak power of 0.67 W/kg (PS1, Figure 8 D) to extend the stifle joint for an active push-off. At the end of the swing phase, the flexor muscles of the stifle absorbed energy, controlling the rate of stifle extension just before ground contact (PS2, Figure 8 D). By opposition to the stifle, an extensor moment was generated around the hip during the first half of the stance phase (MH1, Figure 9 C), followed by a flexor
moment. The extensor muscles of the hip generated energy in the first half of the stance phase (PH1, Figure 9 D). During the second half of the stance phase, the flexor muscles of the hip absorbed energy controlling the rate of hip extension (MH2 and PH2, Figures 9 C-D).

b) CCL-deficient limbs

Vertical and braking GRF and JRF were decreased in CCL-deficient limbs compared to normal limbs (Tables 9-10). During the stance phase, the amplitude of movement was decreased in the hock of CCL-deficient limbs and the angular velocity was decreased in both the stifle and hock. Extensor moments at the hock and hip, flexor moment at the stifle and power in all three joints were less than normal (Table 10 and Figures 7-9). During the swing, CCL-deficient limbs displayed less movement at the stifle compared to normal (Table 10). The extension at push-off present in normal and contralateral limbs was absent in CCL-deficient stifles (Figure 8 A). Moreover, affected limbs tended to carry their hip in a more extended position than normal during stance (p-value = 0.07, Figure 9 A).

c) Contralateral limbs

Contralateral limbs dedicated a greater percentage of the gait cycle to the stance phase than other limbs, and had a shorter stride length than normal (Table 9). They exhibited increased propulsive forces and VI than other limbs (Tables 9-10 and Figures 7-9). Contralateral limbs generated three and fifteen times more power at push-off around the stifle joint compared with normal and CCL-deficient limbs, respectively (Table 10). Compared to CCL-deficient limbs, contralateral limbs generated more and absorbed more power at the hock and stifle joints, generated more power at the hip.
Flexor and extensor moments were increased at the stifle and hip joints, as well as vertical and cranio-caudal JRF for all joints of interest (Table 10).
Table 9: Mean (±SD) of animal velocity and acceleration, stance time, stride length, stride time, peak vertical force (PVF), peak braking and propulsive forces, vertical impulse (VI), braking and propulsive impulses for CCL-deficient, contralateral and normal limbs. A, B, C: groups with different letters differ statistically.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>CCL-deficient</th>
<th>Contralateral</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Velocity [m/s]</td>
<td>1.81 (0.24)</td>
<td>1.67 (0.13)</td>
<td>1.96 (0.22)</td>
</tr>
<tr>
<td>Acceleration [m/s^2]</td>
<td>-0.34 (0.14)</td>
<td>-0.53 (0.23)</td>
<td>-0.34 (0.15)</td>
</tr>
<tr>
<td>Stance Time [%]</td>
<td>40.04 (4.96) A</td>
<td>49.67 (6.38) B</td>
<td>43.32 (3.47) A</td>
</tr>
<tr>
<td>Stride length [m]</td>
<td>0.97 (0.14) AB</td>
<td>0.92 (0.11) A</td>
<td>1.04 (0.07) B</td>
</tr>
<tr>
<td>Stride time [s]</td>
<td>0.54 (0.04)</td>
<td>0.53 (0.02)</td>
<td>0.53 (0.06)</td>
</tr>
</tbody>
</table>

**GRF (peaks)**

<table>
<thead>
<tr>
<th></th>
<th>N/kg</th>
<th>%BW</th>
<th>N/kg</th>
<th>%BW</th>
<th>N/kg</th>
<th>%BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVF</td>
<td>3.41 (1.67) A</td>
<td>34.8 (19.1) A</td>
<td>6.66 (0.51) B</td>
<td>68.0 (8.3) B</td>
<td>6.32 (1.03) B</td>
<td>54.5 (10.5) B</td>
</tr>
<tr>
<td>Peak braking</td>
<td>0.19 (0.17) A</td>
<td>1.9 (1.8) A</td>
<td>0.54 (0.24) B</td>
<td>5.5 (2.5) B</td>
<td>0.55 (0.18) B</td>
<td>5.6 (1.8) B</td>
</tr>
<tr>
<td>Peak propulsion</td>
<td>-0.52 (0.24) A</td>
<td>-5.3 (2.5) A</td>
<td>-0.96 (0.25) B</td>
<td>-9.8 (2.54) B</td>
<td>-0.72 (0.23) A</td>
<td>-7.42 (2.3) A</td>
</tr>
</tbody>
</table>

**GRF (impulses)**

<table>
<thead>
<tr>
<th></th>
<th>N/kg</th>
<th>%BW</th>
<th>N/kg</th>
<th>%BW</th>
<th>N/kg</th>
<th>%BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>0.45 (0.22) A</td>
<td>4.6 (2.3) A</td>
<td>1.07 (0.19) B</td>
<td>10.9 (1.9) B</td>
<td>0.89 (0.13) C</td>
<td>9.1 (1.3) C</td>
</tr>
<tr>
<td>Braking impulse</td>
<td>0.01 (0.01) A</td>
<td>0.10 (0.11) A</td>
<td>0.04 (0.02) B</td>
<td>0.38 (0.25) B</td>
<td>0.04 (0.02) B</td>
<td>0.39 (0.15) B</td>
</tr>
<tr>
<td>Propulsive impulse</td>
<td>-0.05 (0.00) A</td>
<td>-0.47 (0.22) A</td>
<td>-0.09 (0.03) B</td>
<td>-0.80 (0.33) B</td>
<td>-0.06 (0.02) AB</td>
<td>-0.56 (0.19) AB</td>
</tr>
</tbody>
</table>

*normal limbs results are for average of bilateral values for healthy dogs.*
Table 10: Peak of mean (±SD) kinematic and kinetic values for the hock, stifle, and hip joints for CCL-deficient, contralateral and normal limbs. Kinematics included maximum joint extension, flexion, and range of motion (ROM), and joint angular velocity during the stance and the swing phases of the gait cycle. Kinetics included net joint moment, power, and reaction forces (JRF) in the vertical and cranio-caudal directions. For each joint, mean values with different letters for each variable (row) differ significantly.
Figure 7: Mean (± SE) of (A) hock angular position, (B) angular velocity (C) net joint moment, (D) power, and joint reaction forces (JRF) in the (E) vertical and (F) cranio-caudal direction for normal (solid black), CCL-deficient (gray dash) and contralateral (black dash-dot) limbs during one gait cycle. Gray dot lines indicate standard error. ▲ Indicate the end of the stance phase of the gait cycle. MHo, PHo1, and PHo2 are the peak of hock net extensor moment, power absorption, and generation, respectively.
Figure 8: Mean (± SE) of (A) stifle angular position, (B) angular velocity (C) net joint moment, (D) power, and joint reaction forces (JRF) in the (E) vertical and (F) cranio-caudal direction for normal (solid black), CCL-deficient (gray dash) and contralateral (black dash-dot) limbs during one gait cycle. Gray dot lines indicate standard error. ▲ Indicate the end of the stance phase of the gait cycle. MS1 and MS2 are the peak of stifle net flexor and extensor moment during stance, respectively. PS1 and PS2 are the peak of stifle net power generation and absorption, respectively, during stance and swing.
Figure 9: Mean (± SE) of (A) hip angular position, (B) angular velocity (C) net joint moment, (D) power, and joint reaction forces (JRF) in the (E) vertical and (F) cranio-caudal direction for normal (solid black), CCL-deficient (gray dash) and contralateral (black dash-dot) limbs during one gait cycle. Gray dot lines indicate standard error. ▲ Indicate the end of the stance phase of the gait cycle. MH1 and MH2 are the peak of hip net extensor and flexor moment during stance, respectively. PH1 and PH2 are the peak of hip net power generation and absorption during stance, respectively.
3.3. Association between surface electromyography, kinetics and kinematics of the
hind limb in healthy trotting Labrador Retrievers

The third objective of the first general goal was to evaluate the EMG activity of
major hind limb muscle groups by means of surface EMG and evaluate concurrently
kinetics and kinematics of the stifle joint in dogs trotting over a treadmill.

3.3.1. Materials and methods

3.3.1.1. Subjects

We recruited 5 adult client-owned Labrador Retrievers among the local pet
population. For each dog, absence of neurological, orthopedic or medical disease was
documented based on history, physical, orthopedic and neurological examinations, and on
radiographs of both hip and stifle joints under sedation.

3.3.1.2. Measurements

The right limb was divided into the foot, crus and thigh segments. Measurements
of the hind limb segments length and girth were performed with the use of a non-
stretchable tape. Length of the thigh was defined as the distance between the greater
trochanter and the most distal point of the lateral femoral epicondyle. Length of the crus
was measured between the most distal point of the lateral femoral epicondyle and the
lateral malleolus of the fibula. Length of the foot was defined as the distance between the
lateral malleolus and the fifth metatarsophalangeal joint. Circumference of the thigh was
measured in the midshaft region of the femur. Circumference of the crus was measured at
the level of the tibial crest. Lateromedial widths of the stifle, tarsal, and metatarsal joints were measured with a caliper at the level of the lateral femoral epicondyle, lateral malleolus, and fifth metatarsophalangeal joint, respectively. The segment mass, location of the center of mass, and the mass moment of inertia were calculated using equations designed to estimate body segment parameters in Labrador Retrievers (Ragetly et al 2008).

3.3.1.3. Gait data collection

Dogs were acclimated via two training sessions to trot on a land treadmill until a consistent gait was observed for ten minutes. Reflective surface markers were fixed onto the skin to identify the thigh, crus and foot segments of the hind limbs for kinematic analysis. Markers were placed over the cranial dorsal ilial spine, greater trochanter, most distal point on the lateral femoral epicondyle, lateral fibular malleolus, lateral fifth metatarsophalangeal joint, sacro-coccygeal joint, and on the dorsum aspect of the front foot (Ragetly et al 2010). Kinematic data were collected using a 6-camera optical motion capture system (Vicon Motion Systems, Inc., Lake Forest, CA) synchronized with the two force plates embedded under a split-belt treadmill (173 cm x 41 cm per belt, Bertec, Corp., Columbus, OH). Force and kinematic data were collected at 100 Hz after calibration of the systems.

Surface EMG data were recorded for the lateral aspects of the quadriceps (vastus lateralis, vastus intermedius, and rectus femoris muscles), hamstrings (biceps femoris, semimembranosus, and semitendinosus muscles), and gastrocnemius (lateral head of the gastrocnemius, peroneus longus, superficial and deep digital flexors and the caudal part of
the biceps femoris muscles) muscle groups. Kinematic and electromyographic sensors were placed on both hind limbs to prevent bias in gait due to the presence of tape to hold the motion markers and EMG electrodes. The recording electrodes (single differential surface EMG sensor, DE 2.1, Delsys, Boston, MA) were placed over clipped areas of each muscle group, parallel to the line of action of the muscle fibers. The placement of the electrodes was standardized based on anatomical landmarks. An electrode was placed 5 cm lateral to the cranial midline aspect of the thigh to study the quadriceps muscle group. A second electrode was placed 5 cm lateral to the caudal midline aspect of the thigh to study the hamstring muscle group. Both of these electrodes were located midway of the line connecting the greater trochanter and the lateral femoral condyle. The muscular activity of the gastrocnemius muscle group was recorded via a third electrode. This third electrode was placed at the proximal third of a line connecting the lateral femoral condyle and lateral malleolus of the fibula, 5 cm lateral to the caudal midline aspect of the crus. A ground electrode (red dot, 3M Health Care, St Paul, MN for dog 1, Dermatrode HE-R, American Imex, Irvine, CA for all other dogs) was placed on the lateral aspect of the stifle joint in dog 1, over the sacrum in dog 2, and on the medial side of the proximal crus in dogs 3 to 5. The skin was cleaned with isopropyl alcohol and double-sided tape was used to affix the electrodes to the skin. Conductor transmission gel (Conductor transmission gel, ref 4238, Chattanooga, Hixson, TN) was carefully applied to both silver detection bars on each EMG sensors and medical tape was used to provide further adhesion of the EMG electrodes to the skin (Elasticon, Johnson & Johnson Inc., Skillman, NJ). All instrumentations were performed by one investigator to improve reproducibility. During trotting, myoelectric signals were relayed from the surface EMG sensors to an input
module attached to the dog’s harness. The myoelectric signal was amplified (Bagnoli Amplifier, 16 channel EMG system, Delsys, Boston, MA) with a 10,000 gain. The sampling rate of the EMG signals was 1,000 Hz.

In order to synchronize the kinematic and kinetic data with the EMG data, a synchronization pulse was sent from the EMG system to the motion analysis computer when data acquisition was started using two distinct methods. The first method was via the use of a 7th surface electrode (dogs 1 and 2) which was taken off from the subject shortly after data collection began, resulting in a sudden increase of random noise in the signal for that electrode. The second method was using the built-in triggering feature (Trigger Module SP-U02, Delsys, Boston, MA) of the EMG system (dogs 3 to 5). A rising trigger signal signified the beginning of EMG data collection.

