Cranial Cruciate Ligament Rupture in the Dog: Gait Analysis Utilizing an Electronic Walkway System and an Investigation in the Role of Steroid Hormones on Collagen Homeostasis

by

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Abstract

Canine CCL rupture is one of the leading orthopedic problems in the United States and other parts of the world. It has been suggested that gonadectomy of the male is a risk factor for trauma to the CCL, implying that sex hormones play a role in CCL development and maturation. Understanding how sex hormones affect CCL growth during puberty could provide an informed basis for determining the appropriate age for gonadectomy in young animals. Using prepubertal male rabbits, the present study investigated effects of sex hormones on collagen content of the CCL. The results showed that gonadectomy caused the loss of collagen content, affected steroid hormone receptor (AR and ESR1) protein expression and slightly altered MMP-1, -2 and -9 protein levels in the CCL.

In order to investigate gait abnormalities caused by orthopedic issues such as CCLR, a normative database was needed using a pressure walkway system. Normal Labrador Retrievers (n=56) were walked across the walkway system which recorded temporal-spatial variables, including SrL, SrT, ST, ST%, TPI applied by each limb, and NS. This study established a protocol for the collection of temporal-spatial gait analysis variables by use of a portable walkway system and determined reference values for variables and symmetry ratios.

Subjective clinical gait analysis has been shown to be unreliable indicating that an objective assessment is needed. A pressure walkway system was used to identify measurements that could distinguish between normal gait and lameness associated with surgical stabilization of a CCLR by TPLO. This study indicates that trend lines for unaffected/affected hind limb ratios

for NS and TPI from dogs recovering from TPLO surgery were within 1 SD of normal values at days 80 and 91 for NS and TPI, respectively. The time course of recovery documented provides a baseline for future studies to assess rehabilitation protocols after TPLO surgical repair or to compare alternate treatments of CCL insufficiency.

Since this pressure walkway system is considered a new technology in the veterinary field, a comprehensive guide to its use was also produced. The manual outlines use of the system from set up, to data collection, to interpretation of the data.

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List of Abbreviations

ACL	Anterior cruciate ligament
AR	Androgen receptor
CCL	Cranial cruciate ligament
CCLR	Cranial cruciate ligament rupture
DHT	Dihydrotetosterone
E_2	17-β estradiol
ERK	Extracellular Signal Regulated Kinase
ESR1	Estrogen receptor alpha
F_z	Vertical force; in the z direction
F_x	Craniocaudal force; in the x direction
F_y	Mediolateral force; in the y direction
GRF	Ground reaction forces
LF	Left forelimb
LSS	Lateral suture stabilization
LH	Left hind limb
MPI	Mean pressure index
MMP	Matrix metalloproteinase
NS	Number of sensors activated by each paw strike

OA Osteoarthritis

PVF Peak vertical force

RF Right forelimb

PKC Protein Kinase

P13-K Phosphatidyllnosiol-3-Kinase

RH Right hind limb

RIA Radioimmunoassay

SD Standard Deviation

SrL Stride length

SrT Stride time

ST Stance time

ST% Stance time percentage

TPI Total pressure index

T Testosterone

TEM Transmission Electron Microscopy

TPA Tibial plateau angle

TPLO Tibial plateau leveling osteotomy

3D Three dimensional

2D Two dimensional

VI Vertical impulse force

Chapter 1. Introduction

Goals and objectives

The main goal of these studies was to research cranial cruciate ligament ruptures, one of the leading orthopedic issues in dogs. This goal was accomplished through research and manuscripts on multiple related objectives.

1. Explore the possible role of gonadectomy /removal of sex hormones as a risk factor for predisposition of CCLR.
2. Establish protocols for collection of gait data using a pressure walkway system.
3. Determine reference values for variables and symmetry ratios.
4. Analyze the changes in gait using a pressure sensitive electronic walkway system after TPLO surgery.
5. Produce a manual for using GAITFour, a pressure walkway system.

From these objectives, three manuscripts have either been published or have been submitted to scientific journals. Along with these scientific publications, a manual for using the GAITFour software with the GAITRite walkway system was also written for distribution to clinics and universities that acquire this system.

Canine cranial cruciate ligament rupture: risk factors

Rupture of the cranial cruciate ligament (CCL) is the leading orthopedic disorder of the canine stifle.[1,2] Of the dogs admitted to university hospitals for lameness, 20% of them are diagnosed with cranial cruciate ligament rupture (CCLR).[3-5] The medical and surgical economic impact in the United States in 2003 was estimated at >$1.3 billion.[5] CCLR is believed to occur following degenerative changes within the ligament or conformational abnormalities; only a small percentage of CCLR being attributed to traumatic events. [3,6-8] Studies have shown a positive correlation of CCLR with breed, neuter status, and abnormal conformation. Additional studies have found an increased prevalence with increased weight.[6,9,10] Breed size may influence age of rupture due to faster degeneration of the CCL of larger breed dogs[5,6] which could be attributed to excessive gravitational forces on the dog and ultimately on the ligament. Indeed, the degenerative changes of the ligament found in 5-7 year old large breed dogs are comparable to those observed in 7- 12 year old small breed dogs.[3,6] In dogs <2 years old, breed size, gonadectomized males and females, and increased body weight were the highest risk factors for CCLR.[10]

There is evidence that a decrease in sex hormones would alter ligament remodeling and ultimately affect the predisposition of the anterior cruciate ligament (ACL) to injury.[11] For example, elevated levels of estrogen decrease collagen production of the human ACL in tissue culture.[12] Additionally, expression of an ACL remodeling gene on average is increased in

human females. Therefore, gender differences in ligament remodeling presumably would affect the size, shape or internal structure of the ACL, thereby affecting its ability to sustain loads without rupturing. In the dog, the effect of decreased sex hormones on the CCL of a dog that has been spayed or neutered is not known. However, alterations in sex hormones may affect the size, shape, or material properties of the CCL. CCL remodeling is affected by loads placed on the ligament (Wolff's Law) and, as with many soft tissues, is influenced by sex hormones.[13-16]

A review of more than 10,000 dogs with ACL injuries from 23 vet school clinics reported that neutered dogs had a higher prevalence of CCL injuries than sexually intact dogs.[6] It has also been reported that there was an increased prevalence of injuries in sexually intact female dogs compared to intact male dogs.[6,11] Overall, gonadectomy increased the prevalence of CCL injuries across sizes and breeds of dogs.[11,13]

Currently, most veterinarians in the United States recommend gonadectomy in dogs and cats at 6-9 months of age. However, there does not appear to be any scientific evidence to document that this is the optimal age. Removal of the gonads prior to puberty could have some unknown and undesirable effects due to their role in physical development. During developmental growth, the lack of steroid hormones due to gonadectomy is thought to cause numerous complications that could otherwise be avoided. For example, timing of closure of the physes on the ends of long bones is controlled in part by gonadal hormones. In both cats and dogs, gonadectomy at any age prior to physeal closure has been associated with statistically significant lengthening of the long bone.[17-19] Though no studies have shown this alteration as a cause for lameness[5,10] it is worth considering along with other implications of pubertal or pre-pubertal gonadectomy on the stifle. Through research, it has become apparent that steroid hormones influence more than just the sex organs and sexual behavior.

Canine incidence of CCL ruptures

Canine CCLR is rarely caused by a traumatic event and is believed to be the result of degenerative changes within the ligament or conformational abnormalities.[3,6-8] CCLR has been positively correlated with breed, neuter status, and abnormal conformation.[6,9,10] In order to understand the path to CCLR, studies must be done to isolate contributing factors. A canine model, unlike a human model which is confounded by extrinsic factors, can focus on specific intrinsic factors such as anatomy, genetics, and hormonal status.

Anatomy-the effect of tibial plateau angle and micro trauma

The cranial tibial thrust, a biomechanical force generated by contraction of the gastrocnemius muscle, is a proposed underlying cause for the repetitive micro trauma to the CCL.[24] The magnitude of the cranial tibial thrust depends on the amplitude of the compressive force (70% of body weight at trot) and the slope of the tibial plateau with respect to the axis that joins the centers of motion of the stifle tarsal joints.[25]

The tibial plateau angle (TPA) or the steepness of the tibial plateau has an apparent role in the pathophysiology of CCL deficiency. However, steepness of the tibial plateau as the sole dependent variable is not known to predict the risk of CCL deficiency development in dogs, especially Labrador Retrievers.[26,27]

The role of genetics-breed

Specific breeds of dogs are known to have an increased incidence of CCLR, whereas other breeds appear to be protected against development of CCLR. For example, dog breeds reported to be at increased risk of CCLR include the Akita, American Staffordshire Terrier, Chesapeake Bay Retriever, German Shepherd, Golden Retriever, Labrador Retriever, Mastiff,

Neapolitan Mastiff, Newfoundland, Poodle, Rottweiler, and Saint Bernard.[10,28] This suggests a genetic basis for CCLR in the canine. Indeed, results of a recent study indicated that Newfoundlands likely have an autosomal recessive mode of inheritance[5] and 4 MSATs (microsatellite markers, the most common type of marker for genetic mapping) were found to be significantly associated with CCLR.[29] Larger breed dogs, > 22 kg, also tended to rupture their CCL at a younger age[3,6,9,10,30] which could be due in part to genetic predisposition and regulation of collagenolytic genes.

The effect of hormonal status-gender

A few retrospective studies attempted to isolate contributing factors of canine CCL rupture and discovered a trend in their data. Duval et al. compared over 200 dogs with ruptured CCL to over 800 control dogs <2yrs old,[10] Slauterbeck et al. reviewed over 3000 dogs within a 2 yr. period,[11] and Whitehair et al. gathered data from over 10,000 dogs with ACL injuries from 23 veterinary school clinics.[6] The individual and cumulative results indicate that neutered dogs, both male and female, have an increased risk of CCLR compared to sexually intact dogs.[6,10,11] Whitehair et al. also reported an increased prevalence on injuries in sexually intact female dogs compared to male dogs.[6] These results suggest that hormones, or lack there of, have a potential effect on the prevalence of CCLR in the canine.

Influence of hormones on ACL/CCL tissue

The principal function of steroid hormones is to develop sexual characteristics and behavior but they also have influence on other systems. In general, the effects of testosterone, the steroid hormone produced in the testes of the male, results from the increased rate of protein formation in the target cell called the anabolic effect. In addition to testosterone, small amounts of estrogen are formed in the male, (~1/5 found in a nonpregnant female) likely converted from

testosterone in various tissues throughout the body. Estrogen, the steroid hormone predominately produced in the ovaries of the female, mainly promotes proliferation and growth of specific cells in the body that are responsible for development of secondary sexual characteristics. Pre-pubertal serum blood levels remain low and are nearly equal in both sexes. As puberty approaches, a gradual buildup of hormone levels begins. In males, serum testosterone rises to a slightly higher level than the estrogens, and, in females, the estrogens rise much higher than testosterone levels.

The reason for marked sexual dimorphism and age distribution for both ACL and CCL rupture is unclear. While several hypotheses have been proposed, some investigators have focused on the potential role of female reproductive hormones in the etiology of this disease.

Estrogen

Cell culture and biomechanical studies have evaluated the effect of estrogen on the ACL in several models albeit with conflicting results. Slauterbeck et al. demonstrated a lower load to failure of the CCL in ovariectomized rabbits after 30 days of estradiol treatment (concentrations consistent with pregnancy levels) compared to controls and reported that estrogen may alter ligament strength.[31] Using cell culture methods, Seneviratne examined sheep CCL fibroblasts that were treated with different physiologic doses of estradiol and found no differences in fibroblast proliferation and collagen synthesis. [32] In another study, the authors used 40 age matched rabbits divided into four groups according to estradiol dosage (control, low, medium, and high) and investigated mechanical properties of the CCL.[33] The authors reported that high serum estrogen levels may be one of the pathogenic factors involved in CCL rupture.[33] There is evidence, according to Kapila et al., that estrogen modulates degradation of the extracellular matrix by regulating the expression of various matrix metalloproteinases (MMP).[34] It has been

reported that transcriptional upregulation of MMPs activates multiple pathways, including ERK (Extracellular Signal Regulated Kinase), p38 kinase, PKC (Protein Kinase), and P13-K (Phosphatidyllnosiol-3-Kinase) pathways.[35] Together, these observations suggest that estrogen has a role in collagen degradation.

Although androgen is the predominant sex steroid in males and estrogen is the female hormone, males and females synthesize both sex steroids and express androgen receptor (AR) and estrogen receptor (ER) protein along with aromatase. Aromatase is the enzyme that catalyzes conversion of androgen into estrogen in several tissues. To date, most studies in the literature have investigated the role of estrogen in the female and it is not clear that steroid hormone action in the CCL is due exclusively to androgen or estrogen action in the male. Therefore, experiments were designed to distinguish between the effects of androgen (testosterone, T) versus estrogen (17β-estradiol, E2) on collagen metabolism in the male.

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History of gait analysis technology

The earliest observations of gait date back to Aristotle (384-322 BCE) who reasoned that "if a man were to walk on the ground alongside a wall with a reed dipped in ink attached to his head the line traced by the reed would not be straight but zig-zaged, because it goes lower when he bends and higher when he stands upright and raises himself."[1] Such an early example of gait analysis is a component of a rich history of scientists evaluating the way animals walk.

The mathematical form of modern gait analysis emerged in Europe during the Renaissance. Girolamo Cardan (1501-1576), an Italian mathematician and medical professor, was the first European to use complex numbers to study probability and the properties of three-dimensional angles. In 1545, he published his greatest mathematical work *Ars Magna* that delineates the methods for solving cubic and quadratic equations.[2] Also contributing to this growing field of applied mathematics was Giovanni Alfonso Borelli (1608-1679), who is mainly credited with the first experiment in gait analysis and considered the father of biomechanics.[3] His most notable works, *De motu animalium (On Animal movement I)* and *On Animal movement II,* used mathematics to support his theories on the relationship between animals and machines.[3] Borelli also recognized that forward motion entailed movement of a body's center of gravity forward, which was then followed by the swinging of its limbs in order to maintain balance.[3] Though these concepts were correct, there were basic mistakes in his ideas of the physical laws concerning forces. In 1688, Sir Isaac Newton (1642-1727) properly formulated the laws of physics in *Philosophia Naturalis Pricipia Mathematica,* which focused on the heavenly bodies, not the human body.[4] While working in Netherlands, Hermann Boerhaave (1668-1738) applied Newtonian mechanics to human movement.[5] Although there was preliminary exploration of the field during the 17th century, the understanding of gait biomechanics mainly progressed in mid-

nineteenth century by major contributors the Weber brothers, Willhelm Eduard (1804-1891) and Eduard Fredrick Willhelm (1806-1871) who published *Mechanics of the Human Walking Apparatus*.[6] The elegant experiments conducted by the Weber brothers only required a stop watch, measuring tape, and a telescope. The use of such few and simple tools allowed the Weber brothers to conclude that step length and cadence change with walking speed.[6]

As one of the first modern gait analysts, French physiologist Jules Etienne Marey (1830-1904) and American photographer Eadweard Muybridge (1830-1904) advanced the understanding of human and animal locomotion. Convinced the human body was subject to the same laws as the rest of nature, Marey believed in the plausibility of appropriate measurements deducing the relationship between body and movements.[7] He collaborated with his student Gaston Carlet (1849-1892) who developed a pressure transducer built into the sole of a man's shoe to record forces exerted on the floor.[7] In an effort to understand locomotion, he expanded gait analysis to non-human subjects. Marey adapted Cartlet's device to detect pressure in the cannon bone of the horse. Marey and colleagues used this piece of equipment to conduct several experiments, many of which concluded that during gaits faster than a walk (i.e. trot, canter, gallop) there was a suspension period, or a short period of time when none of the horse's hooves were on the ground.[7] Based on this revelation, Muybridge engineered multiple cameras triggered in succession to capture the entire gait of the horse.[8]

Pioneering in his experimental methods, Marey furthered Muybridge's work by inventing chronophotography.[7] This revolutionary concept used the shutter of a single camera configured to open and close at fixed time intervals, allowing the plate on the camera to record successive positions of the body during movement. In collaboration with George Demeny (1850-1918), Marey experimented with different types of markers to identify body parts.[7] In addition, Marey

implemented the concept of the single frame multiple exposure camera as an alternative for body and limb measurement of locomotion. Another of Marey and Demeny's inventions used Cartlet's technology to develop a pneumatic force plate which measured the vertical component of ground reaction forces.[7] Although this device introduced excessive time lags in recording, it was useful in relating events during the gait cycle.

The first investigator to conduct three dimensional analysis of gait was a German mathematician, Otto Fischer (1861-1917). In collaboration with Willhelm Braune (1831-1892), an anatomy professor that worked in inertial parameters of the human body, Fischer used continuous exposures of a subject walking in the dark with Geissler tubes strapped to their body. With this device, points were measured on the images from each of the cameras on the respective side of the subject, and a full 3D reconstruction of the true position of the point was calculated. In addition, Fischer calculated the trajectory of the center of mass for each body segment and the entire body. Using a full inverse dynamics approach, he was also measured the joint moments for the lower limbs during the swing phase of gait.

Whereas Bruane and Fischer's work on kinematics became dogma for several decades, the development of the force plate dominated gait analysis techniques. Jules Amar (1879-1935), a rehabilitation specialist working in France during and after World War I, developed the first force plate that incorporated three components of ground reaction forces. The mechanism of the device involved compressing rubber bulbs and, similar to Demeny's approach, included pneumatic transmission of these compressions. Amar's biomechanical system was further developed in the United States in the 1930s. Wallace Fenn, a Rochester engineer, produced a purely mechanical force plate to measure the horizontal component, while Herbert Elftman made a full, three component mechanical force plate at Columbia in 1938. Elftman is credited with

developing the practice of measuring ground reaction forces, the pressure distribution under the foot, and the theoretical analysis of the force, moment and energy changes in the leg during walking.[9-11] The next development in force plate technology was in the late 1940s by engineers Cunningham and Brown at the University California.[8] This updated version of the force plate used strain gauges, which proved to be very successful, thus leading to it commercial availability in the early 1970s. Since then, there has been little development in the basic principles of operation of this device.

Current gait analysis technology

One of the past challenges to clinical gait analysis is the hundreds of hours required for analysis and the limited options for output to acquire clinically meaningful data.[12] In the last 25 years, technological advances in computer assisted gait analysis have aided our ability to quantitatively and expeditiously define temporospatial gait characteristics and therefore gaining a better understanding of canine locomotion. With the ongoing advancement of computer technology, biomechanists have been able to develop systems that integrate methodologies using kinematic (motion) analysis and kinetic (forces) analysis, simultaneously in the same system.

Subjective evaluation of canine gait has been used for many years. However, our ability to perceive minute details during the gait cycle can be very difficult, and in some respects impossible, even for the most talented gait specialist. During subjective evaluation, a clinician can only perceive a few kinematic variables at a time and cannot determine the forces involved in gait. But a modern kinematic or kinetic analysis system can capture, analyze and store hundreds of observations per second. Therefore, in order to fully understand gait, these tools must be utilized.

Kinematics

Kinematics is the study of relative motion that exists between rigid bodies.[13] This is usually accomplished through a three dimensional (3D) or two dimensional (2D) video system that incorporates multiple cameras. 3D kinematic analyses give the most accurate and comprehensive information but the systems can be expensive. Kinematic 2D analyses are less expensive but are limited with their inability to record out of plane movements. To identify specific anatomical points on the dog, markers are placed on anatomic landmarks associated with the location of the joint center. There are two types of markers: (1) a marker that produces delineation in color that is recognized by the system and (2) a marker that reflects light back to the image source in order to be tracked by the system. Conventional kinematic analysis systems track the markers through different planes of space over time to quantify position and reveal their respective x, y and z positional values. The system can then use these values in mathematical equations to calculate linear and angular velocity, displacement and acceleration of segments and joints in space. These data provide information regarding the structure of musculoskeletal system and lameness, and can be used for evaluation of surgical and medical treatment protocols. It is an excellent tool to quantify motion, but its accuracy is dependent upon proper methodological procedures.

Kinetics

The kinetic approach to gait evaluation assesses the forces generated during and resulting from the gait cycle.[13] This analysis quantifies these forces by isolating and measuring kinetic variables such as peak vertical force (PVF), horizontal force, vertical impulse (VI), strain within various tissues, rates of loading and pressure distribution. Most systems used are ground-based kinetic systems that measure variables resulting from the stance phase of gait. There are two

main types of these kinetic systems, force plates and pressure mats. Both of which will be discussed further in the following section. Other systems utilize tools such as strain gauges measure the strain of different tissues, while accelerometers measure body accelerations to calculate temporal and dynamic stride variables. Therefore, there is much versatility depending on the variable required for kinetic evaluation.

Force Plates

As discussed earlier, force plates measure orthogonal forces exerted on the ground during stance phase of the gait cycle. The three orthogonal forces measured are vertical (F_z), craniocaudal (F_x) and mediolateral (F_y). The most popular of these measurements that uses the force plate is the PVF and the VI, which measure some aspect of vertical force. Many researchers have used PVF and VI to study surgically repaired stifles and other functional discrepancies of the stifle.[14-19] Further application of these measurements include assessment of drug therapy efficacy [20-22] and research recovery after experimental injury.[23] Scientists have also utilized multiple force plates to analyze locomotion in normal and lame dogs.[24,25] As indicated, force plates have been used in a variety of veterinary settings and have proven to be a useful tool.

Pressure systems

The latest tool for kinetic analysis is pressure systems. The pressure system normally consists of a multiple pressure sensors in a grid formation that as a foot strikes, maps the contact spatially. Pressure mats come in different sizes. Some are the size of a individual pad on a dog's paw[26] while others can be as long as 26 ft. When compared to force plate systems, the longer pressure mat systems allow multiple gait cycle readings; simultaneous, consecutive and collateral foot strikes to be recorded in a single pass over the walkway. This leads to fewer trials required to generate an adequate amount of data for statistical comparison.[27] When the animal moves

across the mat, graphics display pressure distribution from each paw appear on a computer screen.

Recent comparisons between a force plate and pressure mat, data have indicated that the pressure mat was a viable alternative for measuring VI, thus supporting the usefulness of the pressure mat in a clinical setting[27,28]. Furthermore, one study concluded that although there were significant differences between the pressure mat and the force plate, PVF was consistent over time with the use of the pressure mat.[28] A recent trial using a pressure mat to collect normative data from orthopedically normal Labrador Retrievers concluded that symmetry ratios from the pressure mat may also be useful to detect dog lameness and document progress of recovery.[29] Overall, due to its multiple capabilities, pressure mats combined with laptop computers provide a convenient, portable kinetic data collection system.

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Chapter 2. Steroid hormone regulation of collagen concentrations of the cranial cruciate ligament in the male rabbit model

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Objective—To investigate the effects of gonadectomy on collagen homeostasis using the male rabbit as a model.

Animals—30 prepubertal (16 wks) male New Zealand White rabbits

Procedures—Rabbits were randomly assigned into 5 groups: i) intact animals receiving placebo; ii) castrated animals receiving placebo; iii) castrated animals receiving testosterone (T) supplementation; iv) castrated animals receiving dihydrotestosterone (DHT) supplementation; and, v) castrated animals receiving 17 β-estradiol (E_2) supplementation. Placebo and steroid hormones were administered by slow release pellets whereas intact control animals were sham-operated. After 21 days of hormone supplementation, serum concentrations of T and E_2 were determined by radioimmunoassay (RIA). Collagen concentrations were determined using the Sircol Collagen Assay kit. Expression of steroid hormone receptor and matrix-metalloproteinase (MMP) proteins were analyzed using Western Blotting procedures. The size of collagen fibers was determined in ultraphotomicrographs. Data were analyzed by one-way ANOVA followed by a Tukey Kramer's test for multiple comparisons and differences with $p<0.05$ were considered significant.