3.3.1.4. Data processing

Kinematic and electromyographic data from only the right limb of each dog were analyzed. A minimum of four non consecutive trotting gait cycles (stance and swing phases) were analyzed for each subject. The selection of gait cycle was based on the combination of GRF, joint angular position, and EMG data. Ground reaction forces were measured by the force plate under the belt of the treadmill on the right side. A full gait cycle is represented by two peaks: the first peak represented the stance phase of the hind limb (swing phase of front limb) while the second peak represented the stance phase of front limb (swing phase of hind limb, Figure 10).

The GRF and kinematic data were filtered with a low pass 4th order Butterworth filter with a cut-off frequency of 15 Hz. The GRF and kinematic data were combined with
dog-specific morphometric data to obtain an inverse dynamics solution for net joint muscle moments (N.m/kg) and net joint muscle powers (W/kg) of the stifle joint in the sagittal plane based on techniques reported previously (Ragetly et al 2010). The EMG signals were first down sampled to 100 Hz, then rectified and filtered using a low pass 4th order Butterworth filter with a cut off frequency of 18Hz. The threshold values for onset and offset of muscle activation were determined manually base on the following criteria: (i) the threshold value was set higher than the smallest peak of the filtered EMG signal in a gait cycle, which correspond to baseline noise, (ii) the threshold value and (iii) timing of muscle activation determined per muscle group were consistent across all gait cycles selected in a subject. The threshold value was determined after reviewing all gait cycles for a given subject and was the highest value from all gait cycles. EMG activity relative to the stance and swing phases and stifle joint biomechanics are illustrated schematically in Figure 11 D using a visualization method utilized by Robert et al (1999). The average electrical activity (grey boxes) represents the mean timings for start and end of activity bursts that were present in all dogs. Three bursts of activity were present only in certain dogs for the quadriceps and hamstring muscles (dashed boxes). The range of electrical activity (whiskers bar) represents periods of the gait cycle before or after a burst of electrical activity where muscle activity was not concurrently present in all dogs.

All calculations were performed using a custom computer program (written in MATLAB, MathWorks, Inc., Natick, MA). Analyses of the association between waveforms of stifle angle, moment and power, and EMG activation pattern of the studied muscle groups were performed.
Figure 10: Mean (± SE) vertical ground reaction forces during the stance (first peak) and the swing phases (second peak). The light grey line represents toe off.
3.3.2. Results

The right limbs of five adult Labrador Retrievers were studied. The studied population was composed of three neutered males and two spayed female, with a mean age and body weight of 48.0 ± 39.4 months and 26.9 ± 4.1 Kg, respectively. Excessive noise and artifacts were present in some dogs and the EMG data recorded for the quadriceps muscle group of subject 5 had to be rejected because of poor quality. Trotting velocity of the subjects ranged from 1.93 to 2.61 m/s with a mean velocity of 2.45 ± 0.29 m/s. The mean stride duration was 0.48 ± 0.05 s. The stance phase lasted an average of 45 ± 1.5 % of total gait cycle and corresponded to a swing phase of 55 ±1.5%.

In this study, EMG activity of the quadriceps muscle group was highly variable between subjects (Figure 11). The activity of the quadriceps muscle group began on average at 99 % of the gait cycle (swing phase) and lasted on average until 32 % of the gait cycle (stance phase). A second burst of electrical activity was documented during mid-swing in three of the four dogs with valid quadriceps data (subjects 2, 3 and 4), with mean start and end times at 64 and 84 % of the gait cycle, respectively. A third short burst of electrical activity was observed in subject 2 only at push off/early swing.

EMG data indicated that there was one common burst of activity of the hamstring muscle group presents in all dogs and one burst presents only in certain dogs. In all dogs, one common burst began on average at 89 % of the gait cycle (swing phase) and stopped on average at 18 % of the gait cycle (stance phase). In three of the five dogs (subjects 2, 4 and 5), activity of the hamstring muscle was also noted during the
swing phase, starting and ending on average at 62 and 81 % of the gait cycle, respectively.

EMG activity of the gastrocnemius muscle group consisted in two bursts interrupted by a silent gap in all dogs. The first burst started at the end of the swing phase of the previous step (average of 95 % of the gait cycle) and ended in the middle of the stance phase (average of 17 % of the gait cycle). The timing of the second burst was more variable. A second activity pattern for the gastrocnemius muscle group was found approximately from take off to mid-swing, starting and ending on average at 53 and 64 % of the gait cycle, respectively.

Based on kinematic and kinetic analysis, the stifle joint presented a biphasic pattern of flexion and extension during the stance and the swing phases (Figure 11 A). A net flexor moment and net power generation were first documented across the stifle joint from ground contact until mid-stance (Figure 11 B-C). From mid-stance to mid-swing, a net extensor moment was observed at the stifle joint (Figure 11 B). During mid-stance, the extensor muscles of the stifle absorbed energy, controlling the rate of stifle joint flexion, and then generated energy to extend the stifle joint for an active push-off (Figure 11 A-C). At 60 % of the gait cycle, the extensor muscles of the stifle absorbed energy, controlling the rate of stifle flexion just before extension of the joint (Figure 11 A-C). At the end of the swing phase, the flexor muscles of the stifle absorbed energy, controlling the rate of stifle extension just before ground contact (Figure 11 C).

Bursts of muscle electrical activity correlated well with changes in the sense of the movement of the stifle with contraction of the hamstring and gastrocnemius
muscles consistently occurring during flexion of the joint (e.g., 1 to 17 and 53 to 75\% gait cycle), but also at the end of the swing phase (89 to 100\% gait cycle). Activity of the quadriceps muscle group was documented during extension of the stifle in the stance and swing phases (20 to 32 and 64 to 84\% gait cycle), but also during flexion of the joint in the weight bearing phase of the gait cycle (1- 19\% gait cycle).
Figure 11: Mean (± SE) of (A) angular position, (B) net joint muscle moment, and (C) net joint muscle power of the stifle joint. (D) Patterns of muscular activation for the quadriceps (n=4), hamstring (n=5) and gastrocnemius (n=5) muscle groups: Grey boxes = bursts of electrical activity present in all dogs; Dashed boxes = bursts of electrical activity present in specified dogs only (# activity in dog 2; * in dogs 2, 3 and 4; $ in dogs 2, 4 and 5) (Robert et al 1999). Horizontal wiskers bars represent the range of activity before or after a burst of electrical activity. Vertical gray line in all plots indicates average toe-off time.
3.4. Multivariate analysis of morphometric characteristics to evaluate risk factors for cranial cruciate ligament deficiency in Labrador Retrievers

The second general goal was to identify individual dogs that are susceptible to CCL disease using an equation combining conformation factors (Ragetly et al 2011).

3.4.1. Materials and methods

3.4.1.1. Subjects

For this part of the study, the same dogs as in part 3.2. were recruited except two dogs without CCL deficiency. These dogs were excluded because hip dysplasia on radiographs prevented the completion of all morphometric measurements. Group 1 included 24 normal rear limbs from 12 adult pure-bred Labrador Retrievers over six years of age and without history or current orthopedic disease of the hind limb joints based on history, physical examination, radiographs and CT. According to Reif and Probst only six percent of dogs with CCL rupture were eight years of age or older (Reif and Probst 2003). Therefore, we recruited Labrador Retrievers of at least six years of age with no evidence of CCL rupture and these dogs were defined as non-predisposed due to their low risk to develop CCL disease in the future. Moreover, the owners were contacted by phone two years after data collection to confirm absence of CCL disease at that time. Therefore, these limbs are considered as being at low-risk for CCL disease. Group 2 consisted of nine sound contralateral limbs of non-traumatic unilateral CCL-deficient dogs between 3 and 7 years of age. These contralateral limbs were considered as predisposed to CCL disease (Duval et al 1999; Cabrera et al 2008; Moore and Read
The owners were called up to five years after data collection to investigate potential occurrence of contralateral CCL deficiency in the long term. The diagnosis of CCL disease was confirmed either by arthroscopy or by exploratory arthrotomy at the time of surgical treatment of the CCL disease. Each limb in this study was analyzed separately and classified as low-risk to develop CCL disease (group 1) or predisposed to CCL disease limb (group 2). Signalment and body weight were recorded prior to complete physical and orthopedic examinations in each dog.

3.4.1.2. Radiographic measurements

The set of radiographs obtained in all dogs included: medio-lateral and caudo-cranial radiographs of each tibia extending proximally to the femoral diaphysis, an extended ventro-dorsal view of the pelvis, and medio-lateral projections of each femur. All radiographic measurements were made on digitized radiographic views (General Electric Advantage Workstation, General Electric Medical Systems, Milwaukee, WI). Tibial length, proximal tibial width, and distal tibial width were measured as previously described (Mostafa et al 2009). The tibial plateau angle (TPA) (Slocum and Slocum 1993), patellar ligament angle (Dennler et al 2006; Schwandt et al 2006), and the angle between the diaphyseal and proximal tibial axes (Osmond et al 2006) were also assessed on the mediolateral view of each tibia. The femoral condyle length, femoral length, femoral width and stifle angle were also evaluated based on techniques previously reported (Mostafa et al 2009). The caudo-cranial view of the tibia was obtained to evaluate joint alignment and tibial torsion (Apelt, Kowaleski and Dyce
An extended ventro-dorsal view of the pelvis was used to assess the conformation of the hip joint (angle of inclination (Hauptman, Prieur and Butler 1979; Rumph and Hathcock 1990; Sarierler 2004) and Norberg angle (Towle et al 2005)), the alignment of the patella (quadriceps angle) (Towle et al 2005; Kaiser et al 2001), the angulation of the femur (femoral varus angle) (Dudley et al 2006), and the femoral anteversion angle (FAA, Figure 12) (Bardet, Rudy and and Hohn 1983).

### 3.4.1.3. Computed tomographic measurements

Computed tomographic examination of each dog was performed under general anesthesia. Dogs were positioned in dorsal recumbency with the pelvic limbs extended and parallel to each other. Images were acquired on a helical scanner at 120 kVp, 100 mAs with a helical pitch of 1:1 and a display field of view (DFOV) of 34.2 cm, mean slice thickness was 4 mm with a 1 mm overlap between slices (General Electric High Speed F/X Scanner, General Electric Medical Systems, Milwaukee, WI). The data were reconstructed to form a 3-dimensional image from the hip joint distally to the metatarsal bones. The 3-dimensional images were used to obtain measurements via both black-and-white and transparent bone protocol. The tibial length, tibial torsion, tibial crest alignment were measured, as well as the widths and heights of the femoral condyles and intercondylar notch as previously described (Mostafa et al 2009). Tibial and femoral lengths, the inclination angle, Norberg angle of each hip, as well as the quadriceps angle were also measured. Femoral torsion was evaluated based on the anteversion angle, femoral head trochanteric angle, and femoral condyle trochanteric angle (Mostafa et al 2009).
3.4.1.4. Dual energy X-ray absorptiometry (DEXA) measurements

The total area, mineral density, lean content, lean density, fat content, lean and fat percentage were measured for the whole body of each dog with high resolution DEXA (QDR 4500 fan beam X-ray bone densitometer, Hologic Inc, Bedford, MA). The lean content of the quadriceps, hamstring, and gastrocnemius muscles was measured and normalized by the radiographic length of the corresponding tibia to palliate size differences between dogs (Mostafa et al 2009).

3.4.1.5. Logistic regression

The optimal set of conformation parameters was selected using logistic regression on the body weight of the dog, radiographic, CT, and DEXA data, with predisposed/low risk as the binary-dependent variable and conformation parameters as candidate explanatory variables. The average of the left and right normal hind limbs was used as the low risk group. A stepwise model building procedure was used to select the optimal set of conformation characteristics that could potentially and accurately model predisposition to CCL disease. A forward and backward stepwise process was used, with variables entering the model if p<0.05 and leaving the model if p>0.1. Conformation parameters measured from the predisposed hind limb were compared with the conformation parameters from hind limb of the low risk group. Conformation parameters are continuous measures and the cutoff which maximized Youden's index (sensitivity+specificity−1) was used to classify dogs as predisposed or at low risk to CCL disease (Evans, Horstman and Conzemius 2005).
Receiver Operating Characteristic (ROC) curve analysis was used to assess the diagnostic properties of conformation parameters obtained from femur and tibial radiographs, hind limb CT images, and DEXA studies. In a ROC curve the true positive rate (sensitivity) is plotted in function of the false positive rate (100-specificity) for different cut-off points. Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. The area under the curve (AUC) is a commonly used summary of test accuracy that ranges from 0.5 (a diagnostic test that cannot distinguish between disease and non-diseased) to 1 (a cutoff that has perfect sensitivity and specificity) (Evans, Horstman and Conzemius 2005). The AUC under the ROC curves were obtained for conformation parameters using MedCalc 11.0 (MedCalc Software, Broekstraat 52, 9030 Mariakerke, Belgium). All data were expressed as mean ± SD. Statistical significance was set at P < 0.05.
Figure 12: The femoral anteversion angle (FAA) equal the value of the tangent of “a” divided by “b”. The long axis of the femoral shaft is drawn on the lateral (A) and cranio-caudal (B) femoral views between two points centered in the proximal femoral shaft. These two points were identified by bisecting the two femoral cortices, respectively, at one-fourth (25% line) and one-half (50% line) of the total femoral length. A The distance “a” from the center of the femoral head to the extended axis of the femoral shaft was measured on the lateral view of the femur. B The distance “b” from the center of the femoral head to the extended axis of the femoral shaft was measured on the cranio-caudal view of the femur.
3.4.2. Results

Labrador Retrievers enrolled in the normal group (group 1) were older (100.7 ± 16.5 months) than dogs with CCL predisposition (group 2, 58.6 ± 19.6 months). The average body mass did not differ between groups and it was 35.6 ± 8.3 kg. Gender distribution did not differ statistically between groups, consisting of 62 % female and 38 % male dogs. The normal group was composed of four females spayed, three intact females, two castrated males and three intact males. Six females spayed and three castrated males composed the group with CCL predisposition. On long-term follow up, four of the nine (45 %) predisposed limbs have developed CCL deficiency (within a median of 35.5 months, range 5 to 54 months), two predisposed limbs (22 %) were still unaffected, and the status of three predisposed limbs was unknown (33 %). Absence of CCL disease on long term follow up was confirmed for all non predisposed limbs.