Results—Following gonadectomy and hormone supplementation, animals exhibited differential serum T and E_2 concentrations. Collagen concentrations were decreased and fiber diameters were increased in the absence of sex hormones, in association with differential ESR1 and AR receptor protein expression. Although there were differences in expression of MMPs, the differences were not statistically significant.

Conclusions and Clinical Relevance—Sex hormones may play a role in CCL homeostasis in the male. Physiological changes triggered by the lack of sex hormones following prepubertal gonadectomy potentially predispose animals to orthopedic injuries.

Abbreviations

AR	Androgen receptor
CCL	Cranial cruciate ligament
CCLR	Cranial cruciate ligament rupture
DHT	Dihydrotetosterone
E_2	17-β estradiol
ESR1	Estrogen receptor alpha
MMP	Matrix metalloproteinase
RIA	Radioimmunoassay
T	Testosterone
TEM	Transmission Electron Microscopy

Introduction

Rupture of the cranial cruciate ligament (CCLR) is the leading cause of orthopedic disorders of the canine stifle,[1] and twenty percent of dogs admitted to university hospitals for lameness are diagnosed with this condition.[2-4] The economic impact associated with the treatment of CCLR in the United States in 2003 was estimated at greater than $1.3 billion.[4] The pathogenesis of CCLR has been linked to degenerative changes within the ligament as may result from conformational abnormalities and traumatic events.[2,5-7] Other studies have demonstrated a positive correlation between CCLR, body weights and neuter status.[5,8,9]

Earlier reports highlighted the possibility that sex hormones may impact CCL integrity. A review of data from more than 1,000 dogs presenting with cranial cruciate ligament (CCL) injury at 23 veterinary clinics across North America suggested that neutered dogs, both male and female, had a higher prevalence of CCL injuries than sexually intact animals.[5] Furthermore, the prevalence of injuries in sexually intact females was higher than in intact male dogs.[5,10] These reports were supported by observational studies indicating that gonadectomy increased the prevalence of CCL injuries in both sexes and in all sizes and breeds of dogs.[10,11] Altogether, these observations imply that the incidence of CCLR may be related, at least in part, to steroid hormone action.

The mechanism(s) by which sex hormones affect CCL integrity are not clear but previous studies have suggested that CCL remodeling is subject to sex hormone action.[12-14] For example, elevated E_2 levels in female human subjects were found to decrease collagen production in anterior cruciate ligament (ACL) cultures.[15] Also, expression of the matrix metalloproteinase group of enzymes (MMPs), which play a role in collagen metabolism, is regulated by steroid hormones.[16-19] Together, these studies raise the possibility that gender differences affecting

ligament remodeling alter the size, shape and internal structure of the CCL, and hence, its ability to sustain loads.[20] Thus, altered ligament remodeling occasioned by changes in sex hormone levels potentially predisposes the CCL to injury.[20]

Although castration has historically been performed as early as four weeks,[21] veterinarians in the United States now recommend that gonadectomies in dogs and cats be performed between the ages of 6 and 9 months.[22] Incidentally, removal of the gonads prior to the attainment of puberty may result in sexual and developmental anomalies and/or predispose to disease conditions.[23, 24] In particular, absence of the hormones E_2 and relaxin have been associated with increased incidence of heart and lung fibrosis,[25] whereas the interaction between E_2, relaxin and progesterone is thought to contribute to degeneration of specific synovial joints in female subjects.[26] However, there is little or no information on the role of sex steroid hormones in collagen homeostasis in the male. Therefore, the present study was designed to investigate the effects of gonadectomy on collagen homeostasis in the CCL, using the male rabbit model.

Materials and Methods

Experimental Procedure: Thirty 16-week-old male New Zealand White rabbits[a] were obtained and allowed three days to acclimate to new housing conditions. On the fourth day after arrival, animals were anesthetized by intramuscular administration of ketamine[b] (15mg/kg) and dexdormitor[c] (0.5 mg/kg). To establish base lines values for serum sex hormone concentrations, blood was drawn from the marginal ear vein and separated to obtain serum, which was stored at -20 ºC until analyzed. Animals were randomly assigned into 5 groups (n=6 per group) as follows: i) intact animals receiving placebo; ii) castrated animals receiving placebo; iii) castrated animals receiving testosterone (T) supplementation; iv) castrated animals receiving dihydrotestosterone

(DHT) supplementation; and, v) castrated animals receiving 17 β-estradiol (E_2) supplementation. Intact control animals were sham-operated. Placebo and slow-release pellets containing steroid hormones[d] were implanted subcutaneously in the lateral side of the neck with a suture subsequently placed over the incision. Pellets contained 50 mg of T or DHT or 25 mg of E_2 (Fig. 1), and steroid hormone supplementation was for a period of three weeks (21 days). At the end of treatment, animals were anesthetized and blood was collected from the marginal ear vein, followed by euthanasia[e] (150 mg/kg IV). In addition, the right CCL was aseptically harvested, snap frozen in liquid nitrogen and stored at -80ºC until Western blotting analysis (n=6/treatment group). The left CCL was collected and processed for electron microscopy for determination of collagen fiber diameters (n=3/treatment group) or the collagen assay for determination of collagen concentration (n=3/treatment group). All procedures on rabbits were approved by the Institutional Animal Care and Use Committee (IACUC) of Auburn University.

Radioimmunoassays: The serum concentrations of T and E_2 were determined using a tritium-labeled T and E_2 radioimmunoassay[f] (RIA) that utilized T and E_2 antibodies[g] (antitestosterone-6-BSA and antiestradiol-6-BSA, respectively). The T assay has an interassay variation of 7.8% and the lower limit of detection was 0.01ng/ml, whereas the E_2 assay has an interassay variation of 8.5%, and the lower limit of detection was 0.005 ng/ml. Serum T and E_2 levels were calculated as ng/ml.

Collagen Assay: The concentrations of soluble collagen concentrations in the CCL were determined using a collagen assay kit[h] and following manufacturer's instructions. This assay is based on the binding of sirius red, an anionic dye, to the side chains of amino acids present in collagens fibers (1 – IV). Ligament samples (50 mg) were placed in digestion solution containing a protease inhibitor cocktail (10µl/ml) and pepsin (1:10) in 0.5 M acetic acid at pH

3.0 and incubated overnight on a rocker at room temperature. The following morning, fresh solution was added and incubation was continued for 4 hours. At the end of incubation, 100 µl of the pepsin digest was mixed with 1 ml of the Sircol dye and incubated for 30 minutes at room temperature. The collagen-dye complex was then precipitated by centrifugation at 12,000 rpm for 10 minutes. The supernatants were drained off and discarded and the collagen-dye pellet was dissolved in 1 ml of alkali buffer provided by the manufacturer. Aliquots of the dissolved collagen-dye complex (200 µl) for each sample were transferred to a 96-well plate and measured at a wavelength of 540nm on a plate reader.[i] Collagen concentrations were calculated based on collagen standards provided by the manufacturer. CCL samples were obtained from three animals per treatment group and the assays were performed in triplicate and the results averaged for each treatment group.

Western Blot Procedures: Expression of steroid hormone receptor and MMP protein levels were analyzed in CCL homogenates from 6 animals per group. Briefly, ligament samples (25 mg) were homogenized in lysis buffer[j] supplemented with 10 µl of protease inhibitor.[k] Protein concentrations in CCL samples were determined using the Bradford protein assay.[l] The 10-20 µg protein samples were applied to 10% glycine-SDS-PAGE gels in a Mini-Protean System[l] and transferred by electrophoresis to nitrocellulose membranes[l] (0.45 µm). Subsequently, nitrocellulose membranes were preincubated for 60 min at room temperature in blocking buffer [5% whole milk in Tris-buffered saline containing 0.1% Tween 20 (TBST)]. The nitrocellulose blots were incubated overnight at 4°C with primary antibodies against ESR1,[m] AR,[n] MMP-1,[n] MMP-2,[m] MMP-9,[m] and β-actin[n] at a dilution of 1:1000 or 1:2000 (β-actin). Subsequently, blots were washed three times for 5 minutes each time and incubated in the appropriate horseradish peroxidase-conjugated secondary antibody[n] at a dilution of 1:2000 in TBST for 90 minutes. The

resulting bands were visualized by chemiluminescence.[n] Relative protein amounts in identified immunoblots were estimated by measuring optical density (OD) of the bands on exposed on autoradiography films[o] using OD software[p] and were normalized to β-actin levels as internal control. Each of the 5 groups had 6 CCL samples, one from each animal in the group. A total of 6 Western blots were performed for each desired protein with one sample from each group along with two positive controls.

Transmission Electron Microscopy: The diameter of collagen fibers was determined in ultraphotomicrographs after CCL samples were processed for transmission electron microscopy (TEM) using standard procedures. Briefly, CCL samples were collected and immediately placed in a 4% paraformaldehyde/PBS and 0.5% gluteraldehyde solution. The samples were subsequently immersion-fixed in 2% O_sO_4/PBS overnight at 4°C. Following dehydration in a graded series of ethanol, samples were infiltrated and embedded with an epoxy resin.[q] Polymerization of capsules was performed at 60°C for 48 hrs. Ultrathin sections (~85-90 nm) were collected on copper mesh grids and photomicrographed using a TEM microscope[r] at a magnification of 45,000. Three photomicrographs were obtained per sample and twenty random fiber diameters were measured on each photomicrograph. Thus, a total of 60 and 180 measurements were obtained per sample and treatment group, respectively.

Statistical Analysis: Data are described as mean ± SD. Data were analyzed by one-way ANOVA followed by Tukey-Kramer's test for multiple group comparisons.[s] Differences of $p < 0.05$ were considered significant. Pearson interclass correlation coefficients were determined for the relationship between collagen concentrations and fiber diameters.

Results

Following gonadectomy and hormone supplementation (Fig 1), the animals exhibited differential serum sex steroid hormone concentrations (Fig 2). For example, serum T levels were low in castrates receiving placebo or E_2 pellets and were greater in animals supplemented with T (Fig. 2A; $p < 0.05$). On the other hand, serum E_2 levels were lower in castrated animals plus or minus T and DHT supplementation but were higher than intact control in castrated animals supplemented with E_2 (Fig. 2B; $p < 0.05$).

The results of the collagen assay demonstrated that absence of sex hormones affected collagen concentrations in the CCL (Fig. 3A; $p < 0.05$). For example, collagen concentrations in CCL from the castrated control group were decreased compared to intact control animals and were recovered partially in animals supplemented with DHT or E_2 and much more so in animals that received T supplementation (Fig. 3A; $p < 0.05$). On the other hand, fiber diameters had the smallest size in the intact control group (Fig. 3B; $p < 0.05$) and were larger in the castrated control group. However, fiber diameters were greater in animals that were administered T, DHT or E_2 but were increased in the castrated control group compared to intact control animals (Fig 3B; $p < 0.05$).

The changes in collagen concentrations and fiber diameters were associated with differential steroid hormone receptor (AR and ESR1) protein expression in the CCL. For example, AR protein levels were greater in the castrated control than in hormone supplemented animals (Fig. 4A; $p < 0.05$). Although ESR1 levels in castrated plus placebo animals tended to be greater than in the T supplemented group but the differences were found not to be significant. Similar to AR, ESR1 levels were greater in the castrated control group compared to animals supplemented with DHT and E_2 ($p< 0.01$). Also, the levels of ESR1 in castrated animals plus placebo were greater compared to intact control (Fig. 4B; $p < 0.05$). Interestingly, it appears that

differences in serum sex hormone concentrations affect MMP protein expression although the differences were not statistically significant ($p > 0.05$). However, MMP-1, -2 and -9 protein levels were similar to those in CCL from intact control animals and in castrated animals that were administered T and DHT, whereas these levels were higher in the castrated plus placebo and E_2 groups (Fig. 5). Also, MMP expression levels appeared to be especially inversely related to collagen concentrations in the castrated control group (Figs. 3A, 5) ($r = -0.7273$; $p < 0.05$). Taken together, these results raise the possibility that steroid hormones, i.e., both androgen and estrogen, are involved in the regulation of collagen fiber development in the CCL of male rabbits.

Discussion

Canine CCL rupture is the leading orthopedic problem in the United States and other parts of the world. It has been suggested that gonadectomy or castration of the male is a risk factor for trauma to the CCL,[8,10] implying that sex hormones play a role in CCL development and maturation. Clearly, understanding how sex hormones affect CCL growth during puberty could provide an informed basis for determining the appropriate age for gonadectomy in young animals. Using prepubertal male rabbits, the present study investigated effects of sex hormones on collagen content of the CCL. Even though animals that were supplemented with T or E_2 had higher serum levels of these hormones than intact controls, the levels were within the physiological range for prepubertal male rabbits.[27] The present results showed that gonadectomy caused the loss of collagen content, affected steroid hormone receptor (AR and ESR1) protein expression and slightly altered MMP-1, -2 and -9 protein levels in the CCL. To expand discussion on CCL insufficiency and fiber diameter, collagen concentrations were in opposite trends to collagen fiber diameters. Nevertheless, the observation of decreased collagen

concentration is important because collagen is known to be the main load-bearing component of ligaments. Furthermore, we observed that following gonadectomy, supplementation with T or E_2 alleviated the loss of collagen content, whereas DHT was not as effective in this regard. Thus, aromatization of T to E_2 appears to be an important regulating factor for CCL homeostasis. Although changes in MMP protein expression levels following gonadectomy were subtle, the large number of MMP isoforms that are physiologically active in several tissues warrants further elucidation of the interaction between sex hormones and MMP activity. Altogether, these results suggest that sex steroids are necessary for CCL homeostasis in the male during pubertal development.

The principal function of sex steroids in the male is to support development of sexual characteristics and behavior and to maintain male fertility. Although T is the primary endocrine product of the testis, E_2, the predominant female hormone, is present in small amounts and measures about 1/5 or 20% of levels found in a nonpregnant female. Conversion of T to E_2 is due to action of the aromatase enzyme which is expressed in several body tissues.[28] However, there is general consensus that, in addition to regulation of reproductive activity, sex hormones affect development of the musculoskeletal system.[28] Because T levels gradually increase along with other changes that occur during pubertal development in the male, it is reasonable to expect that the surge in sex steroid levels, which occur at puberty, will impact physical development. Indeed, there is long-standing evidence that T influences collagen remodeling and structure. [29-32] Also, there is clear evidence that estrogens regulate the collagen content of the ACL[15] and affect the load to failure in female subjects.[33] In this regard, several studies have demonstrated that E_2 has the capacity to modulate changes in collagen turnover and MMP expression.[34] Therefore, an interesting observation from the present study was the increase in AR and ESR1 expression in

CCL from castrated animals. Although the basis for increased steroid hormone expression remains unclear, it is possible that increased AR and ESR1 protein levels represent an adaptive response mechanism designed to augment steroid hormone signaling after gonadectomy.[35]

The MMP family of proteolytic enzymes mediate the degradation and turnover of extracellular matrix macromolecules and collagen fibers.[19] Analysis of MMP levels in the presence and absence of sex steroids is important to the objectives of the present study because estrogens and ER agonists are known to affect collagen content[36] and MMP-2 and MMP-9 activity.[37] However, a major limitation was our inability to measure MMP enzyme activity and to analyze a wide spectrum of collagenases. Although there was a general trend of an increased MMP-1, -2, and -9 protein expression after gonadectomy, these changes did not reach statistical significance. The lack of robust changes in MMP protein levels may be due in part to the small sample sizes, which limit our ability to detect associations or is the result of variations in individual animal responses to steroid hormone treatment and action; it is also likely that other MMPs, not investigated in this study, played a greater role in mediating steroid hormone action in the CCL.[30] We have also considered that a longer period of steroid hormone deprivation than used in the present study (3 weeks) might give a different outcome. Nevertheless, the large number of MMP isoforms that are potentially active in several tissues warrants further elucidation of the interaction between sex hormones and MMP activity.

In the United States, it is a common practice for veterinarians to neuter non-breeding canines before 6 months of age. However, several studies evaluating the effects of early-age neutering on skeletal development seem to suggest that the standard practice may be disadvantageous to canines involved in sports, e.g. greyhound racing or agility.[21,38-40] For example, growth plate closure was delayed in dogs neutered at 7 weeks old, compared to dogs

neutered at 7 months.[21] In concert with growth factors, sex hormones promote the closure of the growth plates at puberty,[39] implying that bones in dogs neutered before puberty may continue to grow. Thus, dogs that were neutered at an early age tend to have longer limbs.[39] The abnormal growth in bone length frequently results in modifications in body proportions and distortions of bone to bone relationships. Also, excessive growth increases the lever arm in the lower limb which exerts stress on the CCL. Together, these observations suggest that structural and physiological changes triggered by the lack of sex hormones following gonadectomy predispose them to orthopedic injuries. Other reports indicate that neutered dogs have a higher incidence of CCL rupture,[10] which is potentially detrimental to canine athlete performance. Thus, additional studies are warranted to evaluate the effects of prepubertal neutering on musculoskeletal function, particularly in canine athletes.

In conclusion, results from the present study support the view that sex hormones play an important role in CCL homeostasis in the male as in the female.[41] From a clinical standpoint, elucidating the role of sex hormones in the maintenance of CCL integrity will help the process of managing traumatic CCL injuries. Although the present study focused on the role of sex steroid hormones, signaling pathways mediated by growth factors, e.g., transforming growth factor-β1 and bone morphogenic protein-1, are known to be involved in the regulation of collagen fibril growth.[42] Thus, additional studies will be required to determine the relative roles and/or the interaction of sex hormones and other physiological factors in the regulation of CCL development prior to attainment of puberty and/or cessation of physical development.

a. Myrtle's Rabbitry Inc., Thompsons Station, TN

b. Fort Dodge Animal Health, Charles City, Iowa

c. Orion Corp, Espoo, Finland

d. Innovative Research of America, Sarasota, FL

e. Euthasol, Virbac AH, Inc., Fort Worth, TX

f. T-Net 370, E_2-Net 317, Perkin Elmer Life and Analytical Sciences, Boston, MA

g. Animal Reproduction and Biotechnology Laboratory, Colorado State University, CO

h. Sircol™ Collagen Assay Kit, Accurate Chemicals and Scientific Corporation, Westbury, NY

i. SPECTRAmax Plus 384, Molecular Devices, Sunnyvale, CA

j. Lysis buffer, Prod # 78510, Thermo Scientific, Rockford, IL

k. Protease inhibitor, Prod # 78410, Thermo Scientific, Rockford, IL

l. Bio-Rad Laboratories, Hercules, CA

m. Abcam, Cambridge, MA

n. Santa Cruz Biotechnology, Santa Cruz, CA

o. Denville Scientific, Metuchen, NJ

p. Doc-It LS software, Version 5.5.4, UVP Inc., Upland CA

q. Ducopan Epoxy Resin, EMS, Hatfield PA.

r. Phillips 301, 60 kV, Eindhoven, The Netherlands

s. GraphPad, Inc., Version 4.0, San Diego, CA,

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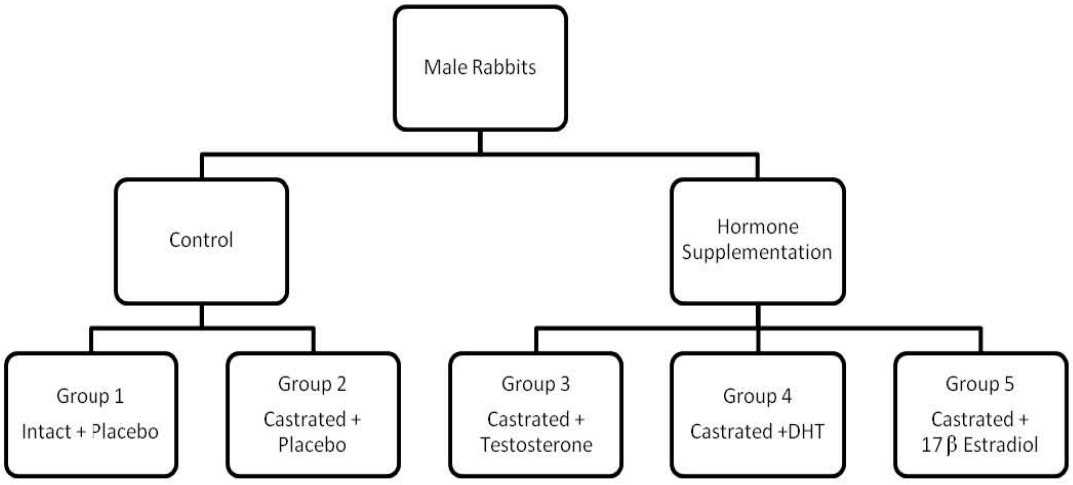


Figure 2-1 Experimental Design

Pubertal New Zealand rabbits were divided into groups (n = 6/group) either as intact controls or were castrated and supplemented with sex steroid hormones testosterone (T), dihydrotestosterone (DHT) or 17β-estradiol (E2). Control intact and castrated animals not supplemented with sex hormones were implanted with placebo pellets.

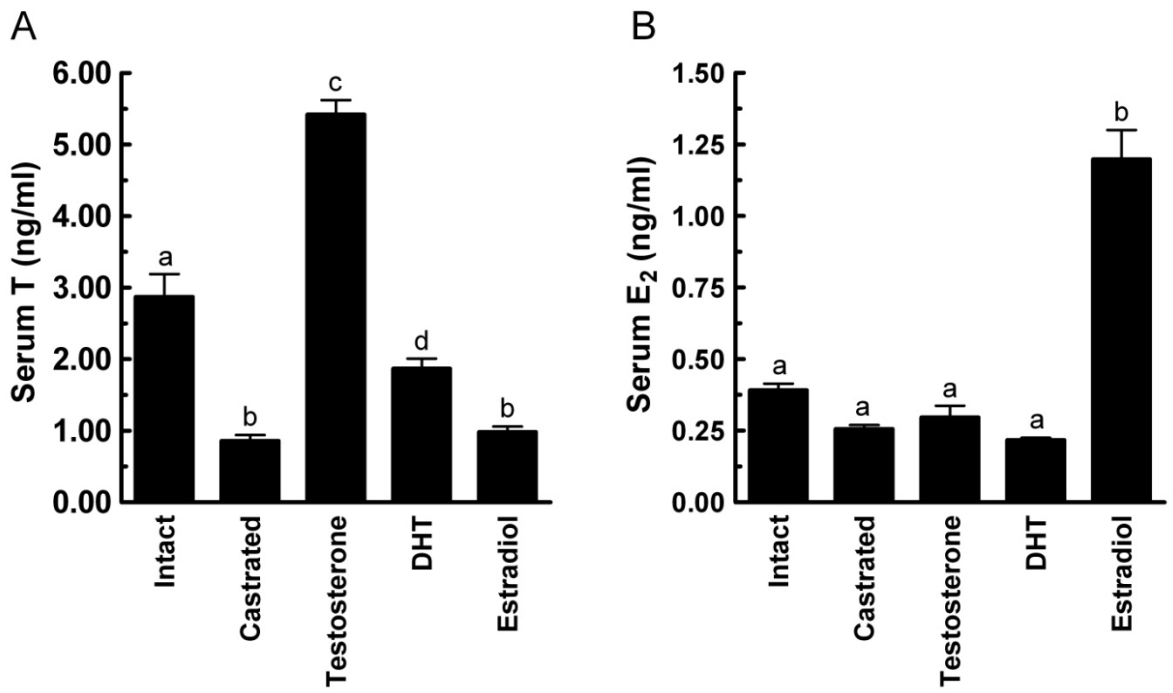


Figure 2-2 Serum concentrations of testosterone (T) and estradiol (E_2) were measured by radioimmunoassay (RIA) for intact and castrated (hormone supplemented and placebo) rabbits (n=6/group). Dissimilar letters indicate significant differences between groups; $p < 0.05$.