The descriptive statistical analysis (mean and standard deviation SD) of each parameters has been reported separately (Mostafa et al 2009). The combination of radiographic conformational parameters that best discriminated limbs predisposed to and at low risk for CCL disease consisted of the tibial plateau angle (TPA) and femoral anteversion angle (FAA). The area under the ROC curve (AUC) generated from this combination was 0.96, with a standard error of 0.047 and a (0.78-1.00) 95% confidence interval. The score equation that combines TPA and FAA was as follows:

\[
\text{Logit(probability of being predisposed)} = -33.49 + 0.37(FAA) + 0.82(TPA).
\]

Predisposed limbs had a score for this equation of 1.11 or more, except for an individual that had a score of -1.96. Limbs at low-risk had a score lower than -1.66, except for an individual that had a score of 1.52. The combined TPA and FAA score
equation had a sensitivity and specificity of 89% ((51.8-99.7) 95% confidence interval) and 92% ((61.5-99.8) 95% confidence interval), respectively, at cutoff determined by maximizing Youden’s index. The value of sensitivity and specificity are cutoff dependent, but emphasize the ability of the conformational radiographic parameters to discriminate between predisposed and at low risk limbs. The sensitivity was 72% ((46.4-89.3) 95% confidence interval) and the specificity was 92% ((59.7-99.6) 95% confidence interval) when using this equation with a -1.5 cut off value to discriminate normal and CCL-deficient limbs in the same population. In order to determine the optimal cutoff, the predicted values from the logistic regression were used, and the cutoff value was picked using that constructed variable.

Scatter plots of TPA against FAA demonstrate the advantage of the multivariate approach to assess predisposition to CCL disease (Figure 13). There are predisposed limbs with TPA values that are in the range of those for limbs at low-risk for CCL disease, so that these TPAs do not individually perform well for distinguishing predisposed from non-predisposed. However, with a similar TPA, most predisposed limbs distinguished themselves from the non-predisposed limbs with a greater FAA (Figure 13). A summary of the mean, SD, and range of TPA and FAA is presented in table 11 for predisposed and non-predisposed limbs to CCL disease.

The combination of radiographic, CT and DEXA conformational parameters that best discriminated limbs predisposed and at low risk to CCL disease was FAA measured on radiographs and whole body fat percentage measured on DEXA (%). Both a large FAA and large fat percentage was observed in predisposed limbs compared with non-predisposed limbs (Figure 14).
Figure 13: Scatter plot of tibial plateau angle (TPA) against femoral anteversion angle (FAA) measured on radiographs. The TPA and FAA are both typically greater in predisposed limbs (black circles) compared with non-predisposed limbs (white diamonds).

Figure 14: Scatter plot of femoral anteversion angle (FAA) measured on radiographs against whole body fat percentage measured on DEXA. FAA and fat content were both greater in predisposed limbs (black circles) compared with non-predisposed limbs (white diamonds).

<table>
<thead>
<tr>
<th></th>
<th>TPA</th>
<th>FAA</th>
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<tbody>
<tr>
<td>Predisposed</td>
<td>28.4 ± 2.0</td>
<td>33.5 ± 3.5</td>
</tr>
<tr>
<td>Non-predisposed</td>
<td>25.2 ± 2.1</td>
<td>26.0 ± 5.0</td>
</tr>
</tbody>
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Table 11: Mean, standard deviation and range of TPA and FAA values presented for predisposed and non-predisposed limbs.
3.5. Kinetic and kinematic analysis of the pelvic limbs in Labrador Retrievers predisposed or at a low risk for cranial cruciate ligament disease

For the third goal of the study, the CCL risk predictive equation was used to assign sound Labrador Retrievers to one of two groups, based on their predictive score for CCL disease. The objective was to examine the pelvic limb mechanics of healthy Labrador Retrievers predisposed to CCL disease and those at low risk to develop the disease. These gait mechanics were examined while trotting overground and on a treadmill.

3.5.1. Materials and methods

3.5.1.1. Subjects

Twenty pure bred adult client-owned Labrador Retrievers were recruited among the local pet population for the study. For each dog, the body weight, age, and sex were recorded. Criteria for inclusion included absence of neurological, orthopedic or medical disease on each dog based on history, physical, orthopedic, and neurological examinations, and on radiographs of both hip and stifle joints performed under sedation. The right limb of each dog was studied and classified as predisposed (group 1) or not (group 2) to CCL disease based on the predictive equation combining the tibial plateau angle (TPA) and the femoral anteversion angle (FAA): $CCL \ risk \ score = -33.49 + 0.37(FAA) + 0.82(TPA)$. Based on these results, limbs with a score greater than -1.5 were considered as predisposed to CCL disease, whereas those with a score lower than -1.5 were considered at low risk for CCL disease. Dogs had two training sessions to walk and
trot on a land treadmill which were scheduled the day before and the day of gait data collection. Each session lasted until consistent gait was observed for ten minutes.

3.5.1.2. Imaging studies

The set of radiographs obtained in all dogs under sedation included: medio-lateral radiographs of the tibia and femur, and an extended cranio-caudal femoral view. The tibial plateau angle (TPA) and femoral anteversion angle (FAA) were measured as previously described (Slocum and Slocum 1993; Bardet, Rudy and Hohn 1983). The fat content was measured in each sedated dog laying in sternal recumbency with high resolution DEXA of the whole body (QDR 4500 fan beam X-ray bone densitometer, Hologic Inc, Bedford, MA).

3.5.1.3. Measurements

Each right limb was divided in three segments: the foot, crus, and thigh. Length of the thigh was measured on the lateral aspect of the dogs as the distance between the greater trochanter and the most distal point of the lateral femoral epicondyle. Length of the crus was defined as the distance between the most distal point of the lateral femoral epicondyle and the lateral malleolus of the fibula. Length of the foot was defined as the distance between the lateral malleolus and the fifth metatarsophalangeal joint. All lengths and the circumferences of the thigh and crus were measured with a single standard plastic non-stretchable metric tape, while sedated dogs were positioned in lateral recumbency. The circumference of the thigh was measured in the mid-shaft region of the femur at a point midway between the greater trochanter and lateral femoral epicondyle. The circumference of the crus was measured at the level of the tibial crest. Medio-lateral
widths of the stifle, tarsal, and metatarsophalangeal joints were measured with a caliper at the level of the lateral femoral epicondyle, lateral malleolus, and fifth metatarsophalangeal joint, respectively. All morphometric measurements listed above were obtained in triplicate on sedated dogs and mean values were used for analysis.

The length, width and girth of each right hind limb segment were used to calculate the body segment parameters of each limb, including segment mass, location of the center of mass along the longitudinal axis of the segment, and mass moment of inertia, using the equations we designed to estimate body segment parameters in Labrador Retrievers (Ragetly et al 2008).

3.5.1.4. Gait data collection

Spherical 14-millimeter diameter reflective markers were affixed to the clipped skin overlying skeletal landmarks over the cranial dorsal iliac spine, greater trochanter, most distal point on the lateral epicondyle of the femur, lateral malleolus, lateral fifth metatarsophalangeal joint, sacro-coccygeal articulation, and the dorsum of the ipsilateral front foot. Markers identified the thigh, crus and foot segments of the right hind limbs in accordance with the aforementioned morphometric model. The landmarks were estimated by palpation of the end points of the bones. Accurate positioning of the markers was verified via manual flexion and extension of the joints after application of the markers. Dogs were then allowed to familiarize themselves with a 10-meter walkway and the treadmill used for gait analysis prior to data collection. Kinematic data were collected using a 6-camera optical motion capture system (Vicon Motion Systems, Inc., Lake Forest, CA) synchronized with a force plate (AMTI, model BP600900, Watertown, MA)
embedded halfway down the walkway for overground trials. Kinematic data were also synchronized with the force plates positioned under the split-belt treadmill that has the unique ability to record the magnitude and location of forces applied to each belt (173 cm x 41 cm per belt, Bertec, Corp., Columbus, OH) for treadmill trials. Force and kinematic data were collected at 100 Hz. A volume of space was calibrated to track markers over the force plate and the treadmill areas. During the overground trials, the same handler led all dogs throughout the study at a comfortable trotting velocity of approximately 2 m/s. During the treadmill trials, the treadmill speed was set to 2 m/s. (Note, after data collection on the treadmill trials was completed, it was determined that the controller program for the treadmill created an incorrect actual belt speed, which averaged 2.47 ± 0.20 m/s.)

3.5.1.5. Data processing

A minimum of two and maximum of five valid trials were analyzed for the right hind limbs during the stance phase of the gait cycle. All calculations were performed using a custom computer program (written in MATLAB, MathWorks, Inc., Natick, MA). Each stance phase started from ground contact of the hind paw onto the force plate/belt and ended with the take off of the same hind paw from the force plate/belt, as defined by visual detection on the curve of vertical GRF. Kinematic and kinetic data were filtered using a forward-backward fourth-order Butterworth low-pass filter with 15 Hz cut-off frequency (determined from residual analysis (Winter 2005)). Actual trotting velocity during each trial (stance phase) was calculated based on the craniocaudal displacement of the sacro-coccygeal marker for overground trials and the combination of the treadmill belt velocity and the displacement of the sacro-coccygeal marker for treadmill trials.
Kinematic data were determined for the hock, stifle and hip joints. These included duration of the stance phase or stance time (s), sagittal-plane angular position (deg) and velocity (deg/s). Joint angles were computed for the anatomic flexor side of each joint, with the value of the angle decreasing during flexion and increasing during extension. Angular velocity was positive during extension and negative during flexion. Kinetic data were normalized by the mass of each dog. These included GRF (N/kg), net joint muscle moments (N.m/kg), net joint muscle powers (W/kg), and net JRF (N/kg) in the sagittal plane for the hock, stifle and hip joints. Ground reaction forces and kinematic data with dog-specific morphometric data were combined to obtain an inverse dynamics solution for the resultant parameters of interest. Extensor net joint muscle moment was positive; flexor net joint muscle moment was negative. Net joint muscle powers were calculated as the product of net joint muscle moment and angular velocity. A positive joint power indicated power generation (concentric muscular contraction) and a negative joint power indicated power absorption (eccentric muscular contraction). Ground reaction forces and net JRF were evaluated in the vertical and cranio-caudal directions (braking and propulsion phases).

The peak values of flexion and extension for angular position and velocity, of flexor and extensor net joint muscle moment, of power generation and absorption, of JRF for vertical, braking, and propulsive forces were documented for each joint of interest, as well as the peak values of GRF for vertical forces and braking and propulsive cranio-caudal forces. The peak values of vertical and braking GRF were defined as the maximum value of vertical and cranio-caudal GRF, respectively, during the stance phase of the gait cycle. The peak values of propulsive GRF were defined as the minimum value of cranio-
caudal GRF during the stance phase. On the opposite, the peak values of vertical and braking JRF were defined as the minimum value of vertical and cranio-caudal JRF and the peak values of propulsive JRF were defined as the maximum value of cranio-caudal JRF during the stance phase. The total impulse values of GRF and JRF, derived from the sum of forces over time (area under the force curve, N.s/kg), were documented. The peak values of extension, extensor moment and power generation were defined as the maximum value of angular position and velocity, net joint muscle moment and net joint muscle power, respectively, during the stance phase of the gait cycle. At the stifle joint, two peaks of power generation were recorded, during early (PS1) and late stance (PS2) respectively. The peak values of flexion, flexor moment and power absorption were defined as the minimum value. The peak and impulse values from each trial were determined and averaged per dogs.