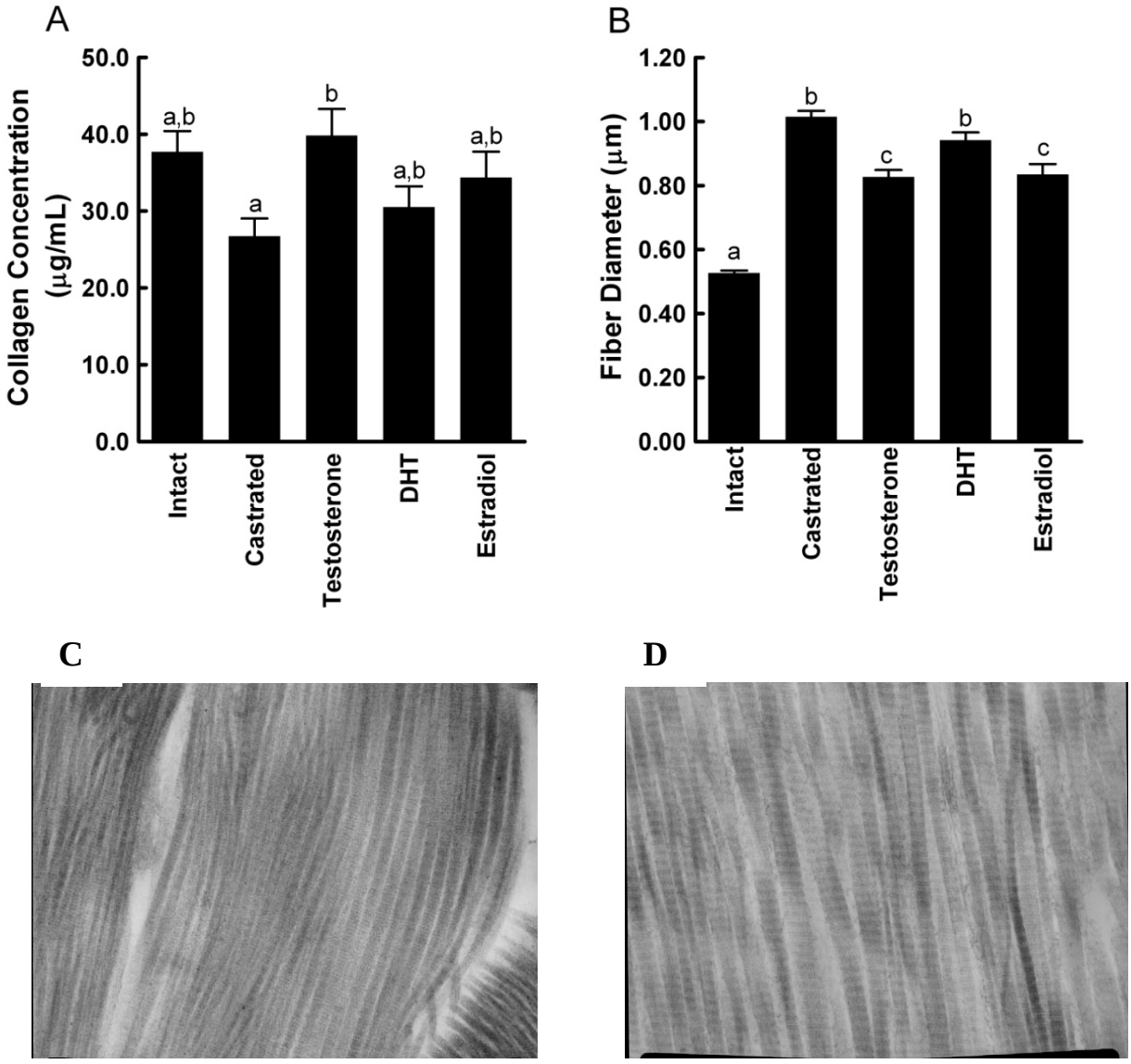


Figure 2-3 Collagen concentration and fiber diameter

Collagen concentrations in the CCL were determined using a Sircol Dye Assay (A). CCL fiber diameters were measured in ultraphotomicrographs after processing for transmission electron microscopy (B). Each of the 5 groups, intact and castrated (hormone supplemented and placebo) consisted of 6 rabbits. Dissimilar letters show significant differences; $p < 0.05$. Ultramicrograph images of cranial cruciate ligament from intact group (C) and testosterone group (D). Images were captured @ 45,000x magnification.

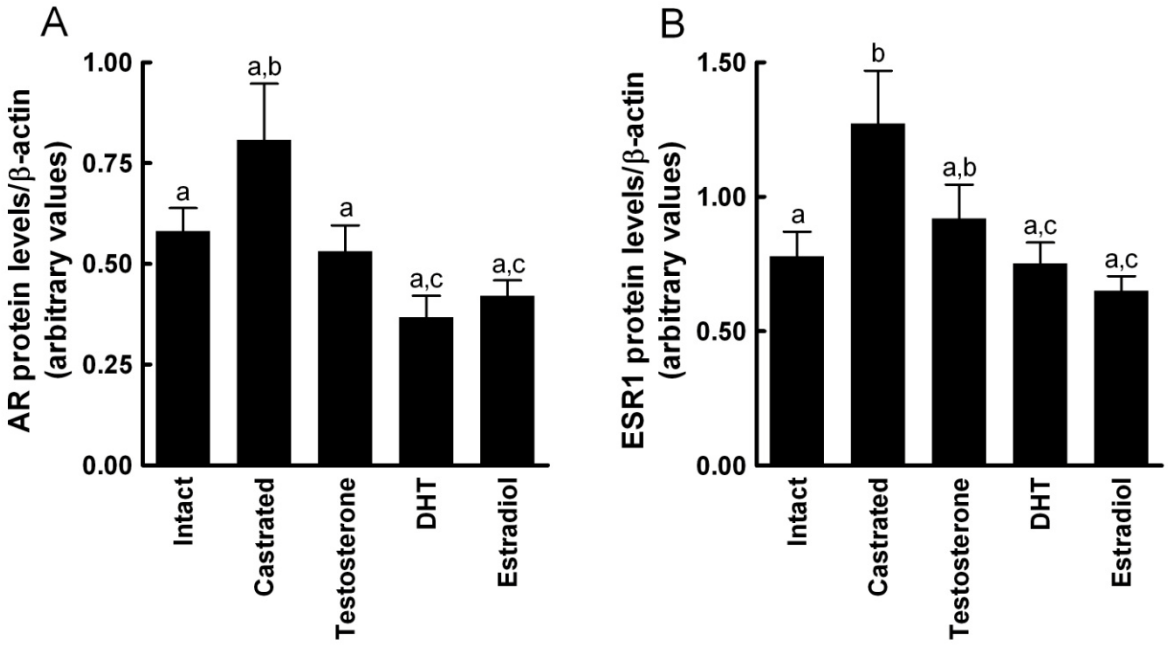
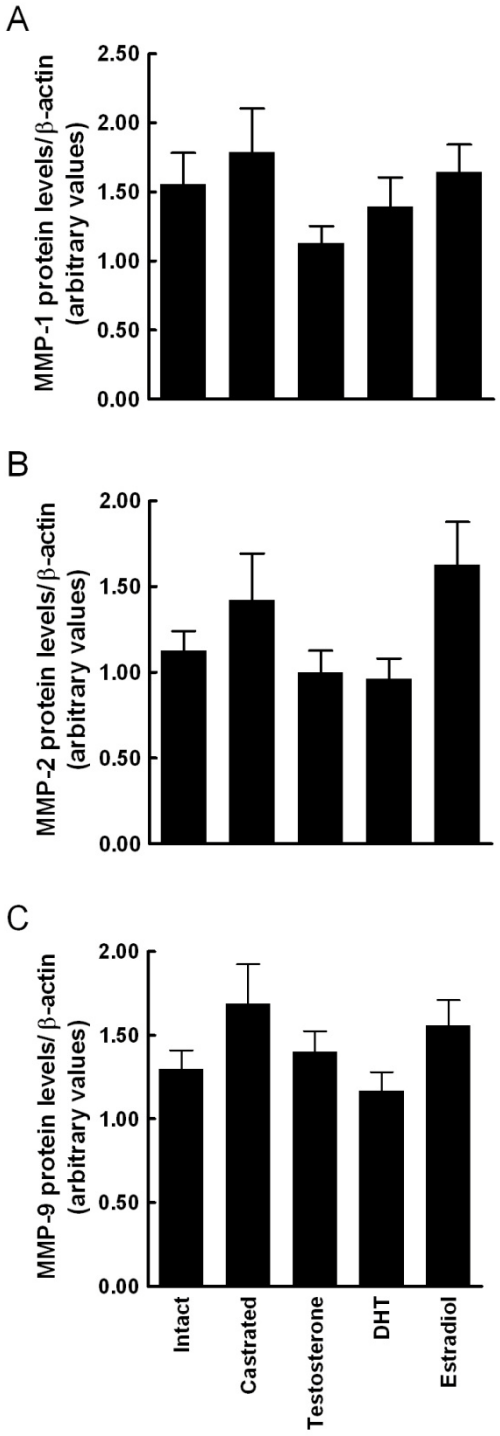


Figure 2-4 Western blot analysis of AR (A) and ESR1 (B) CCL samples from intact and castrated groups (hormone supplemented and placebo) were processed to measure AR(A) and ESR1(B) protein levels by immunoblot analysis. Dissimilar letters show significant differences between groups; $p < 0.05$.

Figure 2-5 West blot analysis of MMPs CCL samples from intact and castrated (hormone supplemented and placebo) groups were processed by Western blotting procedures to determine protein expression levels for MMP-1 (A), MMP-2 (B), and MMP-9 (C). Differences between groups were not statistically significant; $p > 0.05$.



Chapter 3. Temporal-spatial gait analysis by use of a portable walkway system for healthy Labrador Retrievers at a walk

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Objective—To establish a protocol to collect temporal-spatial gait analysis variables using a portable walkway system for Labrador Retrievers at a walk and to determine reference values.

Animals—56 healthy Labrador Retrievers.

Procedures—6 passes across the walkway (3 passes in each direction) were recorded. Inclusion criteria for a pass were a walking gait (60.0- 90.0 cm/s) and minimal head turning. The first 3 passes that met the inclusion criteria were analyzed for each dog.

Results—Mean stride length was 88.4 cm. Mean stance time (ST) of forelimbs and hind limbs was 0.62 and 0.56 sec., respectively. Mean stance time percentage (ST%; proportion of stance time to total gait cycle time) for forelimbs and hind limbs was 55.6% and 50.2%, respectively. Mean total pressure index (TPI) of forelimbs and hind limbs was 27.1 and 17.4, respectively. Mean number of sensors (NS) activated by each paw strike of forelimbs and hind limbs was 17 and 13, respectively. Mean forelimb-to-hind limb symmetry ratios were 1.11 (ST), 1.10 (ST%), 1.62 (TPI), and 1.37 (NS). Symmetry ratios for left limbs to right limbs, left forelimb to right forelimb, and left hind limb to right hind limb were 1.00.

Conclusions and Clinical Relevance—A protocol for collection of temporal-spatial gait variables with a portable walkway system in Labrador Retrievers at a walk was developed, and reference values for variables and symmetry ratios were reported. Further research will determine the extent to which symmetry ratios differ with orthopedic disorders.

Abbreviations

LF	Left forelimb
LH	Left hind limb
MPI	Mean pressure index
NS	Number of sensors activated by each paw strike
RF	Right forelimb
RH	Right hind limb
SrL	Stride length
SrT	Stride time
ST	Stance time
ST%	Stance time percentage
TPI	Total pressure index

Introduction

Gait analysis systems that provide quantitative assessment of lameness beyond subjective analysis have improved the ability of clinicians and researchers to diagnosis lameness.[1–4] Two-dimensional and 3-D motion analysis systems provide various measurement options for the detection of subtle gait abnormalities in dogs.[5,6] Advancements in the medical field, including portable walkway systems, have improved the ability to collect data during sequential footfalls.[7–9]

A portable pressure sensitivenwalkway system has been validated in the human medical field[9–11] and for use in Beagles.[a] The walkway system records temporal-spatial variables, which include SrL, SrT, ST, ST%, TPI applied by each limb, and NS.[b] The purpose of the study reported here was to establish a protocol for the collection of temporal-spatial gait analysis variables by use of a portable walkway system in healthy Labrador Retrievers at a walking gait and determine reference values for variables and symmetry ratios.

Material and Methods

Animals—Fifty-six Labrador Retrievers between 1 and 11 years of age (mean, 3.3 years old) and weighing between 17.7 and 35.5 kg (mean, 27.9 kg) were enrolled in the study. There were 8 spayed and 20 sexually intact female dogs and 13 castrated and 15 sexually intact male dogs. Forty-four of 56 dogs were being trained at an institute[c] as detector dogs, and 12 were being trained for use in competitive field trials. No dog had a history of orthopedic disorders. All dogs were examined by a veterinarian and judged orthopedically sound. The study was approved by the Auburn University Institutional Animal Care and Use Committee.

Equipment—The walkway system[d] used in the study was equipped with a 5.5 X 0.85-m portable mat that had 18,432 encapsulated sensors. The active dimensions of the mat were 4.88 X 0.61 m. A 1.25 X 0.85-m section of inactive mat was placed at each end of the walkway system to provide a transition surface when entering and exiting the system. The mat was calibrated by the manufacturer before purchase. Sensors were batch-tested with an air-actuated plunger that applied a force of 0 to 7 kg to the mat. The walkway system interfaced with a computer and software program[e] for processing and storage of raw data recorded from quadruped gait analysis. The software program interpreted a change in pressure on the sensor and recorded it as a switching level. Additionally, the sensors of the mat had 8 equal switching levels. Accuracy of the switching level of the sensors, spatial resolution, and temporal accuracy at the sampling rate of 180 Hz were ± 0.15 switching level, ± 1.27 cm, and ± 5.55 ms/sample, respectively. Two cameras[f,g] were positioned at a height of 50 cm at opposite ends of the walkway system to simultaneously record movement in both directions. Digital video files of each pass across the walkway system were automatically linked to the data files for footfall verification.

Experimental design—The active and inactive mats were placed on a flat concrete or tile floor. Dogs were walked on the mat until they appeared relaxed and acclimated (approx 4 to 6 passes/dog) to the walkway system and their surroundings. Dogs were walked across the portable walkway system by the same handler. The handler attempted to maintain a constant velocity on a loose leash. A pass was defined as a dog walking the length of the portable walkway system in 1 direction. Each pass consisted of 4 to 5 gait cycles. Two passes were completed across the portable walkway system by walking a dog across the mats, turning the dog beyond the end of the inactive mat, and walking the dog across the mats again. A minimum of 6 passes across the

portable walkway system (3 passes in each direction) were recorded with a total recording time of approximately 3 min/dog. Inclusion criteria for a pass in the data analysis were a relaxed steady walk without the dog pulling on the leash, a velocity between 60.0 and 90.0 cm/s, and no overt turning of the head from midline as observed in the video. The first 3 passes that met the inclusion criteria were analyzed for each dog.

Data processing and analysis—Videos of each pass were reviewed to ensure inclusion criteria were met. The software program[e] was used to distinguish the paw print of each footfall. Paw prints were identified manually as LF, RF, LH, or RH during the first gait cycle; thereafter, the software program automatically replicated the gait pattern on the basis of the manually identified prints. Analysis of each pass by the software program provided a mean velocity, which was calculated by dividing the distance traveled (in centimeters) by ambulation time (in seconds). Additionally, the velocity of individual gait cycles was compared to verify variation within each pass did not exceed > 10%.

Data analysis included mean ± SD values for ST, ST%, SrT, SrL, NS, TPI, and MPI. The ST (ie, stance phase) was the duration of time the paw was in contact with the ground during 1 gait cycle. An ST% (ie, duty factor) was defined as the proportion of stance time to total gait cycle time. The SrT was the amount of time required for a paw to complete a gait cycle, and SrL was defined as the distance between 2 successive strikes of the same paw. An NS was the number of sensors activated by each paw. The TPI was defined as the sum of peak pressure values recorded from each activated sensor by a paw during mat contact, represented by the switching levels and reported as a scaled pressure from 0 to 7 for each sensor. Mean pressure index was defined as the sum of pressure values recorded from each activated sensor during ST divided by NS.

The mean ± SD symmetry ratios of forelimbs to hind limbs, left limbs to right limbs, LF to RF, and LH to RH were calculated for each pass. The software program allowed for a summary of data in a printout after 1 pass or exportation of data for each dog to a spreadsheet[h] for calculation of additional symmetry ratios for each side (ie, LF to LH and RF to RH) and diagonal limbs (ie, LF to RH and RF to LH).

Statistical analysis—A mixed model for a repeated measures ANOVA was used to analyze differences among passes and the mean values of each variable of each dog for limb (ie, forelimb and hind limb [forelimbs to hind limbs, LF to RF, LH to RH, LF to RH, and RF to LH]), side (ie, left and right), and the interaction between limb and side (fixed effects). Comparisons of results were made between individual limbs, left and right sides, and forelimbs and hind limbs for each variable. To measure repeatability, dog and walk were evaluated as random factors in the model. The sum of the covariance parameter estimates and the residual error (as a percentage of the grand means) was used as an indicator of intraobserver repeatability and measurement error for each of the temporal-spatial gait analysis variables and symmetry ratios. A repeatability index was calculated by subtracting the measurement error percentage (error divided by the grand mean) from 100%. Values of $P \leq 0.05$ were considered significant.

Results

Mean ± SD values for temporal-spatial gait analysis variables and symmetry ratios were summarized (Tables 1 and 2). No significant differences were detected among passes for temporal-spatial gait analysis variables. No significant differences were detected among all symmetry ratios for SrT and SrL. No significant differences were detected among symmetry ratios for LF to RF, LH to RH, and left side to right side. Significant differences were detected

among symmetry ratios for forelimbs to hind limbs, LF to LH, RF to RH, LF to RH, and RF to LH when comparisons were made for TPI, MPI, NS, ST, and ST%. Repeatability indices were > 80% for ST%, SrT, and SrL in all limbs. The repeatability index for MPI ratios were between 80% and 89%. Repeatability indices were > 90% for SrT and SrL in all ratios.

Discussion

The use of visual gait analysis alone has been insufficient for gait evaluation in humans.[12] In a study[13] of experimentally induced lameness in dogs, subjective evaluation of gait differed between evaluators and correlated poorly to objective measures of limb function. There are several methods available for obtaining data from objective gait analysis for use by researchers and clinicians. However, each method has limitations. Researchers have used electrogoniometry to measure joint angles in dogs, but the method was cumbersome and failed to provide kinetic data.[14] The use of force plates has become a key advancement in kinetic analysis.[15] Additionally, researchers have evaluated[16] ground reaction forces in healthy dogs with different conformations. Furthermore, that study[16] was followed by studies of abnormal gait in dogs associated with cranial cruciate ligament rupture[6,17–19] or hip dysplasia[20,21] and in response to pain management.[22,23]

Although the use of force plates has become the standard method for measurement of contact time, braking, impulsion, and ground reaction force of each paw independently, several disadvantages have been recognized.[24] For example, the force plate must be located on a level surface and may require the designation of an area dedicated for force plate use and construction of a platform. A single force plate recording supplies data for 1 footfall at a time and does not measure successive footfalls or force distribution from all 4 paws during a single pass;[25] therefore, multiple passes are necessary to collect data for each limb and to obtain proper

positioning of the paw on the force plate.[24] At a walk, dogs have 1 or more paws in contact with the ground at a time,[24] and overlap of paw prints on the force plate causes an inability to distinguish among limbs.[26] This is problematic in smaller breeds because of their typically shorter stride lengths.[24,26] Multiple passes across the plate increases the time required for data collection and also leads to variability associated with repetition.[27]

Investigators found that dogs with undiagnosed cranial cruciate ligament rupture could not be distinguished from clinically normal dogs on the basis of peak vertical force alone through the evaluation of force plate data.[19,28] Thus, a multivariate approach to lameness evaluation was suggested to enhance the accuracy of detection of cranial cruciate ligament rupture.[19,28] In another study,[24] investigators compared ground reaction force values from a force plate and a pressure-sensitive walkway in dogs. Findings of that study[24] indicated the use of a multivariate approach was possible with a pressure walkway system that collected sequential footfalls and multiple variables. This method[24] decreased the number of recordings required and reduced the variability of results.[26,27]

The portable walkway system[d] used in the study reported here has been validated in the human medical field[9,11,29] and has been used to quantify temporal-spatial gait analysis variables[30,31] for the study of humans with gait abnormalities,[7,32] Parkinson disease,[33,34] and Huntington disease.[35] Similar to reports[7,9,11,29-35] from other investigators, the authors of the study reported here determined that this portable walkway system provided a portable and noninvasive method for the collection of data without the need for a dedicated area or construction of a platform for use of the system.

The protocol included in the present study allowed for the collection of data from sequential foot falls at a walk. The walk was evaluated because it is a symmetric gait[36]; the forces generated in dogs with unilateral lameness are strongly correlated with forces at a trotting gait[4,37]; and these are lower braking and impulsion forces during a walk, which might cause discomfort in dogs with severe lameness and result in failure to use the limb during a trotting gait.[4,37] A consistent velocity is necessary to reduce the within-pass variability that could occur in the temporal (ie, ST) or spatial (ie, SrL) gait analysis variables because of a change in walking velocity.[38] Velocity also must be maintained within a consistent range for the comparison of variables among dogs.[38,39] Four gait cycles are required for the calculation of 3 SrLs and SrTs. Error resulting from external influences in the study reported here was minimized by the use of inclusion criteria.

Analysis of results of the present study indicated that symmetry ratios for healthy Labrador Retrievers were 1.0 when comparing LF to RF, LH to RH, and left limbs to right limbs. No significant differences were detected when a comparison was made between the left and right limbs and between forelimbs and hind limbs. Mean symmetry ratio values (ie, ST, ST%, TPI, MPI and NS) were significantly different when a comparison was made between forelimbs and hind limbs; differences in these variable may have been caused by differences in the distribution of weight on the paws of the forelimbs versus the hind limbs during a walk.[16,40] The greatest repeatability index value in the present study was reported for the symmetry ratios. Repeatability indices for all SrT and SrL symmetry ratios were > 90%.

Symmetry ratios for ST and ST% in the present study were 1.11 and 1.10, respectively, when a comparison was made between forelimbs and hind limbs. These symmetry ratios were not similar to the findings of another study[41] in which investigators reported that contact time for

forelimbs and hind limbs in dogs at a walk was the same. Furthermore, these symmetry ratios do not support the findings of another study[42] that revealed STs for forelimbs could be 1.5 times greater than that of the corresponding hind limbs. However the conclusions of both of those studies[41,42] could be accurate when considering the larger inverse correlation of ST with velocity on the forelimbs when a comparison is made with that of the hind limbs.[39] However, the symmetry ratios for ST and ST% in the present study were similar to those in another study[43] in which investigators reported that the difference in duty factor (i.e., ST%) between the forelimbs and hind limbs is less as the body size of the quadruped increases. An ST% of 1.07 was reported in that study[43] for dogs at a walk.

Researchers in another study reported[40] that the mean force on the forelimbs and hind limbs during a walk is 1.1 and 0.8 times that of the weight of the dog, respectively, when the velocity ranges from 91 to 152 cm/s. The calculated forelimb-to-hind limb force ratio of that study[40] was 1.4 and corresponded to a weight distribution ratio of 58 to 42 between the forelimbs and hind limbs, respectively; this was similar to weight distribution ratios reported in another study.[b] In the present study, the forelimb-to-hind limb symmetry ratio for TPI was 1.62, and this corresponded to a weight distribution ratio of 62 to 38 between the forelimbs and hind limbs, respectively, when the velocity ranged from 60 to 90 cm/s. This ratio is similar to the established[40] forelimbs-to-hind limbs symmetry ratio of 60 to 40 whereby 60% of a dog's weight is distributed over the forelimbs when at rest or during at a walk.[40] The difference in reported values for this ratio may be because of differences in velocity, breed of dog, or differences between the measurement of peak vertical force on the force plate versus TPI on the portable walkway system. Symmetry ratios of both the LF to RH and RF and LH were 1.63 and not significantly different from that of the forelimbs-to-hind limbs symmetry ratio of 1.62.

Additionally, the LF-to-LH and RF-to-RH symmetry ratios were 1.66 and 1.60, respectively, but were not significantly different from that of the forelimbs-to-hind limbs symmetry ratio. Further studies are required to determine whether these symmetry ratios can be used to determine the pattern of pressure redistribution for individual limbs during sequential gait cycles.

The forelimbs-to-hind limbs symmetry ratio for NS was 1.37. Therefore, the paws of the forelimbs support more of the dog's weight but also have a greater ground contact area than do the paws of the hind limbs. An explanation for the difference in ground contact area could be that only a portion of the paws of the hind limbs makes contacts with the ground at a walk.

Researchers have used ground reaction forces of nonconsecutive footfalls to obtain symmetry ratios.[27] In that study,[27] small deviations (attributable to variation among passes) in results were not considered abnormal when limb symmetry was used to establish a reference value. Furthermore, results for dogs that were within 2 SDs for measured temporal-spatial gait analysis variables and symmetry ratios were considered to have a normal gait. In the present study, consecutive footfalls were used and thereby reduced the number of passes and variation among successive passes. The SDs of the symmetry ratios for TPI and NS were greater than the SD for the other symmetry ratios. The larger SDs for these measurements could have been related to the body weight or conformation of the dog. A dog with a heavier body weight or larger conformation would be expected to have larger paws, activate more sensors, and exert a greater TPI than would a dog with a lighter body weight. Furthermore, dogs with heavily muscled forelimbs would be expected to exert a greater TPI on the paws of the forelimbs than that of the paws of the hind limbs. Both the body weight and the extent of muscling of the forelimb would contribute to an increase in the SD of the measured values of the variables and

the related symmetry ratios. Further studies are needed to establish whether symmetry ratios for TPI and NS can be applied to other breeds of dog.

In summary, a protocol for the collection of temporal-spatial gait analysis variables using a portable walkway system for healthy Labrador Retrievers at a walk was developed and reference values for variables and symmetry ratios were reported. It is uncertain whether the results determined for these variables or symmetry ratios are similar to those in other dog breeds. However, this protocol can be used to establish databases for other dog breeds. Kinetic data can vary among and within dog breeds.[44] However, a symmetric gait would be expected to yield similar symmetry ratios in healthy dogs regardless of breed. Therefore, these ratios could prove to be a reliable resource, and the portable walkway system could be considered a useful tool for gait analysis. Further research is needed to determine the extent to which symmetry ratios will change in dogs with orthopedic disorders.

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c. Canine and Detection Research Institute, College of Veterinary Medicine, Auburn University, Auburn, Ala.

d. GAITRite, platinum version, CIR Systems Inc, Havertown, Pa.

e. GAITFour software, version 40f, CIR Systems Inc, Havertown, Pa.

f. Logitech mega pixel Web camera, Logitech, Fremont, Calif.

g. Phillips pixel plus Web camera, Philips Electronics North America Corp, New York, NY.

h. Microsoft Office Excel 2003, Microsoft Corp, Redmond, Wash.

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Table 3-1 Mean ± SD values for temporal-spatial gait analysis variables obtained by use of a portable walkway system[d] in 56 healthy Labrador Retrievers at a walk. *

Variable	LF	RF	LH	RH	Repeatability Index (%)†
Stance Time (sec)	0.62 ± 0.08	0.62 ± 0.08	0.56 ± 0.08	0.56 ± 0.08	77
Stance Time Percent (%)	55.7 ± 3.0	55.4 ± 3.2	50.2 ± 4.0	50.3 ± 4.0	89
Stride Time (sec)	1.11 ± 0.13	1.11 ± 0.12	1.10 ± 0.13	1.10 ± 0.13	83
Stride Length (cm)	88.36 ± 7.20	88.39 ± 7.18	88.63 ± 7.56	88.65 ± 7.69	87
Mean Pressure Index	2.11 ± 0.30	2.10 ± 0.32	1.79 ± 0.26	1.80 ± 0.25	77
Total Pressure Index	27.4 ± 4.2	26.8 ± 4.2	16.7 ± 3.3	17.1 ± 3.5	67
Number of Sensors (NS)	13 ± 2	13 ± 2	9 ± 2	10 ± 2	73

*Variables are derived from 3 passes of each dog walking at a velocity between 60 to 90 cm/s across a portable walkway system.[d] †Within a variable, the value is representative of all limbs.

Table 3-2 Mean ± SD values for symmetry ratios obtained by use of a portable walkway system in 56 healthy Labrador Retrievers at a walk.*†

Variable	Forelimbs: hind limbs	Left limbs: right limbs	LF:RF	LH:RH	LF:LH	RF:RH	LF:RH	RF:LH
Stance Time (s)	1.11 ± 0.07‡§	1.00 ± 0.03‖	1.00 ± 0.03‖	1.00 ± 0.05‖	1.11 ± 0.08‡	1.10 ± 0.07‡§	1.11 ± 0.08‡§	1.11 ± 0.07‡§
Stance Time Percent (%)	1.10 ± 0.06‡§	1.00 ± 0.03‖	1.00 ± 0.03‖	1.00 ± 0.04‖	1.10 ± 0.07‡§	1.10 ± 0.06‡§	1.10 ± 0.08‡§	1.10 ± 0.06‡§
Stride Time (s)	1.00 ± 0.02‖	1.00 ± 0.01‖	1.00 ± 0.01‖	1.00 ± 0.01‖	1.00 ± 0.02‖	1.00 ± 0.02‖	1.00 ± 0.02‖	1.00 ± 0.02‖
Stride Length (cm)	1.00 ± 0.01‖	1.00 ± 0.01‖	1.00 ± 0.01‖	1.00 ± 0.01‖	1.00 ± 0.01‖	1.00 ± 0.02‖	1.00 ± 0.02‖	1.00 ± 0.01‖
Mean Pressure Index	1.17 ± 0.09‡§	1.00 ± 0.05§	1.00 ± 0.07§	1.00 ± 0.07§	1.18 ± 0.10‡§	1.17 ± 0.12‡§	1.18 ± 0.11‡§	1.18 ± 0.11‡§
Total Pressure Index	1.62 ± 0.23‡	1.01 ± 0.07§	1.03 ± 0.09§	0.99 ± 0.12	1.66 ± 0.25‡	1.60 ± 0.28‡	1.63 ± 0.27‡	1.63 ± 0.25‡
Number of Sensors (No.)	1.37 ± 0.12‡	1.00 ± 0.05§	1.01 ± 0.06§	0.99 ± 0.08§	1.38 ± 0.16‡	1.36 ± 0.14‡	1.37 ± 0.16‡	1.38 ± 0.14‡

†A symmetry ratio of 1.0 results when the recorded value of the numerator equals the recorded value of the denominator. ‡Within a variable, values for forelimbs are significantly (P <0.001) different, compared with values for hind limbs. §Repeatability index was > 80%. ‖Repeatability index was > 90%. *See* Table 3-1 for remainder of key.

Chapter 4. Gait analysis with an electronic walkway system to evaluate recovery in dogs after tibial plateau leveling osteotomy

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Objective: To document changes from day 39-89 after surgery using a pressure-sensitive electronic walkway system, and determine which parameter(s) distinguish dogs with normal gait from dogs after tibial plateau leveling osteotomy (TPLO).

Study Design: Prospective, clinical study

Animals: 25 multiple breeds of client-owned dogs (n=25) recovering from TPLO for repair of unilateral cranial cruciate ligament (CCL) rupture (mean body wt, 35.6 kg ± 9.7, mean age 5.2 yr ± 2.9) .

Methods: Owners returned one time between day 39-89 after surgery for gait analysis. Dogs were given a visual lameness grade of 0-4, with 0 = clinically sound and 4 = leg carrying lameness. Dogs were walked across the walkway system. The first 3 passes that met the acceptance criteria were analyzed. Mean values for temporal-spatial measurements, total pressure index (TPI) and number of sensors (NS) activated were calculated; ratios of unaffected vs. affected hind limb were graphed, interclass correlations were calculated and "best fit" trend lines determined.

Results: Trend lines for symmetry ratios (unaffected/affected hind limb) for TPI and NS indicated progressive improvement in the operated limb over the time period studied. Statistical analysis indicated that the TPI symmetry ratio was the most reliable measurement to distinguish between normal gait and dogs recovering from TPLO.

Conclusions: The most reliable lameness indicator was TPI symmetry ratio. Trend lines were within normal range (one SD) for NS and TPI symmetry ratios at day 80 and 91, respectively, after TPLO. Trends suggest that lameness continues to improve to at least day 91 post surgery.

Clinical Relevance: The time course of the documented recovery can serve as a baseline for comparison with future surgical and rehabilitation protocols.

Introduction

Subjective gait assessment in orthopedic disorders can be challenging and there is a need for objective gait analysis methodologies that can be implemented in a clinical setting.[1] Studies of subjective lameness assessment have shown intra- and inter-observer variability and poor agreement with objective assessment.[2-4] Gait analysis using a force plate is considered the gold standard for objective assessment of limb function[5-7] based on individual ground reaction forces (GRFs) or a multivariate approach.[4,8,9] Although force plate analysis has been used to differentiate between lameness associated with orthopedic abnormalities (hip dysplasia, cranial cruciate ligament rupture, etc.) and non- lame dogs, use of the force plate has disadvantages.[4] Ground reaction forces were more accurate in assessing lameness in Labradors with cranial cruciate ligament (CCL) rupture than visual observation.[3] Objective measurement of gait can aid in clinical trials, diagnosis of subtle lameness, post-surgical assessment, and assessing response to rehabilitation therapy.

A recent study suggested that a walkway system incorporating pressure sensors (GAITFour, CIR Systems Inc., Havertown, PA) could be utilized for lameness evaluation by recording multiple consecutive gait cycles.[10] The present study used the walkway system to evaluate dogs recovering from tibial plateau leveling osteotomy (TPLO) stabilization of cranial cruciate ligament (CCL) injury. The goals were to analyze the changes in gait using a pressure sensitive electronic walkway system and determine which measurement(s) would most reliably distinguish between normal gait and lameness associated with surgical stabilization of a CCL rupture by TPLO.

Materials and Methods

Animals

Subjects were multiple breeds of client-owned dogs (n=25) diagnosed with unilateral CCL rupture (mean body weight, 35.6 kg ± 9.7). Breeds included Labrador retriever (n=12), Labrador cross (n=4), American Staffordshire Terrier (n=3), Boxer (n=2), Bull Mastiff (n=1), Great Dane (n=1), Akita (n=1) and Doberman Pinscher (n=1). There were 9 spayed females, 2 intact females, 11 castrated males, and 3 intact males. Dogs ranged in age from 1.3-11.7 yr (mean age, 5.2 yr ± 2.9) Dogs with a unilateral CCL rupture that were stabilized by TPLO, and no prior or concurrent neurologic or orthopedic disorders were eligible for inclusion in the study. There were 14 right hind and 11 left hind CCL ruptures. The study was approved by the Institutional Animal Care and Use Committee at Auburn University.

Surgery

Prior to and after surgery, each dog had a complete physical, orthopedic and a neurologic examination to ensure inclusion criteria were met. The CCL rupture was stabilized with a TPLO by the same A.C.V.S. Diplomate surgeon (R.D.M). A tibial plateau angle (TPA) between 0 and 6° based on post-operative radiographs was accepted as adequate. Owners were instructed to enforce strict cage confinement for 4 wk after surgery. During wks 4-6, dogs were allowed progressively longer leash walks with resumption of normal activity at wk 7.

Gait Analysis

Visual and electronic gait evaluations were performed prior to TPLO surgery and when the owners returned with the dogs one time between 39-89 days after surgery. The visual gait analysis was performed using a 5 point grading scale, where 0=clinically sound, 1=subtle lameness, 2= overt lameness, 3=intermittent leg carrying lameness, and 4=constant leg carrying

lameness. All dogs were visually graded prior to surgery and when they returned by the same surgeon who performed the TPLO. The objective analysis was performed with a pressure sensitive electronic walkway system (GAITFour). Dogs were walked on loose leash at least 6 times ("6 passes") on a 5.50 m mat in either direction. The first 3 passes that met the acceptance criteria were analyzed. Criteria included passes with a forward velocity with <10% variation, no overt head turning from midline, a minimum of 4 gait cycles per pass, and velocities for the 3 passes within 10% variation from each other.

The walkway system was equipped with a 5.50 X 0.85 m portable mat with 18,432 encapsulated sensors that were 1.27 cm on center. The active dimensions of the mat were 4.88 X 0.61 m. A 1.25 X 0.85 m section of inactive mat was placed at each end of the walkway system to provide a transition surface. The mat was calibrated by the manufacturer before purchase at which time sensors were batch tested with an air-actuated plunger that applied a force of 0 to 7 kg to each sensor. The software program interpreted a change in pressure on the sensor and recorded it as a switching level. The sensors had 8 equal switching levels. Accuracy of the switching level of the sensors, spatial resolution, and temporality at the sampling rate of 180 Hz were ± 0.15 switching level, ± 1.27 cm, and ± 5.55 ms/sample, respectively. A camera (Logitech mega pixel Web camera, Logitech, Fremont, Calif.; Phillips pixel plus Web camera, Philips Electronics North America Corp, New York, NY.) was positioned at a height of 50 cm at each end of the walkway system to simultaneously record movement in both directions. Digital video files of each pass were automatically linked to the data files for foot fall verification.

Videos of each pass were reviewed to ensure inclusion criteria were met. The software automatically identified each paw print as LF, RF, LH and RH. Analysis of each pass by the software program provided a mean velocity (distance traveled in cm/ambulation time in sec) and

the velocity of individual gait cycles (average stride length of all limbs/ average stride time of all limbs). The gait cycle velocities were compared to verify that variation within each pass did not exceed 10%.

Analysis included mean ± SD for the following temporal-spatial measurements: stance time (ST), stance % of the gait cycle (ST%), stride time (SrT), stride length (SrL). Number of sensors (NS) and total pressure index (TPI) were also recorded. ST (ie, stance time) was the duration of time the paw was in contact with the ground during 1 gait cycle. ST% (ie, duty factor) was defined as the proportion of stance time to total gait cycle time for each paw or SrT. SrT was the amount of time required for a paw to complete a gait cycle, and SrL was defined as the distance between 2 successive strikes of the same paw. An NS was the number of sensors activated by each paw for each foot fall. TPI was defined as the sum of peak pressure values recorded from each activated sensor by a paw during mat contact. Data from each pass were exported to a spreadsheet (Microsoft Office Excel 2003, Microsoft Corp, Redmond, Wash.) where the mean ± SD symmetry ratios for unaffected versus affected hind limb were calculated. The ratios for each measurement were graphed and "best fit" trend lines were determined.

Statistics

Data comparisons between normal Labrador Retrievers (n=56) reported previously[10] and dogs recovering from TPLO (n=25) were analyzed using logistic regression. A forward and backward stepwise regression model was used with p=0.20 as the criterion for entry into the model. This involved starting with no variables in the model, testing at each stage for variables to be included or excluded without appreciably increasing the residual sum of squares (RSS). The procedure terminated when the measure was maximized, or when the available improvement fell below the criterion for entry into the model. Interclass correlation coefficients were determined.

Results

Gait Analysis

Recording sessions were less than 10 min per dog/visit. Prior to surgery, all dogs had a subjective lameness grade between 1 and 3. Gait analysis was preformed prior to surgery on 23 out of 25 dogs and graphs for TPI and NS are presented in figures 1 and 2. At their return visit, all dogs had a subjective lameness of grade 1 or 2. Trend lines for the gait mat data (unaffected/affected ratios) indicated progressive improvement in the operated limb from 39-89 days after surgery for all measurements, with the exception of stride time ratio. Stride time ratio remained within 1 SD for all dogs at all times. Graphs for TPI ratio and NS ratio are presented in figures 3 and 4. Statistical analysis indicated that TPI ratio was the most reliable measurement to distinguish between lame and orthopedically normal dogs. Interclass correlation coefficients (ICC) were found. ICCs greater than 0.70 were found for ST% ratio and ST ratio (r=1.00), ST ratio and NS ratio (n=0.80) and TPI correlated with visual lameness grade (r = 0.88), ST% ratio (r = 0.90), ST ratio (r = 0.91) and NS ratio (r = 0.95).

Discussion

Gait analysis based on force plate measurements has been reported for dogs with ruptured CCL at various time points after surgical repair.[6,11-14] Multiple tibial osteotomy techniques have been described, including tibial plateau leveling osteotomy (TPLO).[15-17] TPLO stabilizes the stifle by decreasing cranial tibial thrust during weight bearing.[16,18] A previous study in dogs undergoing TPLO after experimental transection of the CCL, evaluated dogs at 8 and 18wks and showed return to normal peak vertical forces (PVF) and vertical impulse (VI) at 18 weeks after surgical repair but not at 8 weeks.[11] In dogs treated with fibular head transposition after

transection of the CCL, PVF did not return to normal even at 10 months.[17] A study using modified retinacular imbrication to repair experimentally transected CCL in dogs found PVF and VI values to be significantly decreased at 4, 8, 12 and 16 weeks but not at 20 weeks post operatively compared with values before transection.[15]

While direct comparison cannot be made between force plate gait analysis and temporal-spatial gait analysis, the data in the present study indicated that symmetry ratio trend lines were within the normal range (one standard deviation) of TPI ratios at day 91 (13 wk), earlier than has been previously reported following TPLO. Our findings also vary from a previous report [12] that showed most dogs did not return to normal function measured by a force plate following TPLO or lateral suture stabilization (LSS) at 6 months after surgery. In that study, with an 80% probability of normal limb function as a cutoff, only 14.9% of dogs undergoing LSS and 10.9% of dogs undergoing TPLO were unable to be distinguished from clinically normal dogs at 6 months following surgery.[12] That study[12] solely assessed Labrador Retrievers, whereas our patient population represented multiple breeds. Furthermore, severity of osteoarthritis and meniscal injury was not assessed during either study. It may be that our population had less osteoarthritis and meniscal damage making it more likely that dogs would return to normal function earlier. However, most dogs with rupture of the CCL, even after surgical repair, will undergo some progression of osteoarthritis (OA).[19] In addition, radiographic severity of OA has not been shown to have any predictive value for limb function.[20]

During a TPLO surgery, the tibial plateau angle (TPA) is altered in order to change the direction of forces exerted on the tibia.[16] In the present study, TPA between 0 and 6° was considered acceptable. A previous study has shown that an altered TPA between 0 and 14° has

no significant effect on GRF in Labradors 4-17 months after TPLO.[21] Therefore, the authors felt the acceptable TPA would not affect the outcome of gait analysis for this study.

Dogs in this study were given conventional post-surgical exercise restriction and did not undergo additional physical rehabilitation following surgery. In dogs with a ruptured CCL, post-operative physical rehabilitation shortened the time to full recovery in comparison to exercise-restricted groups after lateral retinacular stabilization technique.[13] The exercise restricted group was limited to twice daily leash walks (0.3 miles) until week 8; during week 8-16, the twice daily leash walks were increased to a distance up to 1 mile.[13] The investigators found that the PVF of the operated limb was significantly less compared to the normal contralateral limb 6 months after surgery in the exercise restricted group.[13] Another study has shown that swimming can increase the range of motion in the stifle and tarsus at d21 and d35 after extra-capsular repair of the CCL compared to slow and fast walking on a treadmill.[14] Further studies with objective gait analysis need to be performed to determine if TPI ratios return to normal before 91 days in dogs undergoing physical rehabilitation following TPLO.

We used a forward and backward stepwise regression model to determine which of the measurement or combination of measurements could indicate lameness. Of the measurements obtained from the walkway system, statistically, the unaffected/affected hind limb ratio for TPI was the most reliable indicator of lameness. This result can be compared to a report of force plate evaluation which utilized asymmetry indices with a multivariate approach to distinguish between control dogs and dogs with spontaneously occurring CCL rupture or hip dysplasia.[22] Although the data for temporal-spatial ratios did not reach significance in the statistical model for the present study, it remains to be determined if sequential recordings of step length between

contralateral fore- or hind- limbs, hind limb reach (measured between contralateral left or right sides) and stride length for affected limbs are relevant in assessing recovery in individual dogs.

The current study was limited by the inability to have dogs return for additional visits for objective gait analysis. One study has reported subjective data as late as 50 months after surgery,[23] but no long term objective data have been reported. In addition, dogs were not all walked at a uniform velocity, although an attempt was made to walk at a relaxed walk. The velocity within and between each of the passes did not exceed 10% variation for any dog. Inclusion criteria for use of force plate data or kinematic video analysis data at a walk (~1.0 m/s) consists of velocity ±0.15-0.21 m/s and acceleration of ±0.5 m/s^2. This equates to approximate velocity variations of 15-20%.[8,9,24] The authors of the present study decided to use 10% variation to increase the power of the collected measurements. Evans et al.[9] showed that velocity does affect GRF, but that the coefficient of variation was smaller during walking than trotting, 20% and 30%, respectively. In the present study, three passes were averaged to minimize any variation from a dog's normal ratios.

Subjective gait analyses between observers can vary[2] and a need for objective criteria is clear. This variation between observers may be due to the low sensitivity of the grading scale. The clinical grading scale used in the current study is subjective between grades 1 and 2, and objective between grades 2-4, but lacks sensitivity. The data obtained from the present study revealed quantitative differences within grades 1 and 2 lameness scores (Fig. 1 and 2). Similar to force plate evaluations, [2,22,25] the quantitative differences found within lameness grades indicate that the walkway system is an objective way to measure gait and could be considered a more incremental method of gait analysis than visual assessment.

Since the walkway system gives an incremental objective measurement, this method may serve to guide further therapeutic recommendations and to assess efficacy of physical rehabilitation. This information will also be beneficial in objectively assessing subtle lameness, indicated by a visual assessment of grade 1 or grade 2, which might be missed with observation, and in initial orthopedic evaluations for a variety of disease processes. Objective data can also be gathered for clinical trials and in research where lameness grade and gait assessment are outcome measures such as comparing the outcome of TPLO and various other treatments for CCL ligament insufficiency.

This study indicates that trend lines for unaffected/affected hind limb ratios for NS and TPI from dogs recovering from TPLO surgery were within 1 SD of normal values at days 80 and 91 for NS and TPI, respectively. The time course of recovery documented in this study provides a baseline for future studies to assess rehabilitation protocols after TPLO surgical repair or to compare alternate treatments of CCL insufficiency.