3.5.1.6. Statistical analysis

Groups were compared for gender and neutering status distribution with a $\chi^2$ test. Age (months) was compared between groups with a Mann-Whitney test, and body mass (kg) and body fat content (%) with a Student $t$-test. The following kinematic variables were compared between the two groups: stance time, trotting velocity, peak values of joint angular position and velocity during flexion and extension at the hock, stifle and hip joints. The following kinetic variables were compared between the two groups: peak and impulse values for vertical and cranio-caudal GRF and JRF, peak values of net flexor and extensor muscle moments, and peak values of net muscle power generation and absorption at the hock, stifle and hip joints. Moreover, the values
of TPA and FAA were also compared between groups. Normal distribution was tested with a Shapiro-Wilk normality test. For variables with a normal distribution, a Student $t$-test was used when homogeneity of variance was assumed; a Welch’s $t$-test was used when homogeneity of variance was rejected. A Mann-Whitney test was used for parameters without a normal distribution (Systat 11, Systat Inc., Richmond, CA, USA). For all analyses, $p<0.05$ was considered significant. All morphometric, TPA and FAA measurements were done in triplicate by a single observer. The variability of these measurements was assessed by pooling the observer standard deviations for each dog (intraobserver variability) and with the use of coefficient of variation (CV) (Fettig et al 2003; Unis et al 2010).

3.5.2. Results

3.5.2.1. Variability measurements

The intraobserver variability (obtained by pooling the observer standard deviations for each dog) for TPA and FAA was 1.1 ° and 2.1 °, whereas the between dog (interdog) variability was 2.3 ° and 6.6 °, respectively. The average coefficient of variation for the measurement of the TPA and FAA were 0.039 and 0.074, respectively. The intraobserver variability was 0.64 cm and 0.38 cm for the thigh and crus circumference, respectively, and ranged from 0.19 to 0.22 cm for the measurements of segment length, and from 0.04 to 0.09 cm for the measurements of the width.
3.5.2.2. Overground trials

After review of right pelvic limb radiographs, 11 dogs (7 castrated males, 4 females [3 spayed]) were classified as predisposed (group 1, median CCL risk score of 2.4, range -1.2 to 6.4) and 9 dogs (4 castrated males, 5 spayed females) as not predisposed to CCL deficiency (group 2, median CCL risk score of -2.6, range -4.4 to -1.5). Predisposed dogs (median age, 35 months; range, 10.5 - 137 months) were younger than dogs not predisposed to CCL deficiency (median, 77.5 months; range, 24.5 - 130 months). Mean ± SD body mass (group 1 = 26.9 ± 3.4 kg, group 2 = 26.9 ± 3.2 kg) and body fat content (group 1 = 27.4 ± 6.1 %; group 2 =26.2 ± 8.1 %) were not significantly different between groups. Gender and neutering status distribution were not significantly different between groups. Predisposed dogs had a greater TPA (28.6 ± 1.8 °, mean ± SD) and FAA (32.3 ± 6.0 °, median 30.2 °, range 26.3 – 43.3 °) than non predisposed dogs (TPA, 25.7 ± 1.7 °; FAA, 26.4 ± 3.9 °, median 25.6 °, range 21.5 – 32.1 °).

A minimum of three valid trials were examined for each right pelvic limb, with five valid trials examined for 85 % of the subjects. Trotting velocity of the subjects did not differ significantly between groups and the mean velocity was 1.97 ± 0.15 m/s (Table 12). Extensor moment at the hock was increased in predisposed limbs compared to non predisposed limbs (Figure 15 C, Table 13). Predisposed limbs generated more energy around the hock (Figure 15 D, Table 13) and stifle joints (PS1, Figure 16 D, Table 13) compared with non predisposed limbs. Peak values of joint angular position and velocity during flexion and extension of the hock, stifle and hip joints did not differ significantly between groups (Figures 15-17 A and B, Table 13), nor did net joint
muscle moment and power at the hip joint (Figures 17 C and D, Table 13). The value of stance time, vertical and cranio-caudal GRF did not differ significantly between predisposed and non predisposed limbs (Tables 12, Figure 18). The only difference in JRF between groups consisted of a greater impulse value of propulsive craniocaudal JRF at the hip joint in predisposed limbs compared to limbs at low-risk (Table 13).
Table 12: Mean (±SD) of gait velocity (m/s), stance time (s), as well as ground reaction forces (GRF): peak (N/kg) and total impulse (N.s/kg) values of vertical and craniocaudal forces during the braking and propulsive phases for subjects at low-risk or predisposed to CCL disease for overground trials. No statistical differences were observed between groups.
Table 13: Mean (±SD) of peak kinematic and kinetic values for the hock, stifle and hip joints in dogs at low-risk or predisposed to CCL disease for overground trials. Kinematics included peak values of joint angular position and velocity during flexion and extension. Kinetics included peak values of net joint muscle moment and net joint muscle power, and peak and impulse values of net joint reaction forces (JRF) in the vertical and craniocaudal directions. *: for each separate joint, indicates statistical difference between predisposed and non predisposed limbs for given parameter.
Figure 15: Mean (± SE) of (A) angular position, (B) angular velocity, (C) net joint muscle moment, and (D) net joint muscle power at the hock joint for limbs at low-risk (solid) and limbs predisposed to CCL disease (dash) during the stance phase for overground trials. Dot lines indicate standard error. * indicates statistical difference between groups.
Figure 16: Mean (± SE) of (A) angular position, (B) angular velocity, (C) net joint muscle moment, and (D) net joint muscle power at the stifle joint for limbs at low-risk (solid) or predisposed to CCL disease (dash) during the stance phase for overground trials. PS1 and PS2 represent the peak of stifle net power generation during the first and second half of the stance phase, respectively. Dot lines indicate standard error. * indicates statistical difference between groups.
Figure 17: Mean (± SE) of (A) angular position, (B) angular velocity, (C) net joint muscle moment, and (D) net joint muscle power at the hip joint for limbs at low-risk (solid) or predisposed to CCL disease (dash) during the stance phase for overground trials. Dot lines indicate standard error. * indicates statistical difference between groups.
Figure 18: Mean (± SE) of (A) vertical and (B) craniocaudal ground reaction forces for limbs at low-risk (solid) or predisposed to CCL disease (dash) during the stance phase for overground trials.
3.5.2.3. Treadmill trials

The right limbs of twenty adult Labrador Retrievers were studied. Valid kinetic trials could not be generated on the treadmill in three dogs due to invalid cranio-caudal GRF. Therefore, the results presented correspond to 17 dogs only, 10 neutered males, one intact female and 6 spayed female, with a median age of 50 months (range 11.5 to 137 months), and mean body weight of 27.6 ± 2.9 kg. Upon review of the right limb radiographs, seven Labrador Retrievers were classified as non predisposed to CCL deficiency (median CCL risk score of -2.6, range -3.2 to -1.5) and ten dogs classified as predisposed (median CCL risk score of 1.7, range -1.2 to 6.4). The age of Labrador Retrievers in group 1 (predisposed, median age 40 months, range 11.5 to 137 months) was not significantly different than in group 2 (non predisposed, median age 69 months, range 24.5 to 130 months). Mean body mass did not differ between groups (27.6 ± 2.7 kg and 27.5 ± 3.4 kg for predisposed and non predisposed dogs, respectively). Gender distribution was not significantly different between groups. Group 1 was composed of six males and four females, nine neutered and one intact dogs. Group 2 was composed of four males and three females, all neutered. The TPA and FAA was higher in dogs from group 1 than dogs in group 2 (TPA of 28.6 ± 1.9 ° and FAA of 32.0 ± 6.2 ° for predisposed limbs, TPA of 25.9 ± 1.8 ° and FAA of 26.3 ± 3.3 ° for non predisposed limbs).

Three valid trials were studied for the right pelvic limb of each subject except in two dogs, both in the non predisposed group, for who only two valid trials were examined. Trotting velocity of the subjects did not differ between groups and the mean velocity was 2.47 ± 0.20 m/s (Table 14). The stifle was held at a greater degree of
flexion at mid-stance in predisposed limbs (Figure 19 A). In addition, more energy was generated by muscle acting on the stifle in the early stance phase (PS1, Figure 19 C) of these limbs during flexion of the stifle joint. The hock did not reach the same degree of extension in predisposed compared to non predisposed limbs at push-off (Table 15).

Peak values of joint angular position of the hip and of joint angular velocity of the hock, stifle and hip joints did not differ between groups during flexion and extension (Table 15), nor did net joint muscle moment at the hock, stifle (Figure 19 B) and hip joints, and net joint power at the hock and hip joints (Table 15). Stance time, vertical and craniocaudal GRF and JRF did not differ between predisposed and non predisposed limbs (Tables 14 and 15).
Table 14: Mean (±SD) of gait velocity (m/s), stance time (s), as well as ground reaction forces (GRF): peak (N/kg) and total impulse (N.s/kg) values of vertical and cranio-caudal forces during the braking and propulsive phases for subjects at low-risk or predisposed to CCL disease for treadmill trials. No statistical differences were observed between groups.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Non predisposed</th>
<th>Predisposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Velocity</td>
<td>2.40 (0.27)</td>
<td>2.51 (0.14)</td>
</tr>
<tr>
<td>Stance Time</td>
<td>0.22 (0.02)</td>
<td>0.22 (0.02)</td>
</tr>
<tr>
<td>Peak vertical GRF</td>
<td>7.69 (0.52)</td>
<td>7.60 (0.64)</td>
</tr>
<tr>
<td>Peak braking GRF</td>
<td>0.62 (0.24)</td>
<td>0.70 (0.35)</td>
</tr>
<tr>
<td>Peak propulsion GRF</td>
<td>-1.08 (0.29)</td>
<td>-1.05 (0.26)</td>
</tr>
<tr>
<td>Vertical impulse GRF</td>
<td>0.89 (0.14)</td>
<td>0.90 (0.09)</td>
</tr>
<tr>
<td>Braking impulse GRF</td>
<td>0.03 (0.02)</td>
<td>0.04 (0.02)</td>
</tr>
<tr>
<td>Propulsive impulse GRF</td>
<td>-0.08 (0.03)</td>
<td>-0.08 (0.04)</td>
</tr>
</tbody>
</table>
Table 15: Mean (±SD) of peak kinematic and kinetic values for the hock, stifle and hip joints in dogs at low-risk or predisposed to CCL disease for treadmill trials. Kinematics included peak values of joint angular position and velocity during flexion and extension. Kinetics included peak values of net joint muscle moment and net joint muscle power, and peak and impulse values of net joint reaction forces (JRF) in the vertical and cranio-caudal directions. *: for each separate joint, indicates statistical difference between predisposed and non predisposed limbs for given parameter.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Non predisposed</th>
<th>Non predisposed</th>
<th>Non predisposed</th>
<th>Non predisposed</th>
<th>Non predisposed</th>
<th>Non predisposed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hock</td>
<td>Stifle</td>
<td>Hip</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angular position</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>179.0 (10.4)</td>
<td>161.0 (9.2) *</td>
<td>165.3 (17.6)</td>
<td>155.6 (6.3)</td>
<td>136.9 (14.2)</td>
<td>137.9 (5.4)</td>
</tr>
<tr>
<td>Flexion</td>
<td>125.6 (12.1)</td>
<td>116.4 (10.7)</td>
<td>139.3 (7.9)</td>
<td>130.9 (6.7) *</td>
<td>112.0 (16.4)</td>
<td>109.4 (8.2)</td>
</tr>
<tr>
<td>Angular velocity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>675 (174)</td>
<td>593 (104)</td>
<td>165.3 (17.6)</td>
<td>155.6 (6.3)</td>
<td>136.9 (14.2)</td>
<td>137.9 (5.4)</td>
</tr>
<tr>
<td>Flexion</td>
<td>125.6 (12.1)</td>
<td>116.4 (10.7)</td>
<td>139.3 (7.9)</td>
<td>130.9 (6.7) *</td>
<td>112.0 (16.4)</td>
<td>109.4 (8.2)</td>
</tr>
<tr>
<td>Moment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flexor peak</td>
<td>n/a</td>
<td>n/a</td>
<td>0.31 (0.15)</td>
<td>0.46 (0.14)</td>
<td>1.32 (1.23)</td>
<td>1.32 (2.08)</td>
</tr>
<tr>
<td>[N.m/kg]</td>
<td>0.36 (0.13)</td>
<td>0.44 (0.13)</td>
<td>0.94 (0.20)</td>
<td>0.95 (0.23)</td>
<td>0.68 (0.24)</td>
<td>0.94 (0.28)</td>
</tr>
<tr>
<td>[W/kg]</td>
<td>1.66 (0.84)</td>
<td>1.76 (1.01)</td>
<td>1.64 (0.94)</td>
<td>2.93 (1.34) *</td>
<td>2.89 (1.23)</td>
<td>3.12 (2.08)</td>
</tr>
<tr>
<td>2nd generation</td>
<td>n/a</td>
<td>n/a</td>
<td>1.53 (0.91)</td>
<td>1.84 (1.11)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Power</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generation peak</td>
<td>-4.57 (1.86)</td>
<td>-4.90 (2.73)</td>
<td>-2.10 (2.52)</td>
<td>-1.91 (1.02)</td>
<td>-3.13 (1.62)</td>
<td>-3.58 (1.95)</td>
</tr>
<tr>
<td>Absorption peak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JRF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertical peak</td>
<td>-7.58 (0.54)</td>
<td>-7.47 (0.61)</td>
<td>-7.21 (0.53)</td>
<td>-7.09 (0.57)</td>
<td>-6.12 (0.31)</td>
<td>-6.20 (0.62)</td>
</tr>
<tr>
<td>[N/kg]</td>
<td>-0.84 (0.13)</td>
<td>-0.85 (0.09)</td>
<td>-0.79 (0.13)</td>
<td>-0.81 (0.09)</td>
<td>-0.67 (0.12)</td>
<td>-0.70 (0.09)</td>
</tr>
<tr>
<td>Vertical impulse</td>
<td>1.14 (0.39)</td>
<td>1.12 (0.22)</td>
<td>1.32 (0.41)</td>
<td>1.30 (0.22)</td>
<td>1.81 (0.38)</td>
<td>1.71 (0.23)</td>
</tr>
<tr>
<td>Absorption peak</td>
<td>0.08 (0.04)</td>
<td>0.08 (0.03)</td>
<td>0.09 (0.04)</td>
<td>0.09 (0.04)</td>
<td>0.11 (0.04)</td>
<td>0.11 (0.04)</td>
</tr>
<tr>
<td>JRF</td>
<td>-0.86 (0.32)</td>
<td>-1.10 (0.38)</td>
<td>-1.32 (0.48)</td>
<td>-1.69 (0.49)</td>
<td>-1.67 (0.64)</td>
<td>-2.13 (0.52)</td>
</tr>
<tr>
<td>Braking peak</td>
<td>-0.04 (0.02)</td>
<td>-0.05 (0.03)</td>
<td>-0.06 (0.02)</td>
<td>-0.07 (0.03)</td>
<td>-0.08 (0.03)</td>
<td>-0.10 (0.03)</td>
</tr>
</tbody>
</table>

Table 15: Mean (±SD) of peak kinematic and kinetic values for the hock, stifle and hip joints in dogs at low-risk or predisposed to CCL disease for treadmill trials. Kinematics included peak values of joint angular position and velocity during flexion and extension. Kinetics included peak values of net joint muscle moment and net joint muscle power, and peak and impulse values of net joint reaction forces (JRF) in the vertical and cranio-caudal directions. *: for each separate joint, indicates statistical difference between predisposed and non predisposed limbs for given parameter.
Figure 19: Mean (± SE) of (A) angular position, (B) net joint muscle moment, and (C) net joint muscle power at the stifle joint for limbs at low-risk (solid) and limbs predisposed to CCL disease (dash) during the stance phase for treadmill trials. Two peaks of power generation were recorded, during early (PS1) and late stance (PS2) respectively. Dot lines indicate standard error. * indicates statistical difference between groups.
CHAPTER 4: DISCUSSION

In this section, the author presents the discussion for each of the studies successively.

4.1. Body segment parameters measurement and estimation by predictive equations

The main findings of our study about body segment parameters were the followings (Ragetly et al 2008). Thigh and crus of CCL-deficient limbs were lighter than their matched contralateral segments. The thigh of CCL-deficient limbs was also lighter than that of normal limbs. The center of mass (COM) of the crus was located more proximal in CCL-deficient and contralateral limbs than in normal limbs. The normalized mass moment of inertia of the thigh was lower in CCL-deficient limbs than in matched contralateral limbs. The morphometric measurements used in the regression equations to estimate body segment parameters (BSP), i.e. mass, location of the COM and mass moment of inertia, varied with parameter, body segment, and status of the limb.

4.1.1. Age of the dogs

The age of dogs with CCL disease in our study is consistent with the epidemiology of this condition in large breed dogs (Whitehair, Vasseur and Willits 1993). The difference in age between normal and CCL-deficient Labradors in our study was expected because the minimum age requirement for inclusion in our normal group
was based on the decreased risk for CCL disease previously reported in older Labrador Retrievers (Reif and Probst 2003).

### 4.1.2. Body segment parameters

The thigh and crus segments weighed less in CCL-deficient limbs than in unaffected contralateral limbs. The mass of the thigh was also inferior in CCL-deficient compared to normal limbs. These findings are consistent with muscle atrophy secondary to CCL disease (Monk, Preston and McGowan 2006). Although the duration of lameness before presentation varied, the majority of dogs were lame for at least one month; these findings are therefore likely to reflect discomfort and disuse of the CCL-deficient limb. The decreased mass of the thigh also explains the lower mass moment of inertia of the thigh in affected limbs compared to contralateral limbs in dogs with CCL disease. Indeed, the mass moment of inertia of a segment measures resistance to change in angular motion and correlates proportionally with its distribution of mass with respect to the COM. Differences appeared more pronounced in the thigh than in the crus, suggesting that muscle atrophy secondary to CCL disease affects predominantly the muscles of the thigh, rather than those of the crus. Alternatively, the magnitude of changes in the crus may be limited by the amount of tissue naturally present around the tibia compared to the femur. Differences in mass of the crus and mass moment of inertia of the thigh were statistically significant between affected and contralateral limbs in dogs with CCL disease, but not between affected and normal limbs. This could be attributed to the difference in age between our groups and a potential mild atrophy of the muscles in older dogs (Colman et al 2005; Marcell 2003; Braund,
McGuire and Lincoln 1982). However, dogs with CCL deficiency have been found to redistribute their weight so that the contralateral limb bears 87% of the body weight compared to 66% in normal dogs (Rumph et al 1995). Our results are therefore more likely to reflect this compensatory activity, thereby magnifying differences between affected and contralateral limbs in dogs with CCL disease.

The location of the COM of the thigh segment did not differ between groups. Muscle atrophy therefore appears to be uniform, maintaining the repartition of mass along the longitudinal axis of the thigh. The COM of the crus was located in a more proximal position in CCL-deficient and contralateral limbs than in normal limbs. This difference in repartition of the mass along the crus segment is unlikely to be solely due to CCL disease since contralateral limbs displayed the same distribution. These contralateral limbs can be considered as predisposed to CCL deficiency based on the high incidence of bilateral and contralateral CCL disease in dogs (Duval et al 1999; Moore and Read 1995; Doverspike, Vasseur and Harb 1993). Further studies are warranted to evaluate whether a relatively greater proportion of bone and/or muscle in the proximal portion of the crus is a factor predisposing dogs to CCL disease.

The only previous publication of breed-specific morphometric data in dogs was based on musculoskeletal disease-free cadaver specimen of three Labradors and four Greyhounds, whose ages were not reported (Colborne et al 2005). In this study, body segment parameters of the crus and thigh were measured directly on cadavers, using a digital scale and a balance board technique. These techniques were the first methods utilized to measure BSP in man and provide a reference to which new methods of measurements can be compared. Medical imaging techniques such as computed
tomography (Pearsall, Reid and Livingston 1996; Huang and Suarez 1983), magnetic resonance imaging (Cheng et al 2000; Martin et al 1989), DEXA (Durkin and Dowling 2003; Durkin, Dowling and Andrews 2002) and gamma mass scanning (Zatsiorsky and Seluyanov 1983) have consequently been found to provide accurate measurements of BSP in living human subjects. In our study, the crural mass (1.41 ± 0.16 % BM) measured with a non-invasive approach based on computed tomography was similar to that measured directly in the cadaver study of musculoskeletal disease-free Labradors (1.32 % BM) (Colborne et al 2005). The mass of the thigh and the moment of inertia of the segments have not been previously reported, preventing comparison with our results. The COM of the crus (31 % L from the proximal joint) and thigh (42 % L) seem located slightly more proximally in the normal limbs of our study than in the limbs of Labradors described by Colborne et al. (38 % L for the crus, and 48 % L for the thigh). The significance of this difference is difficult to evaluate since direct measurements were not obtained on a representative sample of the population but on three Labradors (Colborne et al 2005). The location of the COM is mostly affected by the geometry of the segment; however the discrepancies between Colborne et al.’s and our studies may also result from inaccuracies associated with the measurement of mass. Two potential sources of error can lead to overestimation of the segment mass using advanced imaging; overestimation of the segmental volume, and use of tissue densities higher than actual values (Martin et al 1989). Overestimation of the volume (3% with CT method and 6.3% with magnetic resonance imaging (Martin et al 1989; Rodrigue and Gagnon 1983)) has been attributed to the difficulty in discriminating the tissue perimeter (Cheng et al 2000). In addition, bone was assigned a constant density regardless of its relative content of cancellous and
compact bone, potentially causing an overestimation of the bone mass in our study. We would expect this overestimation to be magnified in areas where bone predominates over soft tissues, such as the crus. On the other hand, direct measurements in cadavers are affected by post-mortem changes, leading to an estimated 5 to 6 % loss in body fluids, thereby underestimating the mass of segments (Zatsiorsky 2002).

4.1.3. Predictive equations

Although computed tomography allows determination of body segment parameters in living subjects, its application to gait analysis in dogs remains limited by cost and the need for general anesthesia. Data developed in this investigation indicate that mass, location of the COM, and mass moment of inertia for hind limb segments in Labrador Retrievers can be predicted based on simple morphometric measurements of the body. Regression equations generated from direct measurements on cadavers have become the most common method to estimate BSP in humans (Winter 2005; Clauser, McConville and Young 1969; Hinrichs 1985; Schneider and Zernicke 1992; Shan and Bohn 2003). We applied a similar approach to generate regression equations based on simple morphometric parameters in Labrador Retrievers. The mass of individual segments has been expressed as a simple percentage of the body mass and the location of the COM as a percentage of the length of the segment (Dempster 1955). This approach can only provide a crude estimation of parameters and assumes that all individuals have similar body proportions and mass repartition. This assumption is not valid for subjects outside the tested range. Instead, we found that several morphometric measurements (segment length, girth or width) were generally required in addition to
body mass, to generate equations that met validation criteria previously recommended (Buchner et al 1997; Clauser, McConville and Young 1969; Dempster, Sherr and Priest 1964; Staniar et al 2004; Young, Chandler and Snow 1983). These equations have been validated for dogs with morphometric dimensions similar to those of our population but cannot be recommended outside the range of our study. One of the two main sources of uncertainty in inverse dynamic solution in humans has recently been attributed to inaccuracies in estimated body segment parameters (Riemer, Hsiao-Wecksler and Zhang 2008). These inaccuracies are partly due to the fact that anthropometric tables were derived from a relatively small and biased population (Pearsall and Costigan 1999; Rao et al 2006). Similar limitations should be expected in dogs and justify the determination of breed-specific morphometric data. In fact, BSP varied with the status of the limb in our study, preventing breed-specific extrapolation of normal BSP to dogs with orthopedic disease. Accurate determination of morphometric parameters is especially critical to the study of swing phase mechanics, where segmental angular accelerations are the largest, particularly for distal segments (Colborne, Shellard and Morris 2007; Lanovaz and Clayton 2001).

Our study reports the mass, location of the center of mass, and mass moment of inertia in hind limb segments of living normal Labrador Retrievers and in Labradors with CCL disease. This is the first time that morphometric parameters are reported for dogs with CCL disease. Moreover, this is the first time that the mass moment of inertia is reported for hind limb segments in dogs. Computed tomography allowed for non-invasive determination of BSP on living subjects whose gait was concurrently evaluated. However, the application of this approach in small animals is limited by
availability, cost and need for general anesthesia. Regression equations were developed
to estimate the mass, location of the center of mass, and mass moment of inertia per
segment in normal, CCL-deficient, and contralateral limbs of Labrador Retrievers.
These equations are based on parameters that are fast, technically simple and cost
effective to generate. This approach will facilitate clinical studies of the canine gait
mechanics, offering new strategies to investigate the pathogenesis of non-traumatic
joint diseases.

4.2. Inverse dynamic analysis of the pelvic limbs in Labrador Retrievers with and
without cranial cruciate ligament disease

This part of the study describes joint kinetic (net moment, power and reaction
forces) and kinematic data around the hind limb joints in Labrador Retrievers with or
without CCL disease (Ragetly et al 2010). The main findings of this study about inverse
dynamic analysis were the following. Reductions in net moment, power and vertical
and braking JRF at all joints of interest were observed in CCL-deficient limbs
compared to normal and contralateral limbs. To compensate for the CCL disease, an
increased loading and greater mobilization of the stifle extensor muscles of the
contralateral side was observed compared to gait patterns for normal healthy subjects.
4.2.1. Joint moment, power and reaction forces in normal limbs

We present here some interesting findings regarding the kinetics and kinematics in the hock, stifle, and hip joints of normal healthy Labrador Retrievers. A previous publication describing breed-specific inverse dynamics analysis of the hind limbs was restricted to a two-dimensional evaluation of the stance phase in six normal Labrador Retrievers and six Greyhounds (Colborne et al 2005). Dogs in our study trotted at a velocity similar to the normal Labrador Retrievers included in Colborne’s study (1.98 ± 0.14 and 1.98 ± 0.22 m/s, respectively), allowing comparison of data. All data generated from the hock joint in normal limbs were similar between the two studies except for the amplitude of moment and power peaks, which were decreased by half in our study (Colborne et al 2005). This discrepancy may reflect differences in methods, such as definition of the boundaries of the foot segment, determination of the body segment parameters and/or neglecting inertial parameters by Colborne et al. Although every attempt was made to consistently place markers over established landmarks, calculation of kinematic data such as angular position may have been affected by inconsistency in markers. A normalization procedure has been proposed to reduce differences between dogs attributable to marker placement, but was not applied here to allow comparison of our results with those of Colborne et al (DeCamp et al 1993). Beside differences in initial placement, movement during gait, as well as muscular contraction, tendon, and ligament movement would also affect surface marker placement. Further research should focus on developing algorithms to account for skin displacement relative to the underlying bony landmarks in dogs, using the same
approach as previously published with horses (van den Bogert, van Weeren and Schamhardt 1990).