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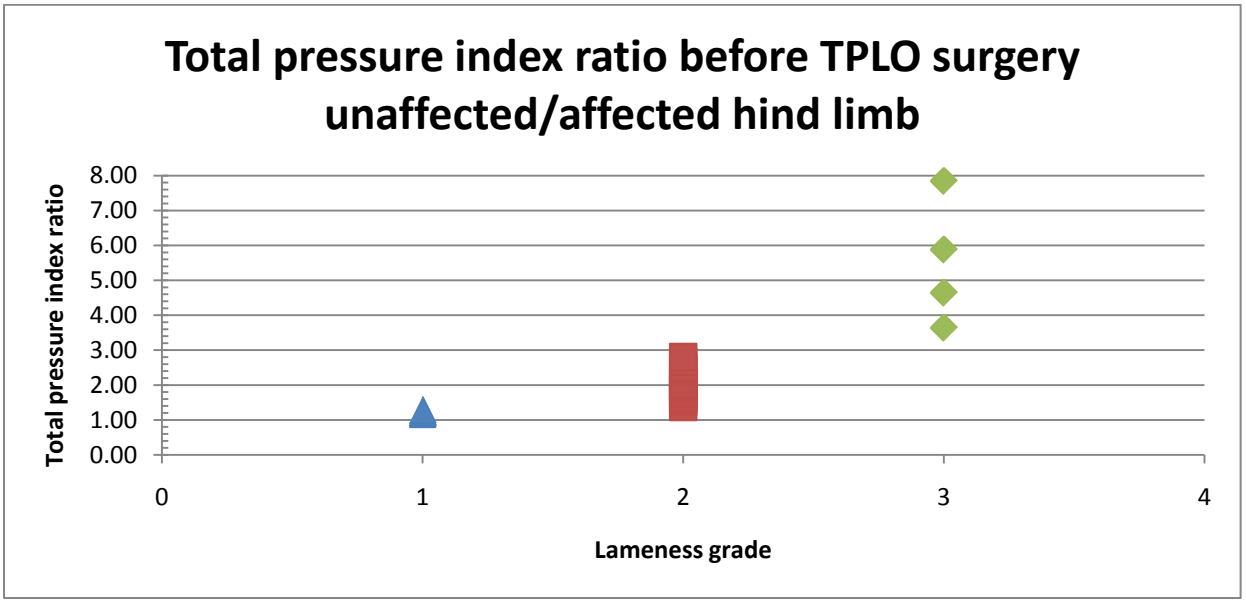


Figure 4-1 Total Pressure Index ratios before TPLO unaffected/affected hind limb

Graph represents individual dogs' (n=25) unaffected/affected hind limb ratios for total pressure index (TPI) where 1.0 is symmetrical. Triangular data points indicate grade 1 lameness, square data points indicate grade 2 lameness and diamond data points indicate grade 3 lameness. Lameness was graded as 0=clinically sound (n=0), 1=subtle lameness (n=4), 2= overt lameness (n=17), 3=intermittent leg carrying lameness (n=4), and 4=constant leg carrying lameness.

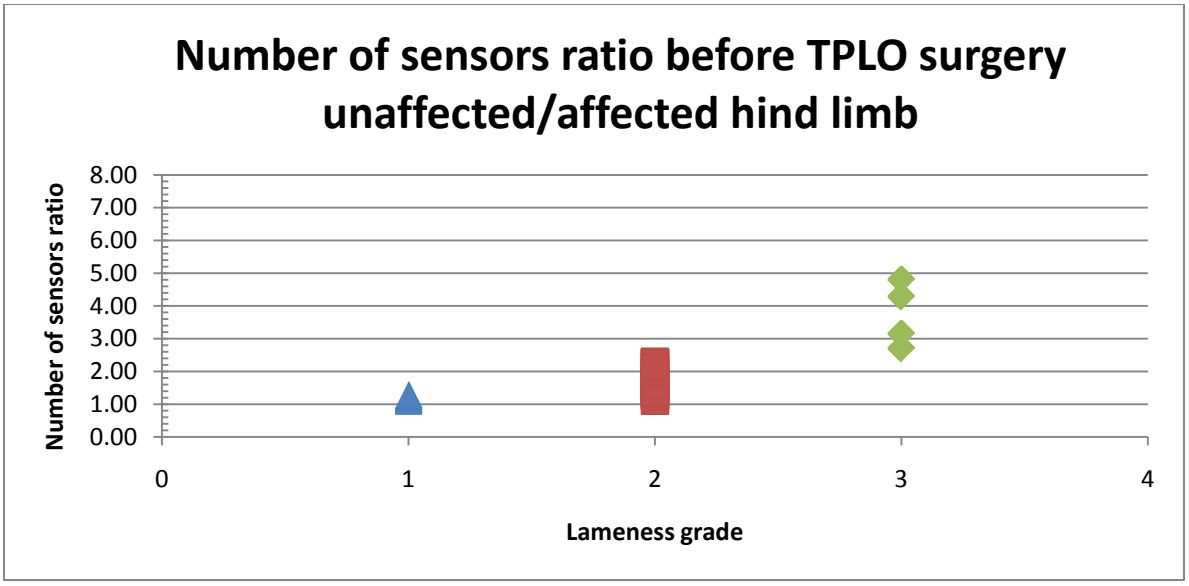


Figure 4-2 Number of sensors ratios before TPLO unaffected/affected hind limb

Graph represents individual dogs' (n=25) unaffected/affected hind limb ratios for number of sensors (NS) where 1.0 is symmetrical. Triangular data points indicate grade 1 lameness, square data points indicate grade 2 lameness and diamond data points indicate grade 3 lameness. Lameness was graded as 0=clinically sound (n=0), 1=subtle lameness (n=4), 2= overt lameness (n=17), 3=intermittent leg carrying lameness (n=4), and 4=constant leg carrying lameness.

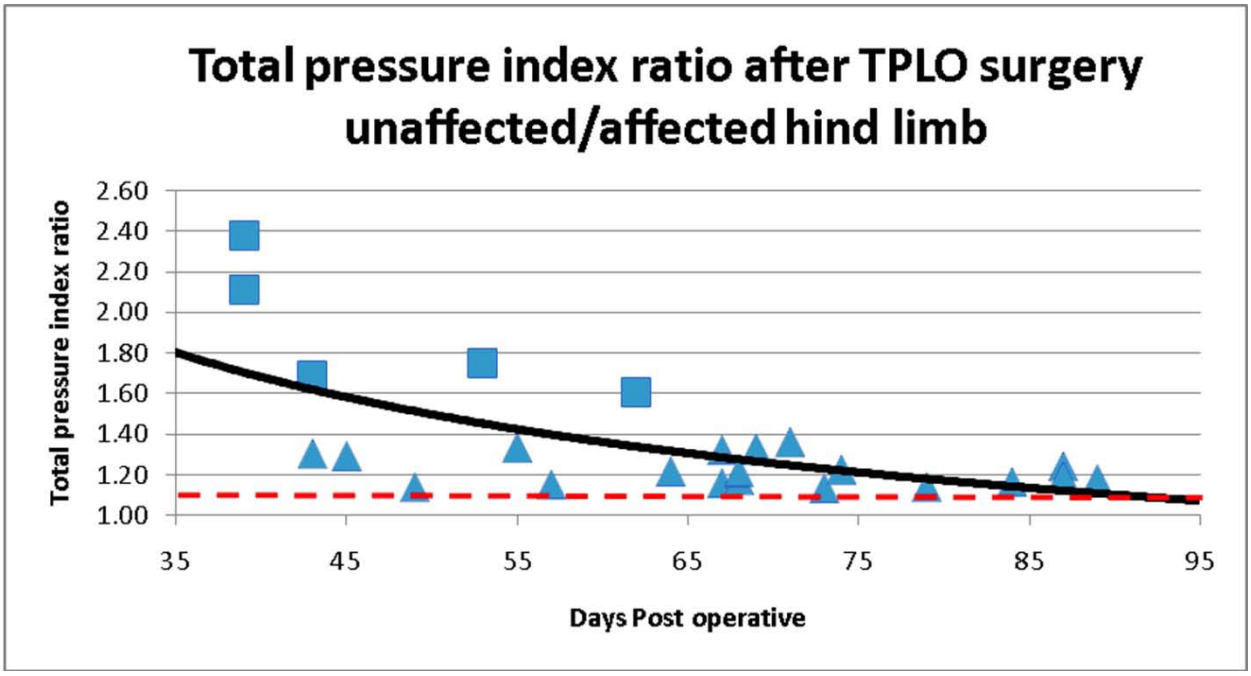


Figure 4-3 Total Pressure Index after TPLO unaffected/affected hind limb

Graph represents individual dogs' (n=25) unaffected/affected hind limb ratios for total pressure index (TPI) where 1.0 is symmetrical. Dashed line represents 1 standard deviation for control dogs.[10] Solid line represents the best fit trend line where $y_{TPI} = 11.499x^{-0.52}$. Triangular data points indicate grade 1 lameness and square data points indicate grade 2 lameness.

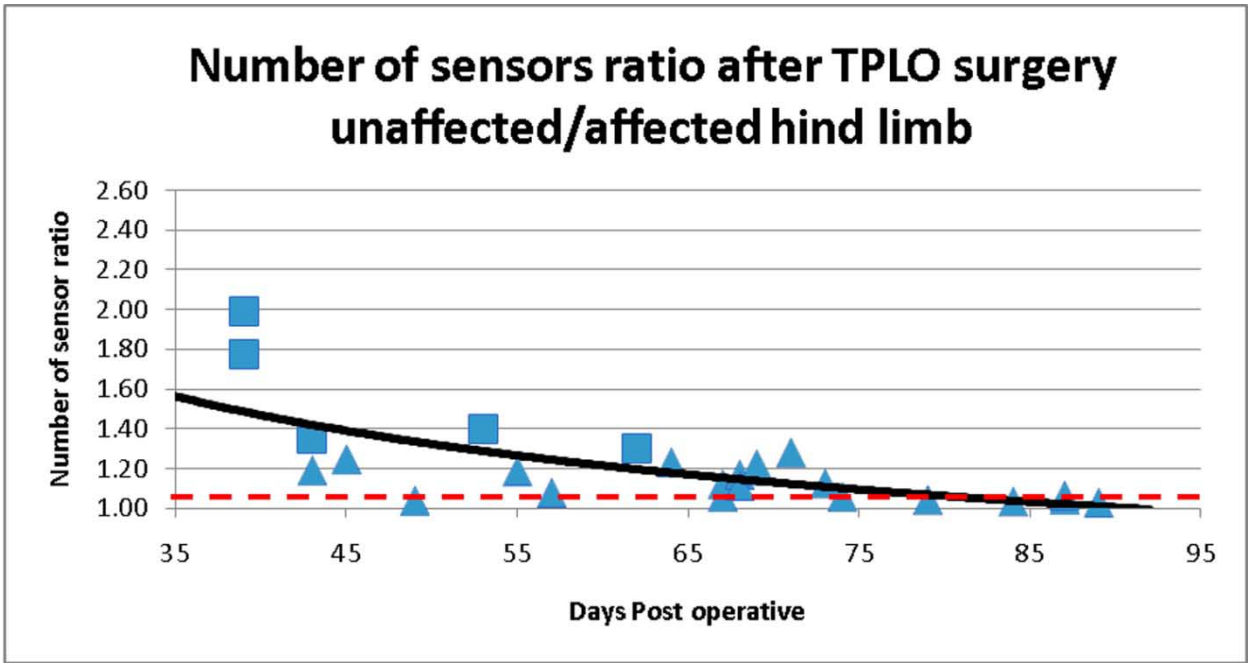


Figure 4-4 Number of sensors ratio after TPLO surgery unaffected/affected hind limb

Graph represents individual dogs' (n=25) unaffected/affected hind limb ratios for number of sensors (NS) activated by each paw where 1.0 is symmetrical. Dashed line represents 1 standard deviation for control dogs.[10] Solid line represents the best fit trend line where $y_{NS} = 8.2613x^{-0.46}$. Triangular data points indicate grade 1 lameness and square data points indicate grade 2 lameness.

Chapter 5. GAITFour Manual

A comprehensive guide to canine gait analysis using the GAITFour portable electronic walkway system

Victoria A Light

2/17/2011

GAITFour Manual Table of Contents

Getting Started

Installing GAITRite software on your computer

To install GAITFour on a “new” or downloading computer:

http://www.gaitrite.com/gaitfour/webinstall.exe

To install GAITFour on another computer (transfer the install.exe file onto a USB drive):

http://www.gaitrite.com/gaitfour/install.exe

To update to the latest version of GAITFour:

http://www.gaitrite.com/gaitrfour/update.exe

Check off the appropriate camera options. (Philips and Logitech, if you have both.)

Minimum facility requirements

24ft x 5ft area for a 16 ft mat (Add approximately 8 ft. to the length of the mat to allow dogs to transition onto the mat and attain steady state gait)

Well lit area for video capture

Electrical outlet- you’ll need 2, one for the laptop and the other to power the mat.

Setting up the mat and computer

Roll mat out.

If applicable, place 3 ft dead mat at each end using duck tape or velcro on the under side of the two mats to keep them stationary.

Plug USB cable of the converter box into a USB outlet on the computer

Plug fire wire cable of the converter box into the mat.

Plug in black power cord to converter box.

Plug other end of black power cord into the three prong extension cord.

Plug the extension cord into the wall.

Place the camera(s) in the far center of the dead mat or where you can see the entire walkway. You can adjust the camera angle when you open them in the software.

If you have two cameras, put the Logitech camera the farthest from the computer and the Phillips camera closest to the computer.

Plug a USB extension cable into the Logitech camera and run the USB extension cable along the very edge of the mat.

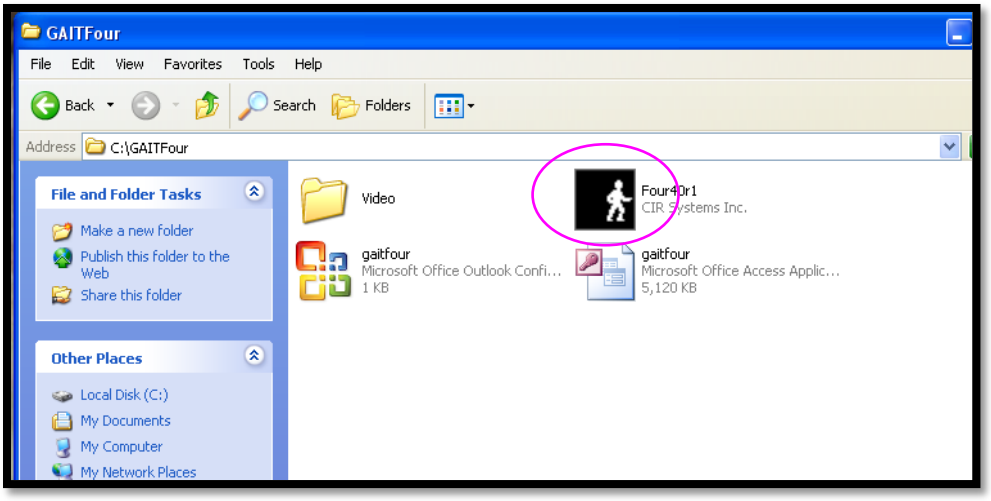
Plug each of the cameras into a USB outlet on the computer.

Opening GAITFour

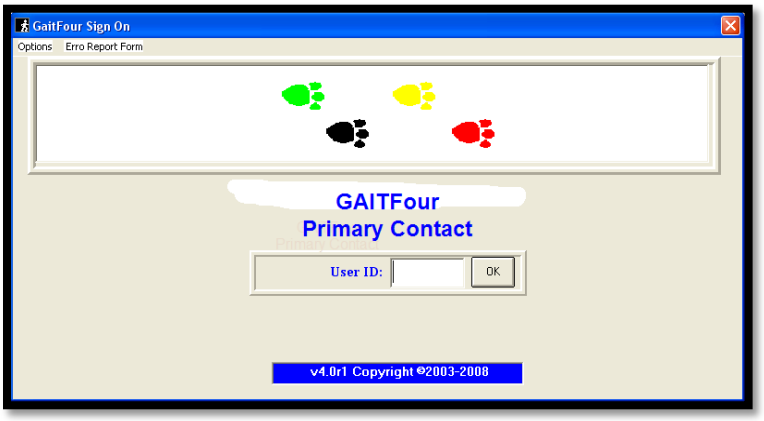
To open GAITFour doubleclick on the GAITRite man icon found on your desktop.

If there is not a shortcut on your desk top, find the GAITFour folder on the c:\ (You can access this by opening “My computer”)

Double click on the GAITRite man icon.

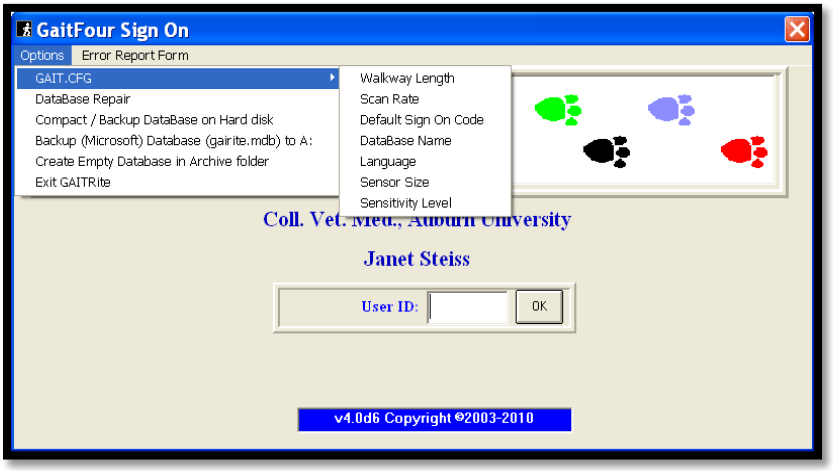


Triple click in the User ID box. The password should have been stored during setup.



Changing the settings

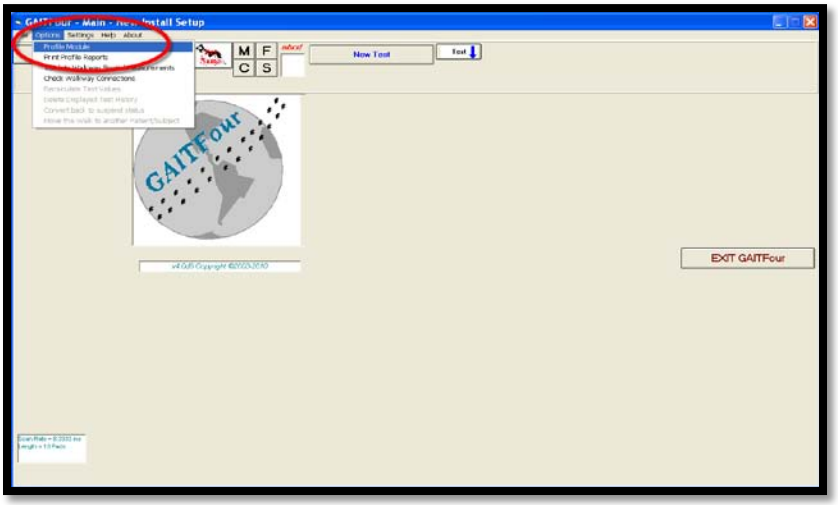
On the sign in page, click <options> then <GAIT.CFG> Here you can change:



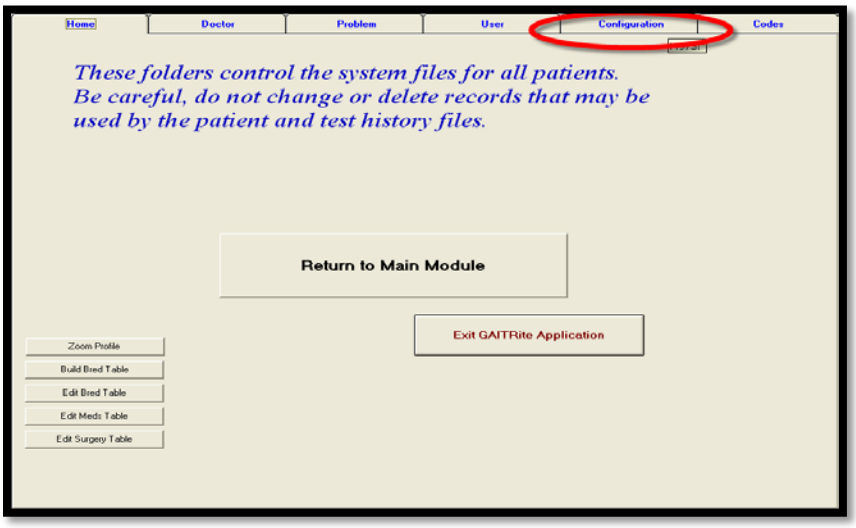
Mat length-commonly this is 14 or 16 ft

Scan rate- it is recommended to run at 120Hz.

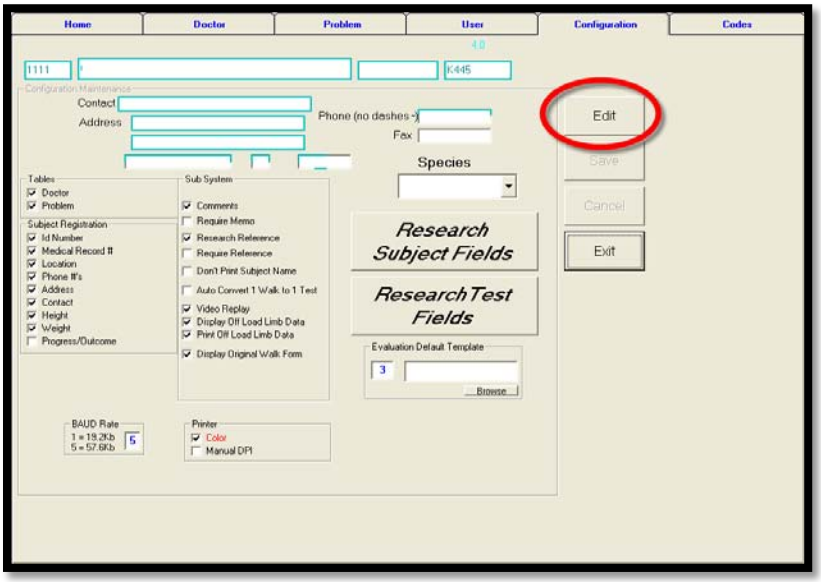
Once you have opened GAITFour, click <Options> then <Profile Module>….



Then <Configuration>.



On this page by clicking <Edit> you can:



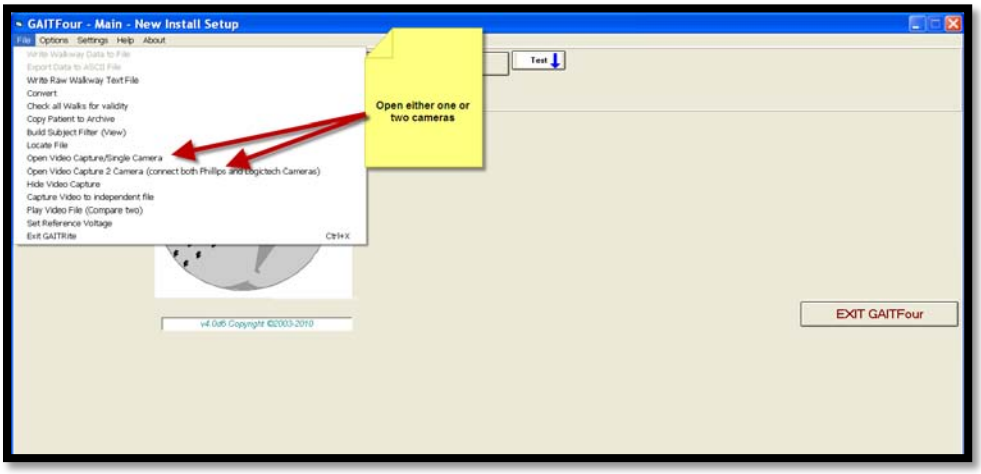
Change Institution Data (Name, Address, etc.)