The contraction of the stifle extensor muscles (quadriceps muscles mainly) (Milis, Levine and Taylor 2004) became dominant at mid-stance and reversed the moment from flexor to extensor, which triggers extension of the stifle and a small but active contribution of the joint during push-off. The slight extension of the stifle observed in our study differs from the flexed position previously reported in trotting Labrador Retrievers but has been described in Greyhounds (Colborne et al 2005) and large mixed breed dogs (Allen et al 1994). Moreover, although the pattern of joint moments throughout the gait cycle was similar to that previously reported during stance (Colborne et al 2005), the amplitude of extensor moment was four times higher in our study. These discrepancies may reflect differences in angular position and velocity of the stifle and / or previously neglected inertial properties. Hip joint moment and power in normal limbs have not been previously reported, preventing any comparison with our results.

4.2.2. Kinetics and kinematics in cruciate-deficient limbs

As expected, CCL-deficient limb kinetics and kinematics differed substantially from normal limbs. The lameness observed on clinical examination of dogs with CCL deficiency correlated with decreased GRF loads similar to values previously published (Rumph et al 1995; Budsberg 2001). Joints of CCL-deficient limbs were loaded with less force in the vertical and horizontal directions, without alteration of stride length and time. Lameness in CCL-deficient limbs manifested itself especially during the
braking phase of the gait cycle and did not affect propulsive forces. The braking /
propulsion ratio shifted from 50:50 % in normal limbs to 33:66 % of the stance phase in
CCL-deficient limbs. Weight bearing generates compression between the tibia and
femur and cranial tibial thrust. The decrease in braking may therefore reflect attempts
to decrease painful mobilization of the stifle, especially hyperextension or cranial
subluxation of the tibia. The second adaptive mechanism observed in CCL deficient
limbs consisted of a lack of extension at push-off. Extension is a position where the
joint is likely to subluxate cranially under load (Korvick, Pijanowski and
Schaeffer 1994). The joint force produced on the canine stifle by weight bearing has
been reported to be almost parallel to the patellar ligament and to produce a
femorotibial shear force in a cranially oriented direction during the extension of the
stifle (Kipfer et al 2008). The observed lack of extension correlated with a decreased
recruitment of the stifle extensor muscles. Our results confirm previous reports that
trotting dogs with CCL disease carry the affected stifle more flexed and have decreased
joint angular velocities than normal (DeCamp et al 1996; Vilensky et al 1994).
These adaptations (decreased flexor and extensor muscle groups contraction, decreased
and slower joint motion, decreased joint loading), may represent the subsequent
reprogramming of the locomotor process associated with canine CCL deficiency and its
painful stimuli described by Vilensky et al. (Vilensky et al 1997). In dogs with
deficient sensory nerve supply and CCL deficiency, full-thickness ulceration of the
femoral condylar cartilage appears sooner and more severely than in CCL-deficient
limbs with intact sensory nerves (Vilensky et al 1997). Vilensky et al. suggested that
sensation of pain and instability result in mechanisms of gait adaptation (decreased
and slower stifle motion) via the central nervous system; thereby delaying the rate of joint breakdown (Vilensky et al. 1994; Vilensky et al. 1997).

### 4.2.3. Kinetics and kinematics in contralateral limbs

Compensatory changes secondary to lameness have been well documented in dogs (Griffon, McLaughlin and Roush 1994; Rumph et al. 1995; Rumph et al. 1993). Dogs with acute CCL deficiency have been found to redistribute their weight so that the contralateral limb bears 87% of the body weight compared to 66% in normal dogs (Rumph et al. 1995). The vertical impulse in contralateral limbs was higher than in normal limbs, but no statistically significant differences were observed in peak vertical forces between normal and contralateral limbs in this study. These findings may correlate to the chronicity of the lameness in our population (Griffon, McLaughlin and Roush 1994; Rumph et al. 1993). Budsberg has previously reported that vertical impulse is a more sensitive indicator of compensation because it provides information about total force applied during the stance phase that may be missed by simply measuring peak magnitude of that force (Budsberg 2001). In this study, compensation was essentially achieved by increasing the time spent with the sound foot on the ground and an increased amount of force applied to all joints of the limb, especially during propulsion compared to normal and CCL-deficient limbs. Propulsion relied heavily on the stifle, as the power generated around that joint at push-off was three-time greater than normal, reflecting an increased stifle extensor muscles contraction. Taken together, our results are suggestive of an increased loading of contralateral limbs compared to normal limbs, associated with greater mobilization of the stifle extensor muscles. These
contralateral compensating limbs can also be considered as predisposed to CCL
deficiency based on the high incidence of bilateral and contralateral CCL disease in
dogs (Duval et al 1999; Doverspike, Vasseur and Harb 1993). The increased stifle
extensor muscles contraction at push-off during stifle extension may increase the
cranial tibial thrust, a force believed to contribute to the pathogenesis of CCL disease
(Arnoczky and Marshall 1981; Slocum and Devine 1983). However, the design of our
study does not allow differentiation of compensatory changes and predisposing factors.

Inverse dynamics gait analysis provides useful insight in animal locomotion as
it allows assessment of muscles moment, power patterns and joint reaction forces
around various joints. This is the first use of an inverse dynamics method to
characterize the gait of dogs with CCL disorder. The results of this study improved our
understanding of gait mechanics in Labrador Retrievers. Characterization of kinetics in
Labrador Retrievers with a low risk to develop CCL disease provides a gold standard to
compare future gait studies. Lameness resulting from CCL disease affected
predominantly the vertical and braking forces, and the extension during push-off.
Kinetic data also identified a greater contribution of the contralateral limbs to propel the
dog forward, especially via the recruitment of stifle extensor muscles.
4.3. Association between surface electromyography, kinetics and kinematics of the hind limb in healthy trotting Labrador Retrievers

The gait mechanics of a population of sound Labrador Retrievers have been characterized with the use of kinetics, kinematics, and surface EMG analysis. The quadriceps was activated in all dogs when the stifle was extending, but also during flexion of the joint in the weight bearing phase of the cycle. Muscular activity of the hamstring and gastrocnemius muscles, flexor muscles of the stifle joint, occurred in all dogs during flexion of the joint during initial weight bearing. Inter-individual variations of electrical activity were observed during the swing phase for the quadriceps and hamstring muscle groups.

Using surface EMG sensors, we obtained similar results regarding muscular activity compared to previous reports using indwelling electrodes in healthy trotting dogs. The vastus lateralis and biceps femoris muscles were previously mainly found active just before ground contact and during 70 and 46 % of the stance phase, respectively. The gastrocnemius becomes active after ground contact for 73 % of the stance phase (Goslow et al 1981). According to Wentink et al, muscular activity is largely concentrated in periods in which a change in the sense of the movement of the limb occurs (Wentink 1976). However, the use of kinematic and kinetic data provided further characterization of the gait mechanics in this study. During the first third of the stance phase, we observed flexion of the stifle joint with a net flexor moment and energy generation, which correlated with the activation of the hamstring and gastrocnemius muscles, flexor muscles of the stifle joint. Co-contraction of the quadriceps, hamstring and gastrocnemius muscles during flexion of the stifle joint
probably balance the flexor motion of the limb in order to avoid collapsing during this
weight bearing phase. During the middle third of stance, energy absorption by the stifle
extensor muscles corresponded with the transition between flexion and extension of the
stifle joint. During the last third of stance, the stifle joint extended and kinetic data
highlighted a net extensor moment with generation of energy around the stifle joint.
From mid-stance to push off, contraction of the quadriceps muscle mainly explained the
observed biomechanics.

The swing phase began with flexion of the stifle joint with concomitant net
extensor moment and energy absorption, corresponding with an eccentric contraction of
the extensor muscles of the stifle. For most dogs, electromyographic activity of the
flexor muscles of the stifle was detected during flexion of the stifle, followed by an
activity of the quadriceps during the transition from flexion to extension of the joint.
Co-contraction of the stifle extensor and flexor muscles was observed during this
transition period. The swing phase ended with an eccentric contraction of the
gastrocnemius and hamstring muscles, probably to stop the extension of the stifle joint
before ground contact. The EMG data correlated well with the kinetic data just before
ground contact. The biceps femoris muscle (part of the hamstring muscle group) has
been previously proposed to decelerate the forward movement of the limb before the
foot touches the ground, by slowing the rate of extension of the stifle (Goslow et al
1981). In this study, EMG helped to characterize co-contraction of antagonistic muscle
groups around a specific joint of interest and revealed which muscles were active when
particular movements were performed. However, EMG does not reveal whether the
muscle contributes to the movement, opposes it or is merely adjusting its length to the altered positions of its attachments (Wentink 1976).

Surface EMG studies are non-invasive and therefore applicable to clinical studies involving client-owned animals. However, several factors affect results such as muscle mechanics, skin impedance, motion artifact, electromagnetic energy, and various types of equipment such as electrocardiograms (Gillette and Angle 2008; Bolton et al 2000). Surface electrodes do not discriminate the activity of individual surface muscles as precisely as indwelling electrodes. Surface EMG inherently may suffer from motion artifacts and from cross-talk and overlap from nearby muscles or part of muscles. Therefore, we elected to present our results as pertaining to muscle groups instead of individual muscles. The activity of the quadriceps was measured with a surface EMG sensor placed on the craniolateral aspect of the thigh. Thus, this sensor is expected to mainly detect the muscular activity of the vastus lateralis muscle. However, we cannot rule out cross-talk with the rectus femoris or vastus intermedius muscles due to initial placement of the EMG electrode or motion artifacts. Similarly, the surface EMG electrode used to measure hamstring activity could have been over the biceps femoris but also the semimembranosus or semitendinosus muscles. Last, the surface electrode positioned to measure the activity of the gastrocnemius could have also recorded the muscular activity of the peroneus longeus, the superficial and deep digital flexor, or the caudal part of the biceps femoris muscles. It was previously reported that these different muscles of the hind limb have different activity patterns (Wentink 1976). Therefore, cross-talk and motion artifact may explain the variation obtained in our EMG data between dogs. Last, another source of variability in our results may be due to
inter-individual variation in muscular activity, especially for the quadriceps and hamstring muscle groups. Individual variations in muscular activity between subjects were also previously observed using indwelling EMG electrodes (Wentink 1976; Gregersen, Silverton and Carrier 1998; Carrier, Gregersen and Silverton 1998).

Electromyograms in humans are typically calibrated against resting activity and isometric maximum voluntary contraction of the muscle of interest. Alternatively, calibration may be adjusted against maximum involuntary contraction (Branch, Hunter and Donath 1989; Besier, Lloyd and Ackland 2003) or a mean value of muscle activation (Kaya, Leonard and Herzog 2003). Calculation in animals can only be based on maximum involuntary contraction, mean or resting activity because maximum voluntary contraction cannot be determined. We elected to normalize our EMG data based on maximum involuntary contraction because this method has been found more precise than maximum voluntary isometric contraction (Knutson et al 1994).

EMG patterns of major hind limb muscles during trotting gait of healthy Labrador Retrievers were characterized and compared with kinetic and kinematic data of the stifle joint. The use of surface EMG highlighted the co-contraction patterns of the muscles around the stifle joint, which were documented during transition periods between flexion and extension of the joint, but also during the flexion observed in the weight bearing phase. Identification of possible differences in EMG activation characteristics between healthy patients and dogs with or predisposed to orthopedic and neurological disease may help understanding the neuromuscular abnormality and gait
mechanics of such disorders in the future. Future studies should also focus on reducing inconsistency in surface EMG data.

4.4. Multivariate analysis of morphometric characteristics to evaluate risk factors for cranial cruciate ligament deficiency in Labrador Retrievers

We applied the statistical method described in an effort to improve the use of radiographs, CT images, and DEXA analysis to identify risk factors for CCL disease, and to determine if a multivariate approach improved discrimination of predisposed limbs from limbs at low risk (Ragetly et al 2011). Our results suggest that the multivariate approach is superior to the univariate one. The combination of percentage of body fat and femoral anteversion angle (FAA) as well as the combination of tibial plateau angle (TPA) and femoral anteversion angle (FAA) were optimal for discriminating predisposed and non-predisposed limbs for CCL disease in Labrador Retrievers.