Select “Display/Print Off Load Limb Data”

Opening the camera(s)

In order to capture a picture for your subject file, you must have the camera open before you create a New Subject.

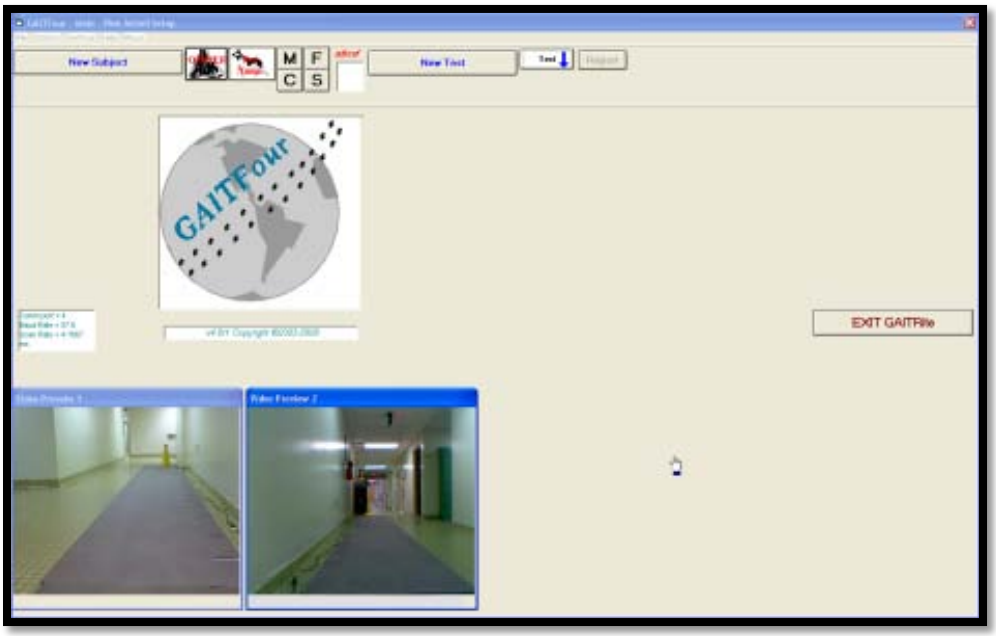
On the front page, select File.



For a one camera set up - Select “Open video capture/single camera”.

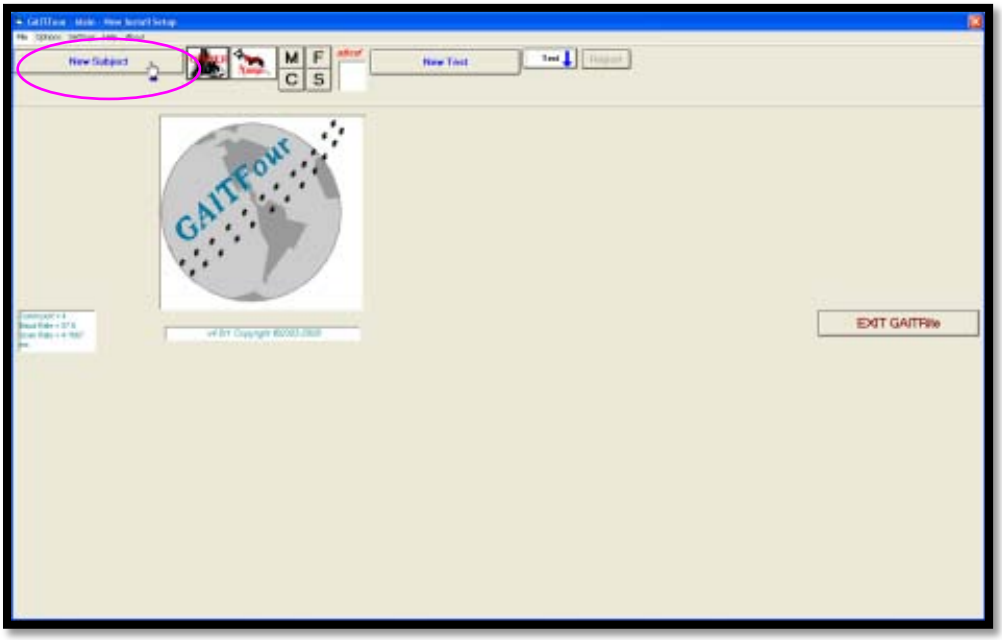
For a two camera set up – Select “Open video capture 2 camera”.

When they are open you will see them at the bottom of your screen.

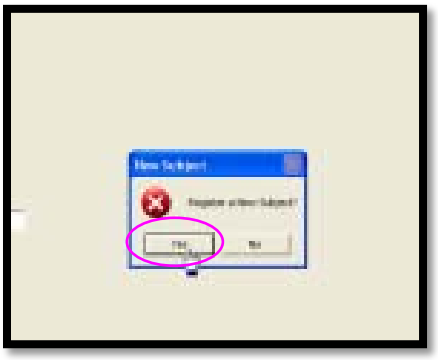


Creating a new subject

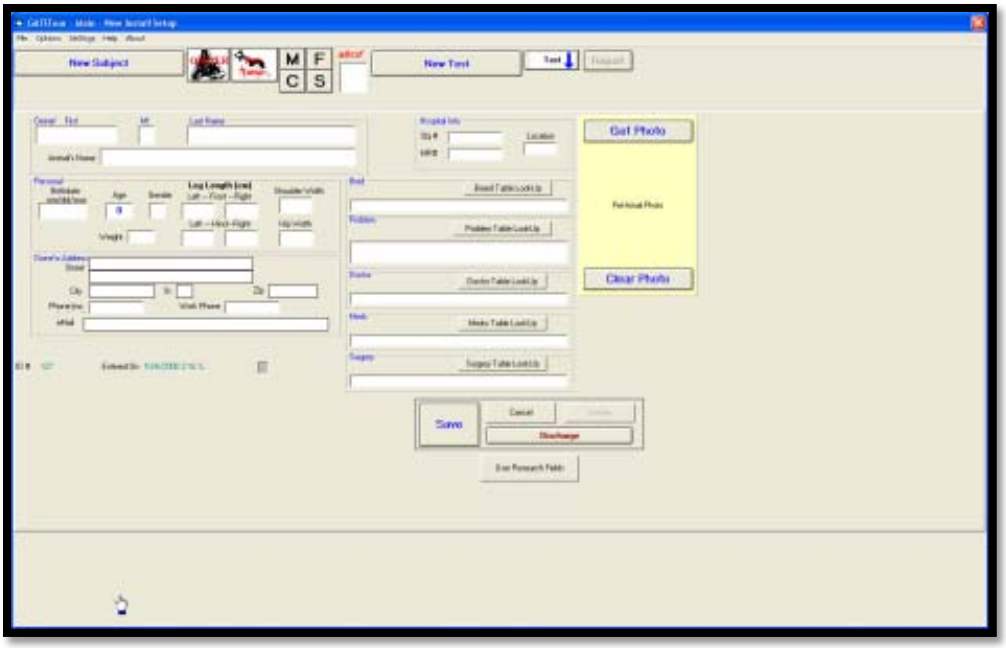
Click on “New Subject”.



A message box will appear asking if you want to register a new subject, select “Yes”.



Enter subject information



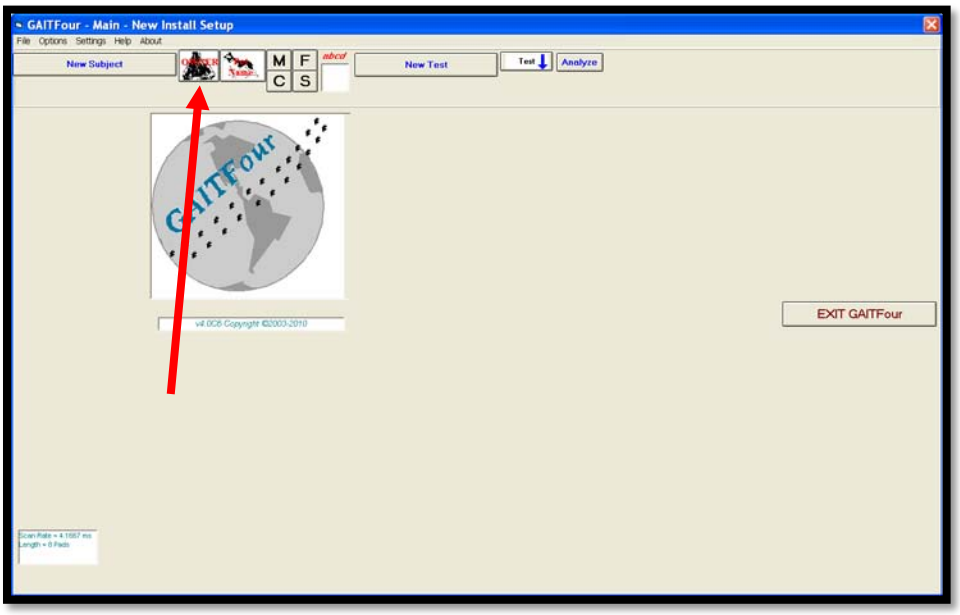
Owner name, animal name, weight, gender and age are required. Other information will help you decipher subjects later but are not required.

(i.e.- breed, subject record #, and owner address and phone number)

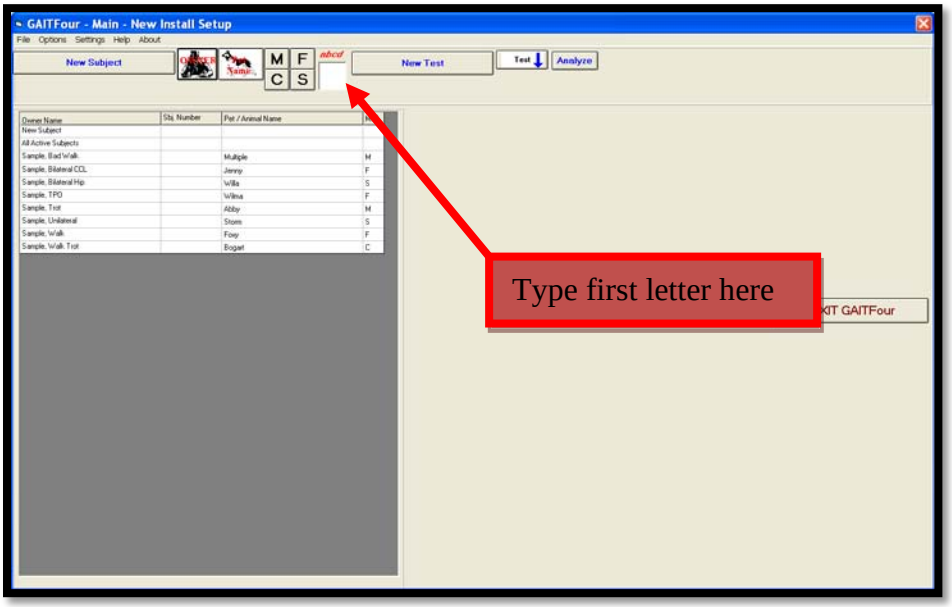
If the camera is open, point it toward your subject and select “Get photo”. (The Phillips camera is the default camera and will capture the photo.)

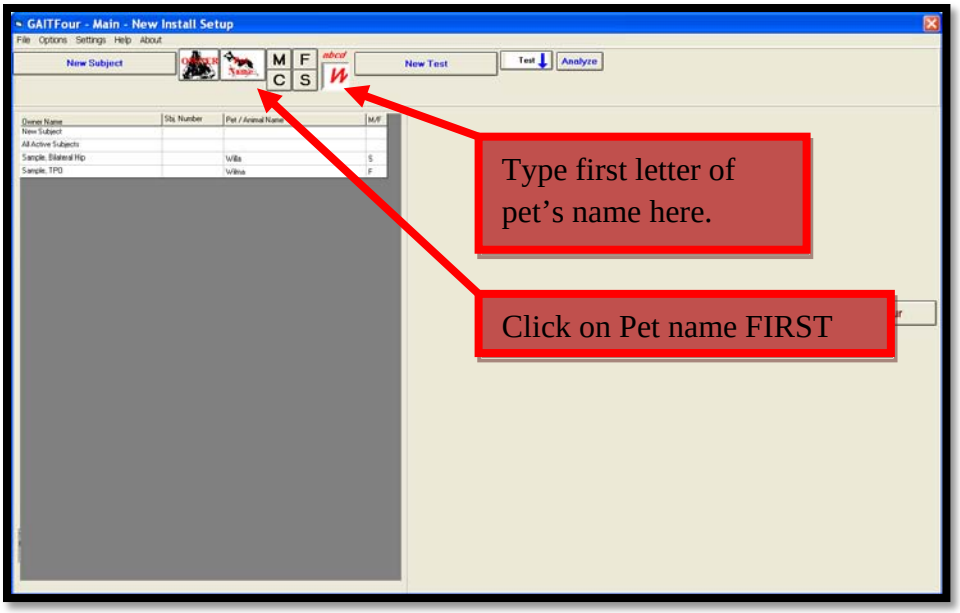
When you are finished entering information, click “Save”.

Opening an existing subject

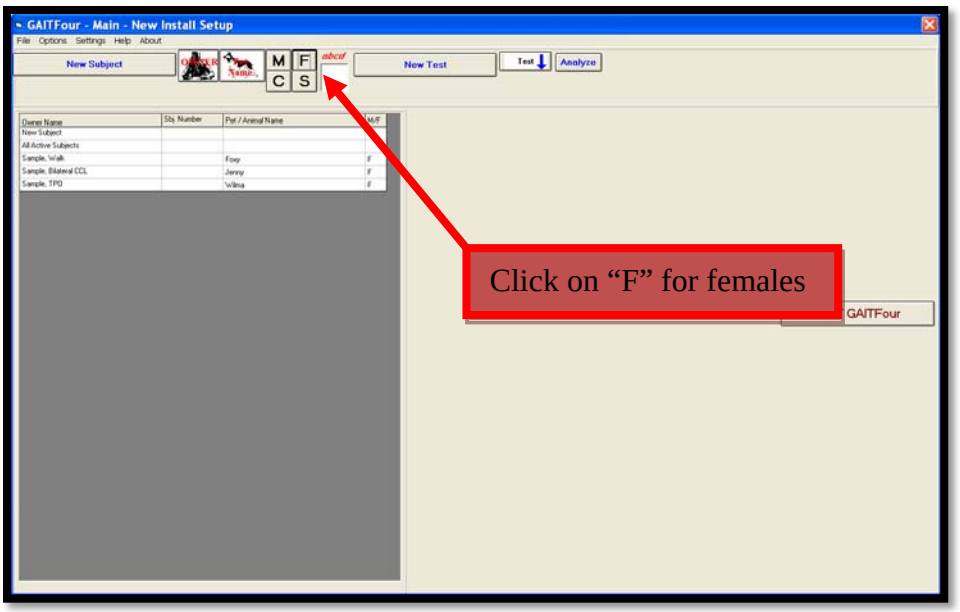


By clicking on “Owner”, you will get a drop down list of all active subjects. At this point you can simply scroll down to find your subject and select them or you can type in the first letter of the owner’s last name to filter through the subjects and then select them.





Another way to filter your subjects is by clicking on Pet name then typing in the first letter of the pet's name.



You can also filter through the M (male), F (female), C (castrated) or S (spayed) buttons.

Capturing data

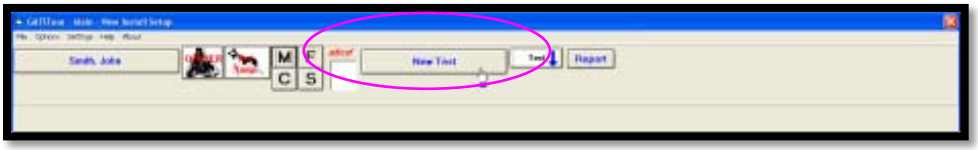
Collecting multiple walks-“auto suspend”

In order to collect multiple walks without immediate analysis, you must open the auto suspend mode.

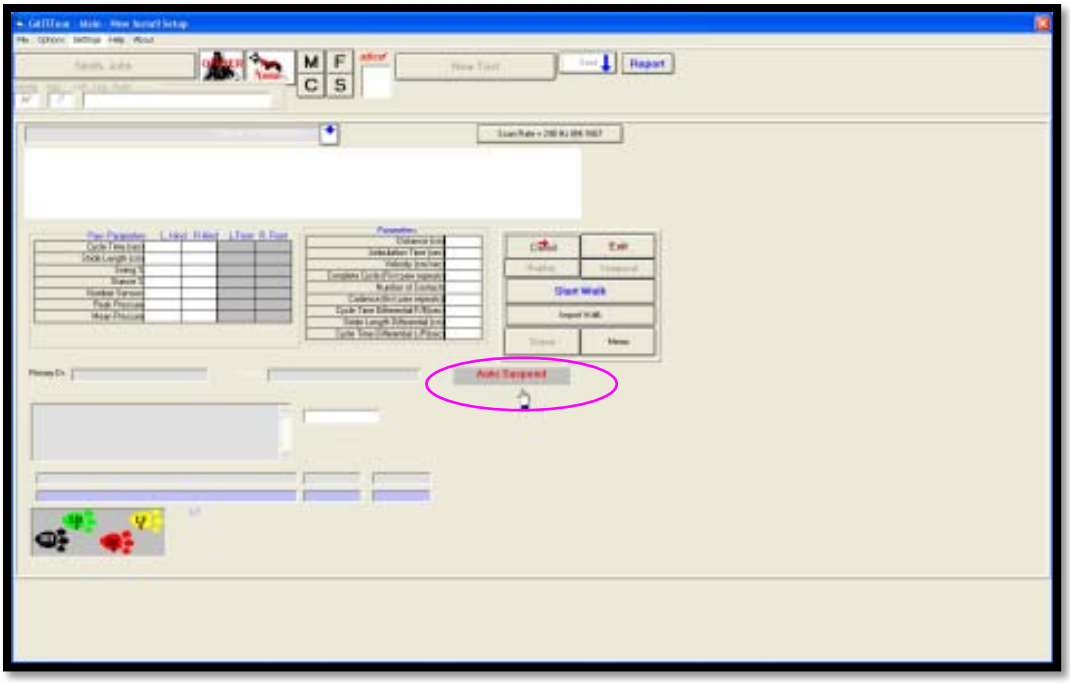
Select “Settings”

Select “Auto Suspend”

To collect a walk, Select “New Test”.

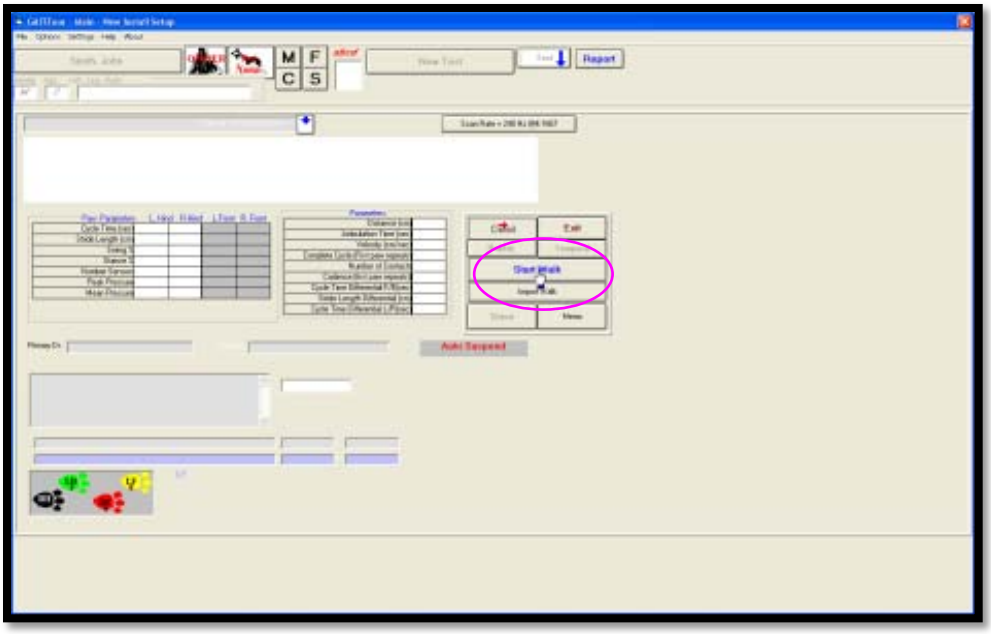


If you have initiated the Auto Suspend mode you will see it below the Start walk button.



If you have not opened the Auto suspend mode, it will not appear below the Start walk button.

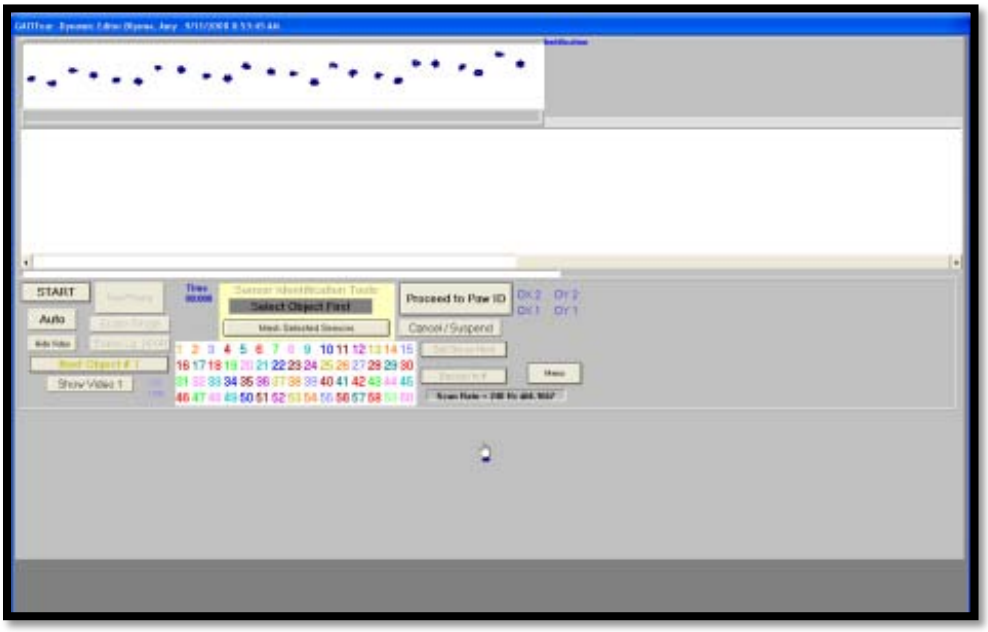
Select “Start Walk” to collect data.



When the data is collected, it will close automatically and you will return to the Start walk page, where you can initiate another walk.

Collecting single walks

If auto suspend mode was not initiated, after the data is collected, GAITFour will take you to the Footfall Identification page.



If you do not want to process the walk at this time, select “Cancel/Suspend” then “Suspend walk to process later”.

If that is the case for most of your walks, you might want to Auto-suspend them. (Refer to #1)

Inclusion criteria- What are good data?

Interpretation for orthopedic diagnosis based on gait analysis is only as good as the data collected. Good data for an objective measurement requires little or no outside influences. In general, it means you want to measure only the dog, not the dog as he pulls you down the hall. To attempt objective gait analysis you must have a basic concept of:

1. The overall anatomy and weight distribution of the dog at various gaits,
2. forces involved in both normal and abnormal motion
3. and that these forces change when velocity (speed and/or direction) changes.

With these concepts in mind, the following inclusion criteria and guidelines were established to help produce good reliable data that can be reproduced and compared.

Inclusion criteria

1. Walk/ Trot at a consistent velocity or steady state gait
2. Loose Leash
3. Head straight
4. “Center” of mat

Forces exerted to increase/decrease speed, change direction or maintain balance can interfere and complicate measurement interpretation. This gait analysis tool does not measure force directly but does measure the influence of these forces. Therefore by limiting excess external influences, your measurements are more representative of the dog and not of how you handle the dog.

Other guidelines

Dogs can be unpredictable. You can't just ask them to walk straight, can you? Maybe not directly, but by using these other guidelines you can establish an environment for success.

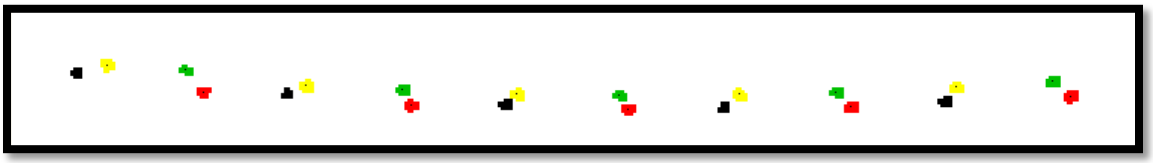
1. Try to set up the mat in a "quiet" location where the dog won't become easily distracted.
2. Start 2 meters before mat to encourage the dog to be straight and at a walk (steady state gait) prior to stepping on the mat!
3. Practice before recording to acclimate the dog to the mat and its surroundings.
4. Encourage through praise when walking next to you without pulling.
5. Do not put constant pressure on the lead. This will encourage the dog to pull against you and will skew the results when recording.
6. Encourage dog to "heel". If they are pulling on the lead, pull back quickly and then release immediately while saying "heel" or "easy".

*The previous statements were found by some handlers to be helpful. This may not work for all handlers or with all dogs.

Examples of good walks:

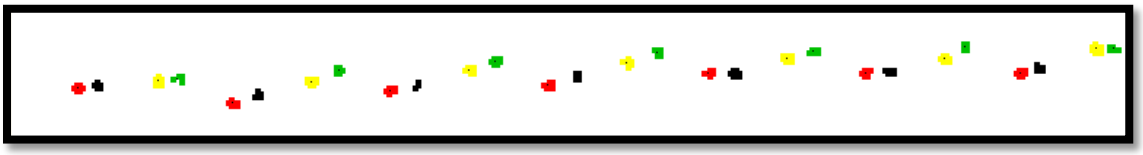
PACE or AMBLE (left to right)

Notice the alternating diagonal paw pattern**:** / \ / \ / \ / \



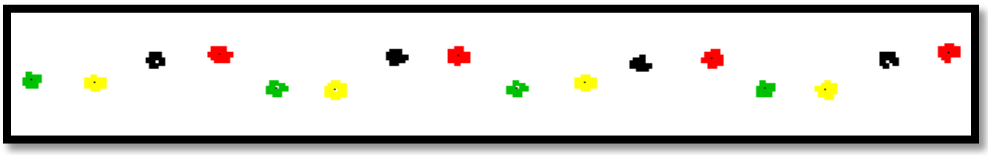
WALK 1 (left to right)

Notice the diagonal paw pattern: / / / / /



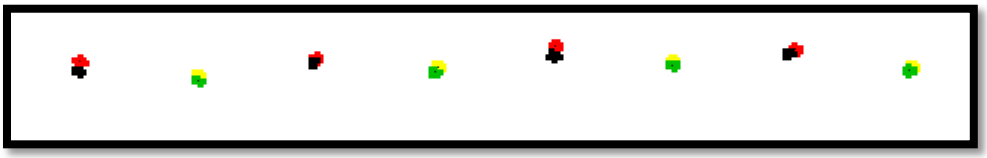
WALK 2 (right to left)

Notice the "wavy" paw pattern: ~ ~ ~ ~



TROT (right to left)

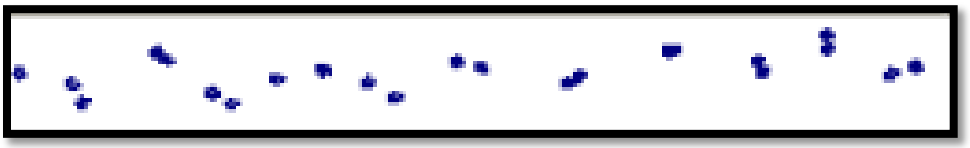
Notice the hind paws are on top of fore paws



Pathology can cause a walk to appear as a trot.

Examples of bad walks:

This dog changed velocity. (walk to a trot-traveling from left to right)



This dog's left front paw stepped off the mat in the middle of the walkway.

This dog did not walk down the center of the mat. (Change in direction affects the amount of force and distribution of pressure)

This dog paused in the middle of the walkway.

Notice the consistent pattern-then the cluster of paws-then consistent pattern again.

This dog walked off the mat and there are not enough paw strikes. (Must have 12 paw strikes or 3 gait cycles)

Processing collected data

Auto identification

Select a subject.

Left click on <Owner>, then desired subject.

Select an unprocessed walk.

Left click on <Test>, then desired data file.

Unprocessed walks are indicated by “Sus” in front of the date/time stamp.

Determine if walk should be processed (See “Inclusion” Criteria in Data Collection section)

Watch video, left click on <Play> and look for

1. Consistent pace (no stopping or changing gait)
2. Loose leash (no pulling)
3. Look at paw strike pattern
4. Consistent pattern (indicates consistent pace)
5. At least 12 consecutive footfalls (3 gait cycles) on the active area of the mat (stays on the mat)

Left click on Auto

Footfalls should be identified automatically and you should be forwarded to the next page where paws are then automatically identified.

If not, this walk data must be manually identified.

Manual footfall identification

If auto identification is not possible, manual can be used.

After selecting subject and unprocessed walk file, left click on <Start>.

Note the size and placement of the “paw strikes” in the small window on the top of the screen. They will turn grey as the paws are about to be placed.

<Next Frame> should be highlighted. If not, left click on it.

At this point you can either continue left clicking on <Next Frame> or hit the <Enter> key on the keyboard (This only works if <Next Frame> is highlighted.)

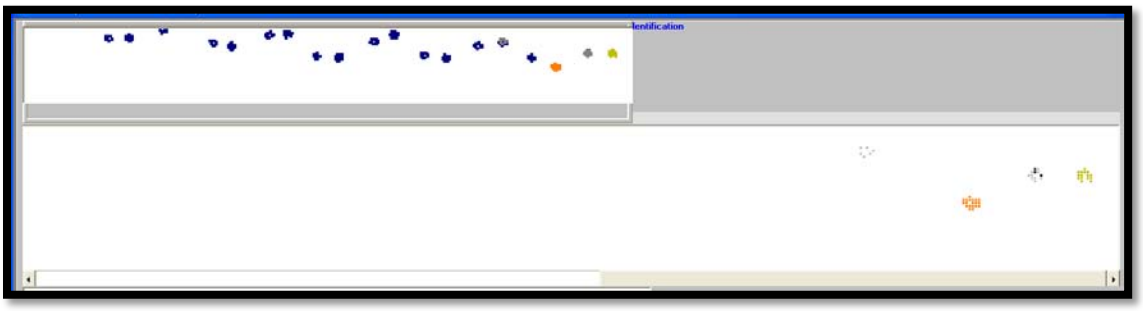
Continue clicking until all of the sensors for a paw have turned on (grey) and then off (black).

Right click in the center of this paw.

Make sure the sensors for the entire paw are highlighted (only the sensors for that paw) and click <yes>.

If all of the sensors were not selected or if too many sensors were selected, left click on <no> and try right clicking in the center of the paw again.

Pay attention to the placement of each paw. Note how the front paw for each side comes down first, followed by the corresponding hind paw.



In this example, the dog is moving from right to left at a walk, the first paw strike is a right front with the second paw strike a left front. The next (3rd) paw strike will be a left hind which is placed spatial in front of the right front paw strike.

Be careful here! The fourth paw strike for this example should be the left front of the next gait cycle, not the right hind. This can be a little tricky so pay attention.

Continue until all paws have been identified then left click on <Proceed to Paw ID>.

Paws should be identified automatically. Verify they are correct.

If automatic paw identification did now work properly, you must manually identify the paw strikes.

Verifying paw identification

Verify left and right sides have been identified correctly.

Correlate the colors of the sequential numbers with the colors of the footfalls.

Determine direction of walk. (left to right or right to left)

If left to right walk, the left paws (front and hind) should be at the top of the top window. If right to left walk, left paws (front and hind) should be at the bottom of the top window.

Verify front and hind paws have been identified correctly.

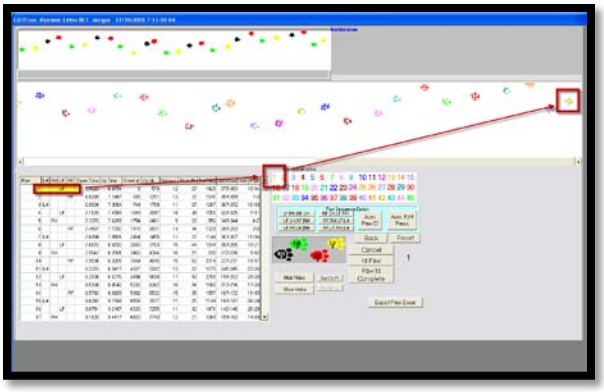
Left click on “Show video”.

Rewind video by left clicking on “|<”

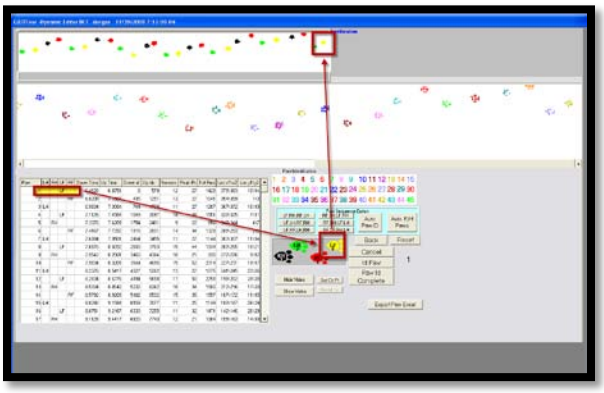
Forward video by left clicking on “>>” until first paw touches active area and identify it. (ex: left front)

Correlate the first paw with the correct color in the middle window

(paw 1 – mustard yellow)



Now verify this paw has been colored correctly in small top window (ex: left front-yellow)



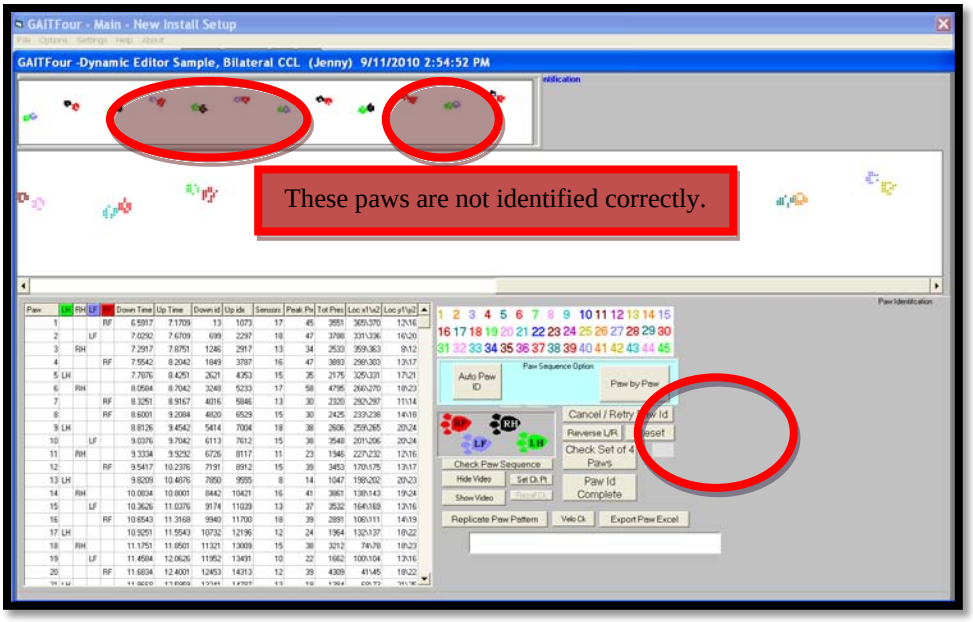
If these are not correct, left click in the correct box (ex: LF) on the paw grid next to the sequential paw number.

This should relabel and color the paw corresponding to the selected paw. (ex: LF, yellow)

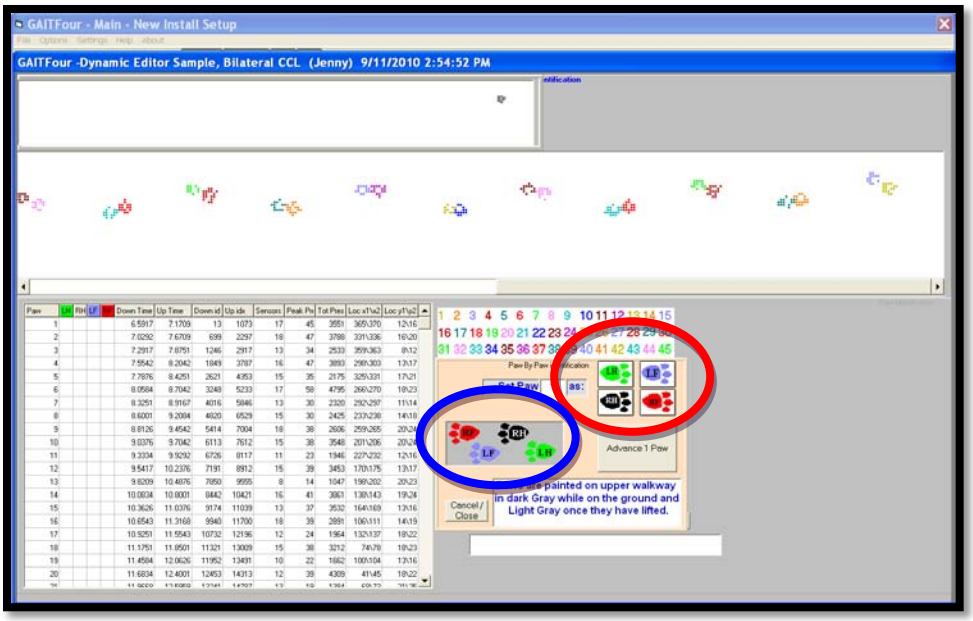
Repeat this for each paw strike until you feel confident the paw pattern is correct.

Paw by paw identification

If the software misidentifies the paws, you can manually identify by using paw-by-paw.

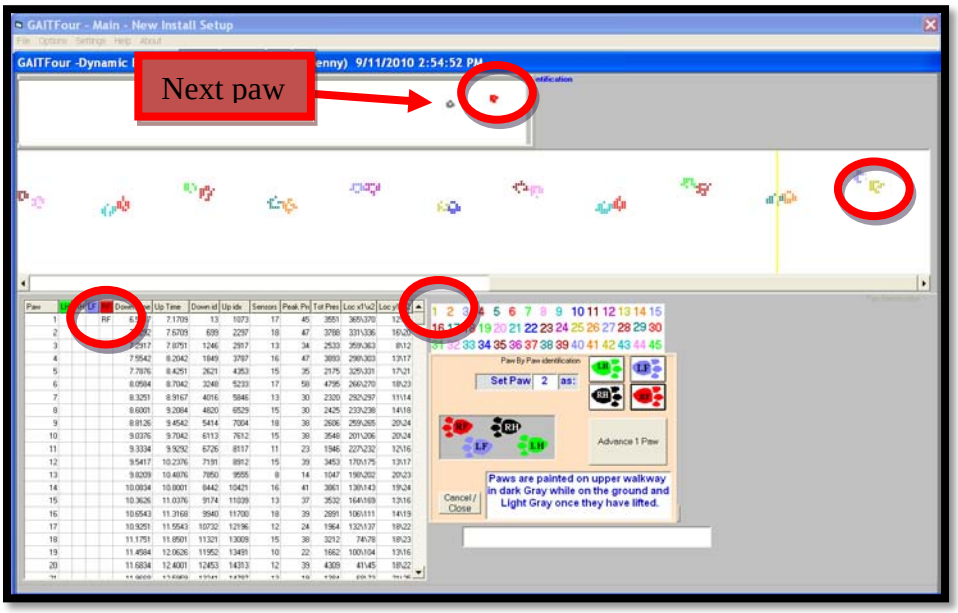


Left click on the “Paw by Paw” button. This will take you to a screen where you can manually identify the paws as they are placed on the mat.



To label the paw, click on the color coded paw print (found in the red circle) that corresponds to what you think the paw should be. You will only be allowed to label them this way, while in Paw by Paw.

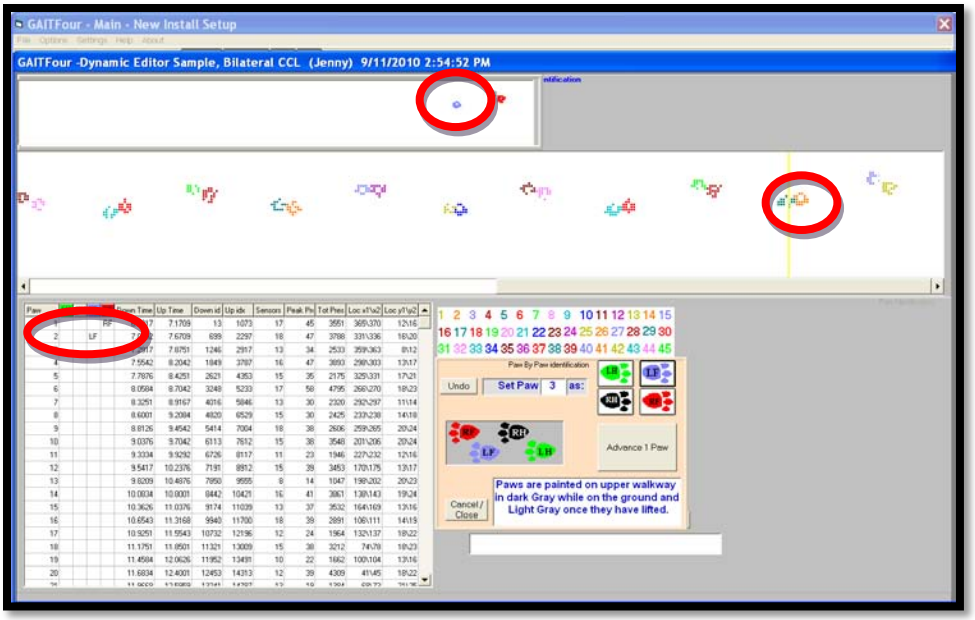
First, notice that the directional paws (found in the blue circle) are facing left. This shows that the dog walked from right to left across the screen.



Since the first sequential paw strike is on the "top" of mat…it must be a right…and since there is a corresponding paw next to it that has not been identified yet…it must be a front.

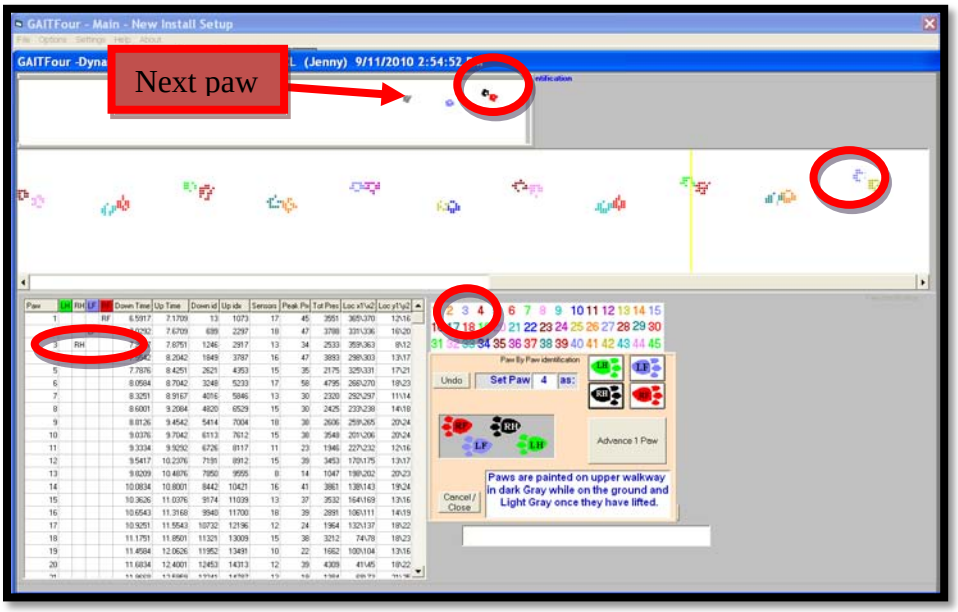
Notice, after left clicking on the RF button, the software "paints" the paw in the top screen and labels it RF in the paw grid to the left.

The paw strike to follow, in this instance paw #2, will appear as a gray paw. The gray color indicates that it has not yet been identified.

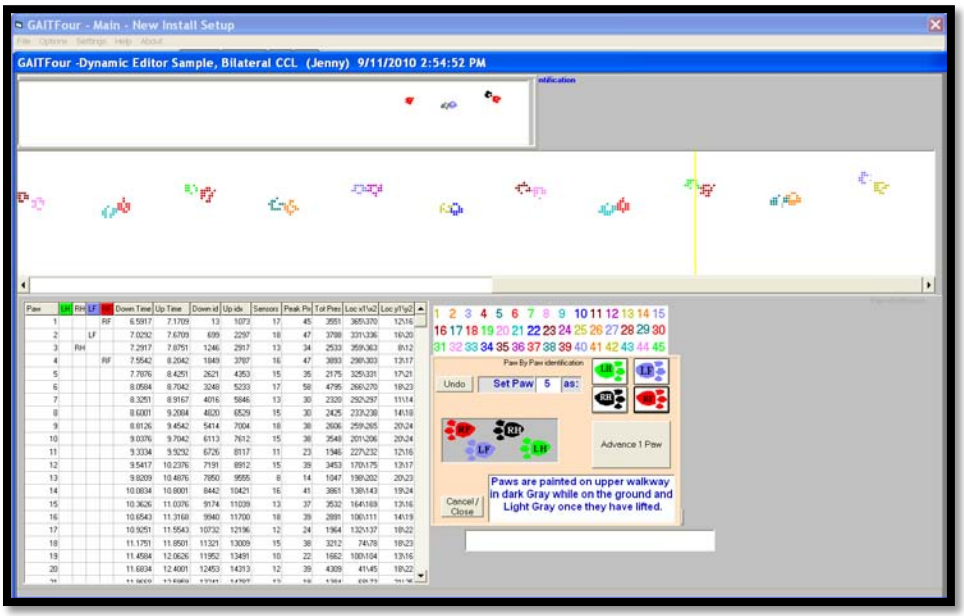


The next paw strike is on the 'bottom" of the screen and very forward of the last paw strike…so it must be a left front. Click on the LF button. Once again it paints the paw and puts LF in the grid.