The combination of radiographic, CT and DEXA conformational parameters that best discriminated limbs predisposed to CCL disease from limbs at low risk was the FAA measured on radiographs and the percentage of body fat measured on DEXA. However, DEXA analysis is not widely spread in the veterinary field and would be difficult to access by general practitioners. Moreover, the measurements of fat content were performed on dogs with unilateral CCL disease which were lame for an average of three months. Therefore, the percentage of fat may not reflect a risk factor for CCL disease, but rather a modification related to decreased activity due to CCL deficiency.
An increased fat mass and percentage of fat has been reported in human suffering from unilateral anterior cruciate ligament disease (Takata et al 2007). Therefore, we focused on developing a model based on conformation characteristics measured on radiographs only, because this technology is available to most general practitioners. Moreover, it was hypothesized that the conformation of the bone should not be modified secondary to CCL disease.

The combination of radiographic conformational parameters that best discriminated limbs predisposed from limbs at low risk to develop CCL disease was the TPA and FAA with increased values observed in predisposed limbs. Model selection can be controversial and even non significant variables can contribute to an improved model fit. However, parsimony is also important to make an interpretable model. The area under an ROC curve is a measure of the accuracy of a diagnostic test with continuous outcomes that is independent of an arbitrary cutoff value (Evans, Horstman and Conzemius 2005). The area under the ROC curve for the combination of TPA and FAA score was interpreted as the probability to correctly discriminate a predisposed limb from a non-predisposed limb.

A greater tibial plateau slope is believed to increase the cranial tibial thrust, thereby increasing the risk of weakening of the CCL secondary to repetitive microtrauma (Arnoczky and Marshall 1981; Hayashi et al 2003; Slocum and Devine 1983). However, the causal relationship between TPA alone and CCL disease remains controversial based on previous publications. According to Morris and Lipowitz, dogs with CCL disease have a significantly greater TPA than those without CCL disease (23.8° versus 18.1 °) (Morris and Lipowitz 2001). However, in another study, no
difference in TPA was observed between Labrador Retrievers with or without CCL disease (Reif and Probst 2003). Similarly, Wilke et al found no difference in TPA between clinically normal Greyhounds (a breed at low risk for CCL disease) and Labrador Retrievers. In fact, the TPA measured in this study was lower in Labrador Retrievers with disease of the CCL than in Labrador Retrievers with intact CCL (Wilke et al 2002). In a more recent study, the median TPA for dogs with unilateral CCL disease (26°) did not significantly differ from that of dogs with bilateral disease (27°) (Cabrera et al 2008). This study suggested that TPA in the range studied (<35 °) did not appear to be a useful predictor of contralateral CCL rupture among dogs with unilateral CCL rupture. Therefore, TPA may be part of a more complex predisposition pattern and is probably not a risk factor alone, which correlates with the findings of our multivariate analysis.

Using the same data set, an increased in overall and distal femoral anteversion angles and decrease of the femoral condyle trochanteric angle were observed in predisposed limbs compared with limbs at low risk for CCL disease. These femoral characteristics were consistent with an internal torsion of the femur distal to the lesser trochanter, which may lead to impingement of the CCL in the intercondylar notch, causing it to weaken over time (Mostafa et al 2009; Aiken, Kass and and Toombs 1995). The impingement resulting of this misalignment of the femoral trochlea may be especially relevant in Labrador Retrievers, a breed reported to have an intercondylar notch naturally narrower than dogs at low risk for CCL disease (Greyhounds) (Comerford et al 2006). Moreover, the CCL controls internal tibial rotation during flexion of the stifle joint (Arnoczky and Marshall 1977; Vasseur 2003). A greater FAA
may also lead dogs to position their tibia in a greater degree of internal rotation further mobilizing the CCL, which may contribute to degenerative changes and fatigue failure. Indeed, progressive mechanical overload has been found to alter the typical crimped structure of the collagen fibrils normally present in intact CCL, with further tensile loading causing disruption of the ligament fascicles leading to increased laxity and progression of osteoarthritis (Hayashi et al 2003).

Therefore, the combination of characteristics (TPA and FAA) identified by the statistical method reported here makes clinical sense. Increased TPA and FAA may modify the stifle joint biomechanics leading to CCL weakness and secondary rupture. Moreover, our results correlated with some previous conformation reports. However, other reports identified an increased DTA/PTA (diaphyseal tibial axis/proximal tibial axis) angle or a less developed tibial tuberosity as risk factors (Mostafa et al 2009;Guerrero et al 2007;Osmond et al 2006;Inauen et al 2009), which highlight the complexity of CCL disease in dogs. Cruciate disease is likely multi-factorial. Furthermore, pathogenesis may differ for different subgroups of cruciate patients, which stress the importance of using this TPA and FAA combination in Labrador Retrievers within the range of our conformation characteristics only (Table 9). The proposed equation should be further validated with a large population in a future study.

In this study, gender distribution did not differ statistically between groups with 62 % female and 38 % male dogs. However, this may reflect a type two error. Moreover, the neutering status was not statistically compared between groups because of the low number of dogs per subgroup. In a future study, the conformation of the tibia and femur should be compared between male and female dogs, and between intact and
castrated animals, in a large group of Labrador Retrievers. A difference in the conformation of the limb has been established in human athletes between male and female, which may contribute to the increased risk of anterior cruciate ligament disease in women (Griffin et al 2006; Conley, Rosenberg and Crowninshield 2007). The relationship between neutering and CCL disease remains controversial in dogs, justifying future investigations to identify potential differences in the conformation of the femur and tibia in neutered animals and female dogs and need for specific logistic score equations (Whitehair, Vasseur and Willits 1993; Slauterbeck et al 2004; Witsberger et al 2008; Duval et al 1999; Lampman, Lund and Lipowitz 2003; Duerr et al 2007).

A multivariate approach of conformation characteristics generated on radiographs was superior to a univariate approach in identifying risk factors for CCL disease in Labrador Retrievers. The combination of radiographic TPA and FAA best discriminated limbs predisposed to CCL disease from limbs at low risk. The proposed equation may help identify dogs predisposed to CCL disease which would benefit from preventive measures such as physical therapy. To establish the validity of this model or the influence of causative factors, large-scale prospective studies are needed to compare the long-term outcome of dogs considered as predisposed or at low risk for CCL deficiency.
4.5. Kinetic and kinematic analysis of the pelvic limbs in Labrador Retrievers predisposed or at a low risk for cranial cruciate ligament disease

4.5.1. Overground trials

The gait mechanics of a population of sound Labrador Retrievers that we assumed were either predisposed or at low risk for CCL disease have been characterized. The net extensor muscle moment of the hock and the energy generated around the hock and stifle joints were increased in limbs predisposed to CCL disease compared to limbs at low risk.

This study highlighted the benefit of using an inverse dynamics method to compute net joint muscle moment and power parameters (Winter 2005). No differences in GRF or kinematics were documented; however, moment and power analysis enabled us to identify differences in gait variables between predisposed and non predisposed dogs. The peak value of net extensor moment at the hock corresponded with the transitions from joint flexion to extension and from power absorption to generation around the hock joint. According to a previous study, the extensor muscles of the hock joint are the main contributors to positive power during the terminal portion of the stance phase (Colborne et al 2005). The observed increased peak value of the net power generation at the hock in CCL predisposed limbs may hence correspond to an increased activity of the extensor muscles of the hock, which consist mainly of the gastrocnemius muscle. The increased net power generation at the stifle during the first half of the stance was concomitant with stifle flexion and a net flexor moment at the stifle. Therefore, the flexor muscles of the stifle (hamstring and gastrocnemius muscles mainly) of limbs predisposed to CCL disease generated more energy than in non predisposed limbs. Alternatively, the activity of
extensor muscles of the stifle (quadriceps muscle mainly) may have been decreased in predisposed limbs.

Our results may provide further evidence of a gastrocnemius predominance over active restraints of the cranial tibial thrust (gastrocnemius-to-hamstring imbalance). A relative predominance of the gastrocnemius over the hamstring muscle group would increase the caudodistal traction of the distal femur over the slope of the tibial plateau, amplifying the load of the cranial tibial thrust on the CCL (Slocum and Devine 1983; Slocum and Slocum 1993). Therefore, increased recruitment of the gastrocnemius muscle observed in predisposed limbs may lead to repetitive microtrauma and weakening of the ligament over time. The mechanism of gastrocnemius dominance has previously been proposed based on comparison of normal versus sound contralateral limbs of Labrador Retrievers with unilateral CCL disease (Mostafa et al 2010). The mass of the gastrocnemius muscle of contralateral predisposed limbs was increased compared with that of unaffected limbs according to densitometric measures (Mostafa et al 2010). Moreover, the ratios of the lean content of gastrocnemius to hamstring muscles and of gastrocnemius to quadriceps muscles were greater in sound contralateral limbs than in normal limbs. Mostafa et al. hypothesized that dominance of the gastrocnemius muscle over other active restraints of the stifle joint may be associated with predisposition to CCL deficiency in Labrador Retrievers (Mostafa et al 2010). In addition, the location of the center of mass of the crus was located more proximally in canine CCL-deficient and sound contralateral limbs compared with normal controls, which may correlate with a relatively greater proportion of muscles (mainly the gastrocnemius muscle) in the proximal portion of the crus (Ragetly et al 2008). However, the design of those previous studies and the
inclusion of CCL-deficient animals precluded differentiation between predisposing factors and compensating behavior.

Future studies should combine electromyography with inverse dynamics analysis in order to further characterize the neuromuscular imbalances affecting the agonist-antagonist relationship between active stabilizers of the hind limb joints and to confirm the functional implications of the observed kinetic differences. If these studies confirm our results, dogs affected by unilateral CCL-deficiency could benefit from a preventive exercise program to decrease the likelihood of subsequent contralateral rupture. Interestingly, the existence of a fast-to-respond reflex arc from mechanoreceptors in the CCL to the hamstring muscle group was demonstrated in cats with intact CCL (Solomonow et al 1987). The electromyographic activity in the hamstring muscle was greatly increased during loading of the CCL. It was hypothesized that the reflex arc allows the antagonist muscle of a joint to act as a torque regulator that is activated on demand during ligament overloading, in order to maintain joint stability. Therefore, specific exercises aiming at strengthening the hamstring muscle group may be beneficial to decrease CCL overloading and subsequent repetitive microtrauma. According to Lauer et al., the activity of the hamstring muscle group was significantly increased when walking on a treadmill at a 5% incline (Lauer et al 2009). This exercise may be part of a comprehensive rehabilitation program for dogs predisposed to CCL disease. The variation of activity of the gastrocnemius muscle in dogs subjected to different type of exercises has not been reported yet according to our knowledge. However, the maximum flexion of the hock was increased and the maximum extension of the hock was decreased during swimming compared to treadmill walking (Marsolais et al 2003). This observation may
correlate with an increased activity of the flexor muscle group of the hock and a decreased gastrocnemius dominance. During sit-to-stand exercises, a similar increased flexion and decreased extension was observed around the hock joint compared to walking; however no statistical analysis was performed (Feeney et al 2007). The effect of a specific rehabilitation exercise program to correct gastrocnemius dominance and strengthened the hamstring muscle group in dogs at risk for CCL disease and its impact in preventing CCL deficiency should be evaluated in a future study.

4.5.2. Treadmill trials

The gait mechanics were also characterized while trotting on a treadmill. The main results of our study indicate that the Labrador Retrievers qualified as predisposed to CCL disease held their stifle at a greater degree of flexion and extended their hock less compared with dogs at low risk for CCL disease. In addition, more energy was generated by muscles acting on the stifle in the early stance phase of predisposed limbs. These results, except for the increased power generation in the early stance around the stifle joint in dogs presumed to be predisposed, are different than the results of the overground trials.

Dogs qualified as predisposed to CCL disease held their stifle at a greater degree of flexion during mid-stance compared with non predisposed animals (130.9° versus 139.3°). Moreover, the muscles acting around the stifle joint generated more energy during stifle flexion in predisposed dogs compare to non predisposed dogs (2.93 W/kg versus 1.64 W/kg). According to previous publications in dogs, the flexion of the stifle joint during stance results from a concentric contraction of the stifle flexor muscles (mainly the
hamstring and gastrocnemius muscles) (Colborne et al 2005; Ragetly et al 2010; Milis, Levine and Taylor 2004). Treadmill trotting stimulated stifle flexor muscles more in dogs qualified as predisposed to CCLD compared to animals at low risk, which resulted in a greater stifle flexion. Similar to our comments made for overground results, a reflex contraction of the hamstring muscle secondary to stimulation of the CCL may be present and more stimulated in predisposed animals compared with dogs qualified at low risk for CCL deficiency (Solomonow et al 1987). Fatigue of the hamstring muscles may lead to CCL overloading and subsequent repetitive microtrauma and failure. Therefore, specific exercises aiming at strengthening the hamstring muscle group, such as walking on an inclined treadmill (Lauer et al 2009), may be beneficial to decrease risk of CCL deficiency.

The greater energy generated by stifle flexor muscles may also be secondary to an increased gastrocnemius activity, another muscle part of the stifle flexor muscles group. This result also confirms previous findings of a suspected increased recruitment of the gastrocnemius muscle in predisposed limbs to CCL disease (Mostafa et al 2010). Alternatively, the activity of extensor muscles of the stifle (quadriceps muscle mainly) may have been decreased in predisposed limbs. Future studies should integrate electromyographic data in order to confirm these findings and allow distinction between antagonist muscles activity.

The hock, stifle and hip joints follow an extension pattern just before take off (end of the stance phase) (DeCamp et al 1993; Ragetly et al 2010). In our study, Labrador Retrievers qualified as predisposed to CCL disease extended their hock less (161.0° versus 179.0°) while trotting on a treadmill compared with dogs at low risk for
CCL disease. This lower extension of the hock joint produced in predisposed limbs may reflect adjustments of the gait and lesser recruitment of the hock extensor muscle (gastrocnemius mainly) (Colborne et al 2005). Alternatively, the activity of flexor muscles of the hock may have been increased in predisposed limbs. Therefore, treadmill trotting may modify the activity of the gastrocnemius muscle in a bimodal way in dogs predisposed to CCL disease: increase its activity during the early stance (while the stifle is flexed) and decrease at push-off during stifle extension.

In the dog, it has been suggested that the joint force produced on the stifle by weight bearing is almost parallel to the patellar ligament and produces a tibiofemoral shear force in a cranially oriented direction during the extension of the stifle, reaches zero at flexion when the patellar ligament is perpendicular to the tibial plateau and shifts to the caudal cruciate ligament with further flexion of the joint (Dennler et al 2006). Increased flexion of the stifle joint may be an adaptation to decrease the cranial tibial thrust by bringing the tibial plateau more parallel to the ground.

4.5.3. Limitations defining subjects predisposed or at low-risk for cranial cruciate ligament disease

One of the limitations inherent to investigations into the pathogenesis of CCL disease consists of defining normal versus predisposed limbs. Numerous factors have been proposed as contributing to the pathogenesis of CCL deficiency. Most of these factors were based on previous studies comparing normal, affected and contralateral predisposed limbs to CCL disease (Griffon 2010). In Labrador Retrievers, 48% of dogs with unilateral CCL disease will develop contralateral deficiency (Buote, Fusco and Radasch 2009). This
high incidence of bilateral and contralateral CCL deficiency provides a rationale for considering the contralateral limbs as predisposed (Duval et al 1999; Doverspike, Vasseur and Harb 1993). However, the effects of lameness in dogs with unilateral CCL deficiency prevent the investigation of muscular conformation or gait characteristics as potential causative factors for bilateral disease. It is hence difficult to define precisely whether a dog is predisposed or not to CCL disease. Defining a normal population is also challenging (Griffon 2010). Comparison between breeds predisposed to CCL disease, i.e. Labrador Retrievers, and breeds at low risk, i.e. Greyhounds, has been used in the past to assess risk factors for CCL disease (Bertram et al 2000; Colborne et al 2005; Comeford 2003). However, this alternative prevents discrimination between interbreed differences and causative factors predisposing to CCL deficiency. To palliate this limitation, older dogs of the same predisposed breed have been included as control because the likelihood to develop CCL disease decreases with time (Reif and Probst 2003; Ragetly et al 2010). However, this alternative introduces age as a variable between the affected and normal population. We developed a predictive equation to assess the risk for CCL disease in Labrador Retrievers (Ragetly et al 2011). The equation was used in this study to classify dogs as predisposed or not predisposed to CCL disease. The reported prevalence of CCL deficiency in Labrador Retrievers is 3.81 to 5.79 % (Whitehair, Vasseur and Willits 1993; Witsberger et al 2008), which is lower than the ratio of predisposed / non predisposed limbs in this study. To establish the validity of this model or the influence of causative factors, large-scale prospective studies are needed to compare the long-term outcome of dogs considered as predisposed or at low risk for CCL deficiency.
Healthy Labrador Retrievers were assigned to either the predisposed or non-predisposed groups based on the equation score combining radiographic TPA and FAA. A significant difference in gait mechanics was observed between the two groups of dogs, which may reflect a difference in gait pattern between predisposed and non-predisposed dogs. However, a higher mean TPA and FAA were measured in predisposed dogs compared to non-predisposed subjects. Therefore, the observed difference in gait mechanics could be secondary to the difference in conformation of the hind limbs in addition to, or instead of, reflecting predisposition to CCL deficiency.

In our study, the intraobserver variability of the TPA measurements was 1.1°, whereas the interdog variability was 2.3°. According to Fettig et al., the intraobserver variability was 1.5°, whereas the interdog variability was 3.2° (Fettig et al 2003). The average coefficient of variation for the measurement of TPA was 0.039, which is similar to values previously published (range 0.043 to 0.057) (Unis et al 2010). Therefore, our variability appears small, which may be due to the use of normal dogs with absence of degenerative joint disease (Fettig et al 2003) and the use of digital images and a computer-based measurement program (Unis et al 2010). No previous studies have investigated the variability of the FAA measurement made on radiographs. Future studies should be conducted to further investigate the amount of intra- and interobserver variability when measuring the FAA similar to what has been done for the TPA measurement. However, based on the predictive equation: \[ CCL \text{ risk score} = -33.49 + 0.37(FAA) + 0.82(TPA) \], the TPA has more influence than FAA due to a greater coefficient compare to the coefficient applied to the FAA (0.82 versus 0.37). Another limitation stems from the use of a non-invasive method. Calculation of
kinematics and kinetics may have been affected by inconsistency in initial placement of the markers and motion artifacts due to relative movement between the surface markers and the underlying bony landmarks (Riemer, Hsiao-Wecksler and Zhang 2008).

4.5.4. **Overground versus treadmill trials**

Although treadmill use offers a convenient and controlled environment for studying canine gait, the equivalence between treadmill and overground locomotion has been the subject of much debate in the human literature (Alton et al 1998; Watt et al 2010; Riley et al 2008; Riley et al 2007). According to a recent study in man, the differences in GRF and kinematic extension and flexion angle values were small between overground and treadmill, typically less than 4 % peak vertical forces and 2 °, which were mostly within the range of repeatability of measured parameters and therefore not considered clinically significant (Riley et al 2007). However, other studies demonstrated that the kinematic and kinetic data differ between the two conditions (Alton et al 1998; Watt et al 2010; Riley et al 2008). The differences between gait parameters was attributed to subjects not being habituated to treadmill locomotion, especially elderly people (Alton et al 1998; Watt et al 2010). Moreover, there may be a greater demand on the subject’s proprioception skills during treadmill trials, which may explain the kinetic and kinematic changes perhaps as a means of avoiding falling off the back of the treadmill and/or keeping up with the belt speed (Alton et al 1998). Brebner et al. found a good concordance of GRF between overground and treadmill trials of sound dogs and dogs with front limb lameness (Brebner, Moens and Runciman 2006). In dogs, the kinematic parameters of treadmill walking and trotting have been
studied in healthy animals and in dogs with orthopedics or neurological disorders (Tashman et al 2004; Marsolais et al 2003; Bockstahler et al 2007a; Holler et al 2010; Gradner et al 2007), but comparison with overground studies are rare and restricted to walk (Gassel et al 2005; Schwartz, Millis and Hicks 2004). After a two-minute walking initiation over the treadmill, consistent elbow and stifl joint kinematics were obtained for treadmill-naïve Greyhounds within only 30 seconds (Owen et al 2004). However, in another study, angular displacements and velocities remained variable in treadmill-naïve Labradors within and between five 2-minute trotting sessions (Clements et al 2005). These observations emphasized the importance of acclimatization, a learning phase during which the subject exhibits subtle changes in gait eventually leading to habituation, characterized by a steady, repeatable gait. In this study, we elected to train the subjects with two 10-minute treadmill sessions, the day before and the day of data collection. According to the handler, a consistent treadmill trotting gait was obtained at the end of the training period. The use of different velocity for overground and treadmill trials prevented us from comparing the two types of trials. Future work should be done to determine the best length of habituation period and to compare overground and treadmill gait mechanics in Labrador Retrievers.

This study is a basis for characterizing a population presumed to be at low risk or predisposed to CCL disease based on their radiographic TPA and FAA. Summarily, for the overground trials, the extensor moment at the hock and energy generation around the hock and stifl joints were increased in predisposed limbs compared to non predisposed limbs. For treadmill trials, trotting Labrador Retrievers qualified as predisposed to CCL disease held their stifl at a greater degree of flexion, extended
their hock less, and generated more energy around the stifle joints while trotting on a treadmill compared with dogs at low risk for CCL deficiency. These outcomes may help develop rehabilitation programs designed to modify the dynamic balance of the hind limb muscles. The effect of a specific rehabilitation exercise program to correct gastrocnemius dominance and strengthened the hamstring muscle group in dogs at risk for CCL disease (for example dogs with unilateral CCL deficiency) and its impact in preventing CCL deficiency should be evaluated in future studies. Moreover, this technique could be applied in future studies to monitor canine patients undergoing rehabilitation programs with regards to the acquisition of gait characteristics approaching those of a population at low risk for CCL disease.
CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

This study addressed our three general goals. First, we further define gait mechanism in Labrador Retrievers with and without CCL-deficiency. Our study reported the mass, location of the center of mass, and mass moment of inertia in hind limb segments of living normal Labrador Retrievers and in Labradors with CCL disease. Computed tomography allowed for non-invasive determination of BSP on living subjects whose gait was concurrently evaluated. Regression equations were developed to estimate the mass, location of the center of mass, and mass moment of inertia per segment in normal, CCL-deficient, and contralateral limbs of Labrador Retrievers. These equations are based on parameters that are fast, technically simple and cost effective to generate. This approach will tremendously facilitate clinical studies of the canine gait mechanics, offering new strategies to investigate the pathogenesis of non-traumatic joint diseases.

Moreover, inverse dynamics analysis provides useful insight in animal locomotion as it allows assessment of muscles moment, power patterns and joint reaction forces around various joints. The results of this study improved our understanding of gait mechanics in Labrador Retrievers. Characterization of kinetics in Labrador Retrievers with a low risk to develop CCL disease provides a gold standard to compare future gait studies. Lameness resulting from CCL disease affected predominantly the vertical and braking forces and the extension during push-off. Kinetic data also identified a greater contribution of the contralateral limbs to propel the dog forward, especially via the recruitment of stifle extensor muscles.
EMG patterns of major hind limb muscles during trotting gait of healthy Labrador Retrievers were characterized and compared with kinetic and kinematic data of the stifle joint. The use of surface EMG highlighted the co-contraction patterns of the muscles around the stifle joint, which were documented during transition periods between flexion and extension of the joint, but also during the flexion observed in the weight bearing phase. Identification of possible differences in EMG activation characteristics between healthy patients and dogs with or predisposed to orthopedic (such as CCL disease) and neurological disease may help understanding the neuromuscular abnormality and gait mechanics of such disorders in the future.

Second we proposed a CCL risk score equation to identify individual dogs that are susceptible to CCL disease. A multivariate approach of conformation characteristics generated on radiographs was superior to a univariate approach in identifying risk factors for CCL disease in Labrador Retrievers. The combination of radiographic TPA and FAA best discriminated limbs predisposed to CCL disease from limbs at low risk. The proposed equation may help identify dogs predisposed to CCL disease which would benefit from preventive measures such as physical therapy. To establish the validity of this model or the influence of causative factors, large-scale prospective studies are needed to compare the long-term outcome of dogs considered as predisposed or at low risk for CCL deficiency.

Third, our study is a basis for characterizing a population presumed to be at low risk or predisposed to CCL disease based on their radiographic TPA and FAA. Summarily, for the overground trials, the extensor moment at the hock and energy generation around the hock and stifle joints were increased in predisposed limbs
compared to non predisposed limbs. For treadmill trials, trotting Labrador Retrievers qualified as predisposed to CCL disease held their stifle at a greater degree of flexion, extended their hock less, and generated more energy around the stifle joints while trotting on a treadmill compared with dogs at low risk for CCL deficiency. These outcomes may help develop rehabilitation programs designed to modify the dynamic balance of the hind limb muscles. The effect of a specific rehabilitation exercise program to correct gastrocnemius dominance and strengthened the hamstring muscle group in dogs at risk for CCL disease (for example dogs with unilateral CCL deficiency) and its impact in preventing CCL deficiency should be evaluated in future studies. Moreover, this technique could be applied in future studies to monitor canine patients undergoing rehabilitation programs with regards to the acquisition of gait characteristics approaching those of a population at low risk for CCL disease.

This study enables further characterization and understanding of the gait of Labrador Retrievers with and without CCL disease. It also provides novel information regarding co-contraction activity of antagonistic muscle groups combined with kinetic and kinematic data of the stifle joint. If validated the proposed equation to identify dogs predisposed to CCL disease will tremendously change the approach to CCL disease. Detection of at-risk individual and preventive measures would be a new leading area of research.
REFERENCES


190


203


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