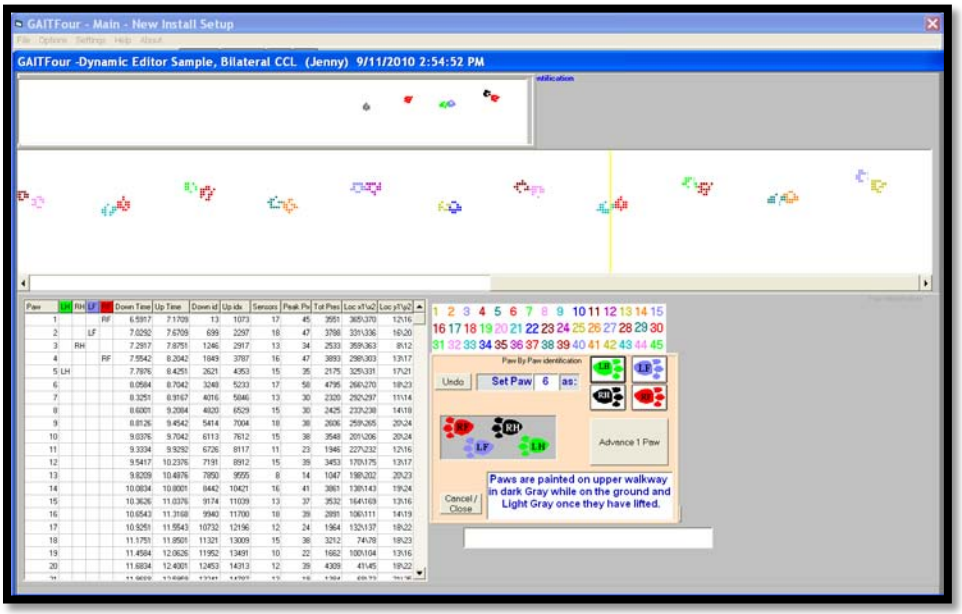
There is also a yellow line that is following your progression. It will be situated just in front of the "farthest" paw. (i.e. the farthest one from the start of the mat.)



The third paw strike in this walk is “behind” the second paw strike…so it must be a hind…and on the “top” …so it must be a right. Click on the RH button.

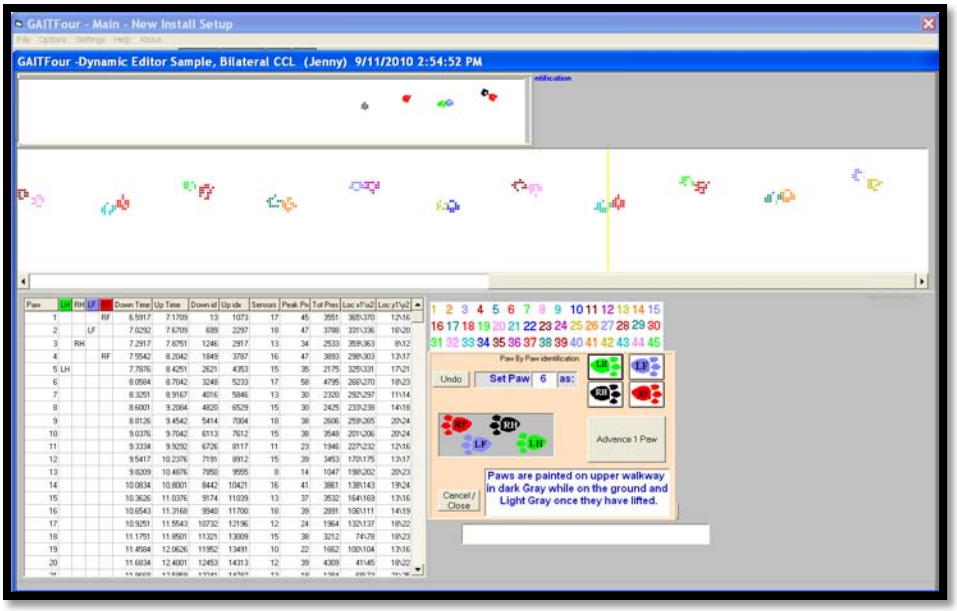


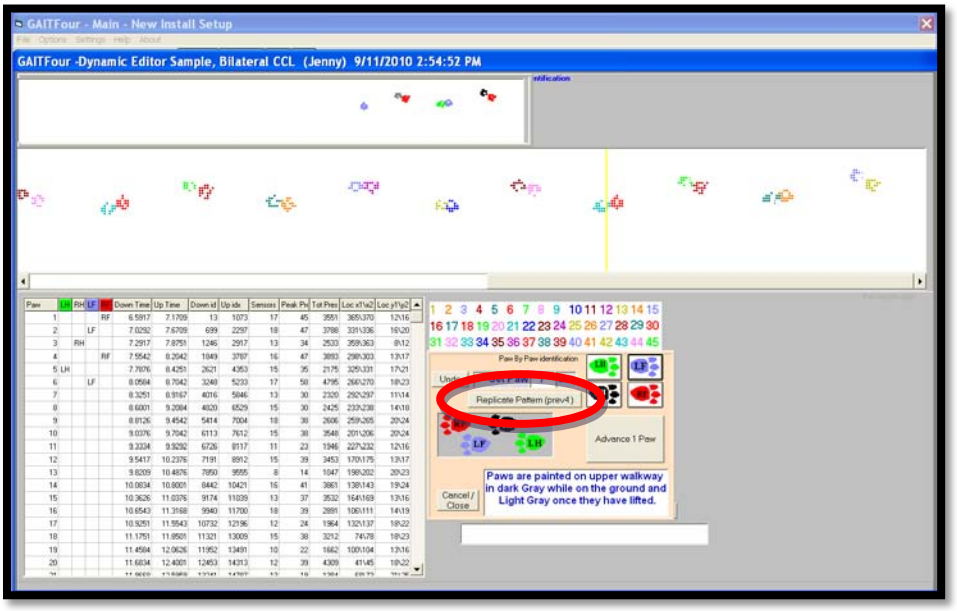
Notice that the Next paw (#4) is in “front” and on the “top”/“right”…it will be a RF.



The following paw (#5) is behind #4 and next to the LF…it will be a LH.

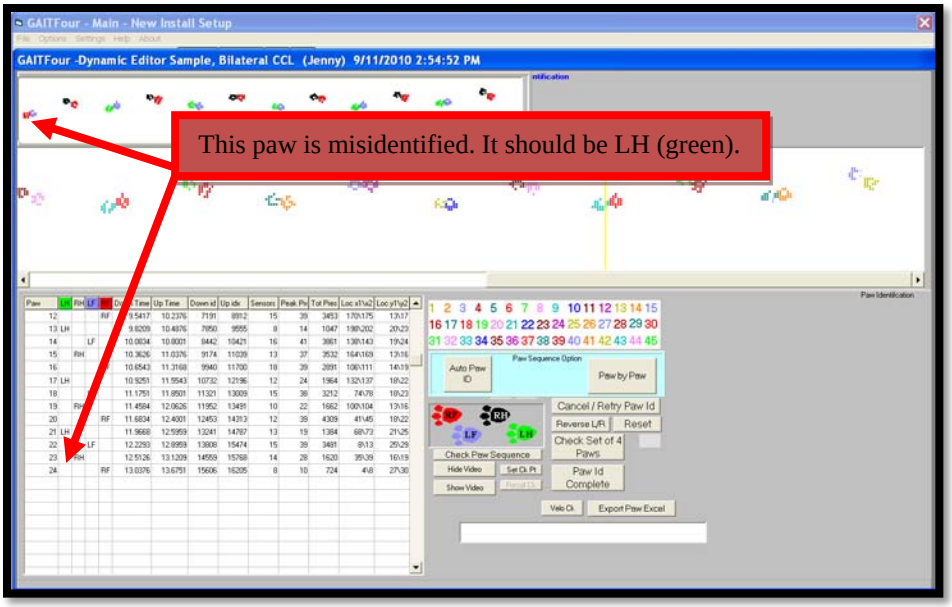
Paw #6 is in front and on the left…so it will be a LF.





After you have identified six paws, a new button shows up. “Replicate Pattern”

By clicking this, the last four paws will be replicated all the way to the end. This saves time, if the dog has a consistent pattern and is in steady state gait.



One drawback to using this feature is that 99% of the time the last paw is misidentified but it's easy to fix. Simply left click in appropriate box on the paw grid that the last paw should be. In this case, paw #24, currently labeled RF should be a LH. Verify Paw Pattern. (Previous sect.)

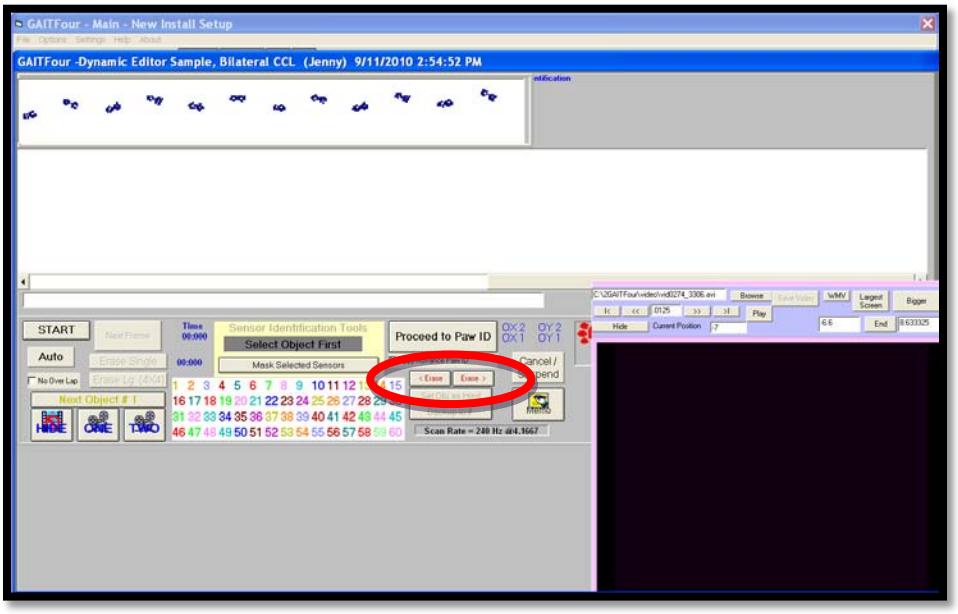
Cleaning up the data

There are a variety of reasons you might need to clean up your data on a single pass

1. The dog walked off the mat at the beginning or end.
2. The dog changed gait at the beginning or end (walk to trot or vice versa).
3. The dog turned his head at the beginning or end.
4. The dog stopped on the mat at the beginning or end

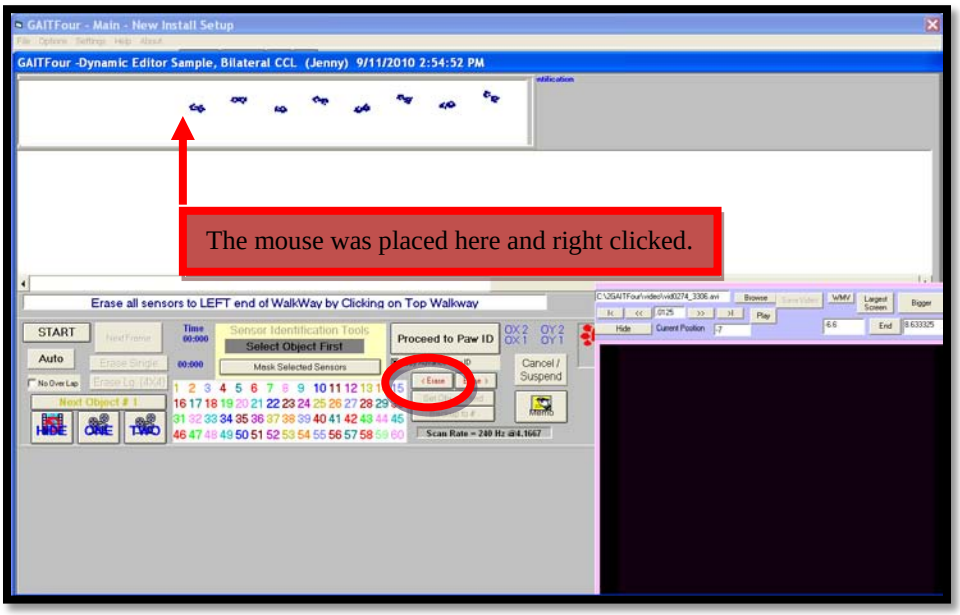
Did you notice the theme in the reasons above? “beginning or end” That was not a typo. Paws cannot be deleted in the middle of the walkway. You can delete as many paw strikes as you like to get good data as long as you have at least 12 paw strikes left to analyze.

Erase to end (Deleting paws before identification)

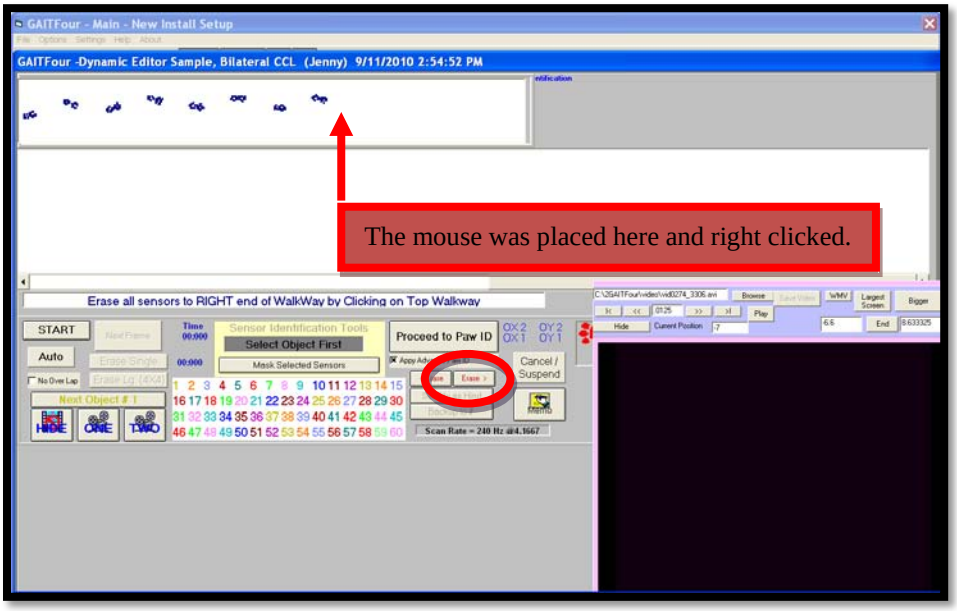


If you already know that you need to delete the first few paws, you can do this before you process the pass by using the “Erase to End” button.

By clicking on the "< Erase" button, and placing your mouse on the top screen and clicking your right mouse button, you will erase everything to the left of your mouse.

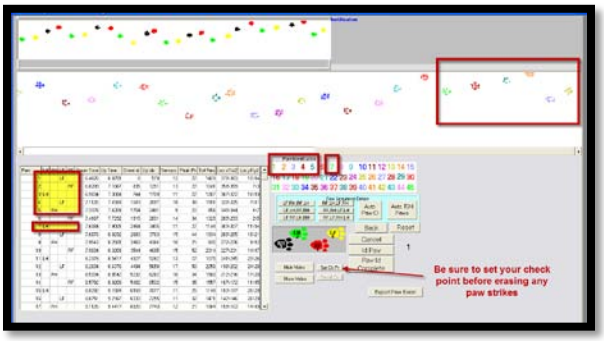


By clicking on the "Erase >" button, and placing your mouse on the top screen and clicking your right mouse button, you will erase everything to the right of your mouse.



Manually deleting paws (One by one-after identification)

After identification, you notice paws that are not entirely on the active area, you can delete them individually. This may mean that additional paws need to be deleted as well.



Note that the paws that need to be deleted in this example are spatially sequential but are not temporally sequential.

To delete a paw strike, first left click on <Set Ck Pt> to set a check point. This can be done after you delete each paw strike.

Now, left click on the left side of the number in the row on the grid for the paw that needs to be deleted.

Repeat this until unnecessary paws are deleted.

If you accidently delete the wrong paw, left click on <Recall Ck> this will take you back to where you set a check point.

The number of paw strikes should be a multiple of four with equal number of paw strikes by each limb

After verification of paw identification and number of paw strikes, left click on <Paw Id Complete>.

Double check yourself before you left click on <Yes> when asked if feet where measured properly.

Before processing the next walk data file, left click on <Save> and then <Exit>.

Manual paw identification

If automatic paw identification did not work properly, left click on <Reset> to clear the paw grid.

Manually identifying paw strikes is similar to verifying them. The exceptions are that neither the small window at the top of the screen nor the paw grid indicates which limb the footfalls represent.

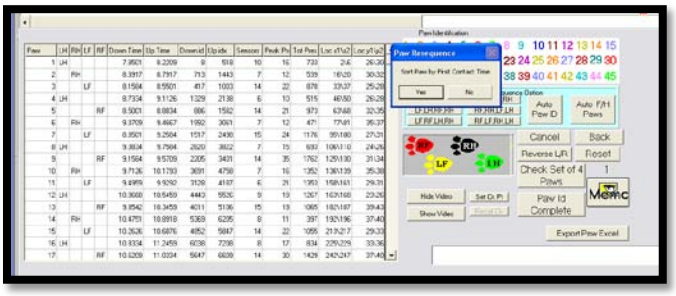
Following the same procedure as the verification process by using the video, identify each paw strike and left click in the representative box for each limb.

Identify at least six paw strikes and then left click on <Repeat Paw Pattern>.

Double check your identification of these paws. Sometimes paws can be switched, especially when working with trotting dogs.

At this point, if you separated paws manually, you could get an “On time sequence error”.

Scroll to the top of the Paw ID grid and left click on top of the “Down time” column label, then <Yes> to sort by first contact time.



When you are confident that the pattern is correct, click <Paw ID Complete>.
Double check before you left click on <Yes> when asked if feet were measured properly.

Before processing the next walk data file, left click on <Save>.

Analyzing data

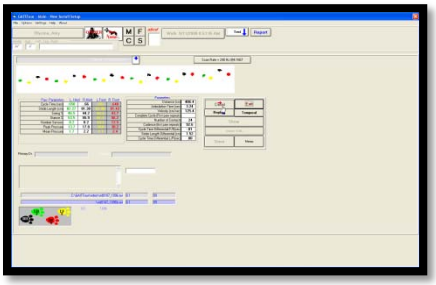
Select the desired subject

Select processed walk file to be analyzed, indicated by “Wlk” next to the date and time stamp.

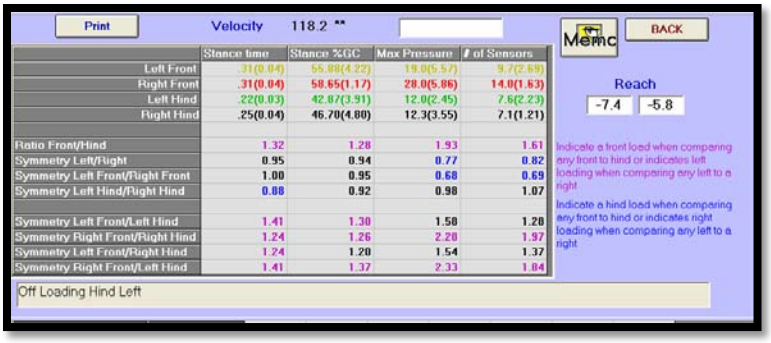
(Files with “SUS” are suspended files that need to be processed.)

Details and summary

To view individual footfall data for each gait cycle, Left click on <Details>



The “Summary” page will be displayed first. It contains a chart with the means (± standard deviation) for each limb and symmetry ratios of 4 of the parameters measured.



The summary page also contains an average velocity (* = 5-10% variation, ** = >10% variation) and a “Reach” measurement.

In the chart, measurements outside of 1 standard deviation are highlighted purple or blue according to + or – direction of the deviation. An explanation of the color is located to the right of the chart.

An off-loading memo box option can be turned on to assist interpretation. (See changing settings)

Printing a summary report

The means, symmetry ratios, picture of paw strikes and graphs can be printed from the summary page by selecting “Print”.

Clinic Name

Clinic's address and phone number

Pt# 1

Bubba

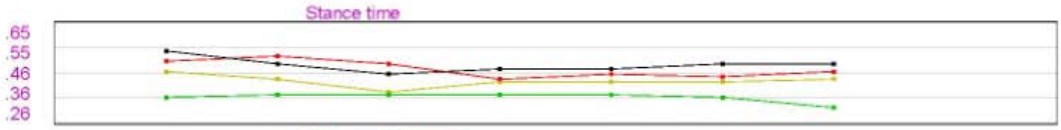
Owner: Owner's name

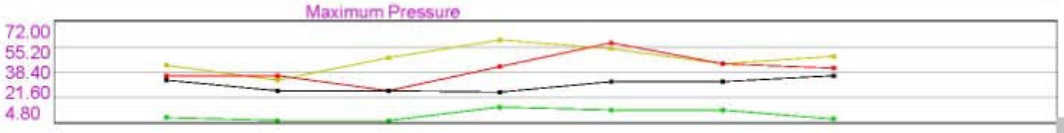
Age	Gender	Left (H/F)	LEG	Right (H/F)	Shoulder	Hip
3	M				0	0

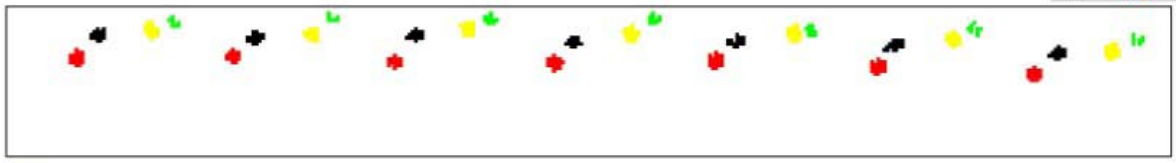
Tested on: 1/7/2009 10:42:17 AM

Velocity: 97.4 **
Reach (L/R): 9.4 / 8.3

	Stance time	Stance %GC	Max Pressure	# of Sensors
Left Front	.42(0.02)	61.57(0.61)	47.3(8.60)	18.6(2.44)
Right Front	.47(0.03)	67.47(2.03)	40.4(9.76)	18.0(3.32)
Left Hind	.36(0.02)	54.13(2.10)	9.7(3.82)	6.6(1.40)
Right Hind	.49(0.03)	70.30(1.28)	30.0(4.28)	13.9(1.57)
Ratio Front/Hind	1.05	1.04	2.21	1.79
Symmetry Left/Right	0.81	0.84	0.81	0.79
Symmetry Left Front/Right Front	0.89	0.91	1.17	1.03
Symmetry Left Hind/Right Hind	0.73	0.77	0.32	0.47
Symmetry Left Front/Left Hind	1.17	1.14	4.88	2.82
Symmetry Right Front/Right Hind	0.96	0.96	1.35	1.29
Symmetry Left Front/Right Hind	0.86	0.88	1.58	1.34
Symmetry Right Front/Left Hind	1.31	1.25	4.16	2.73





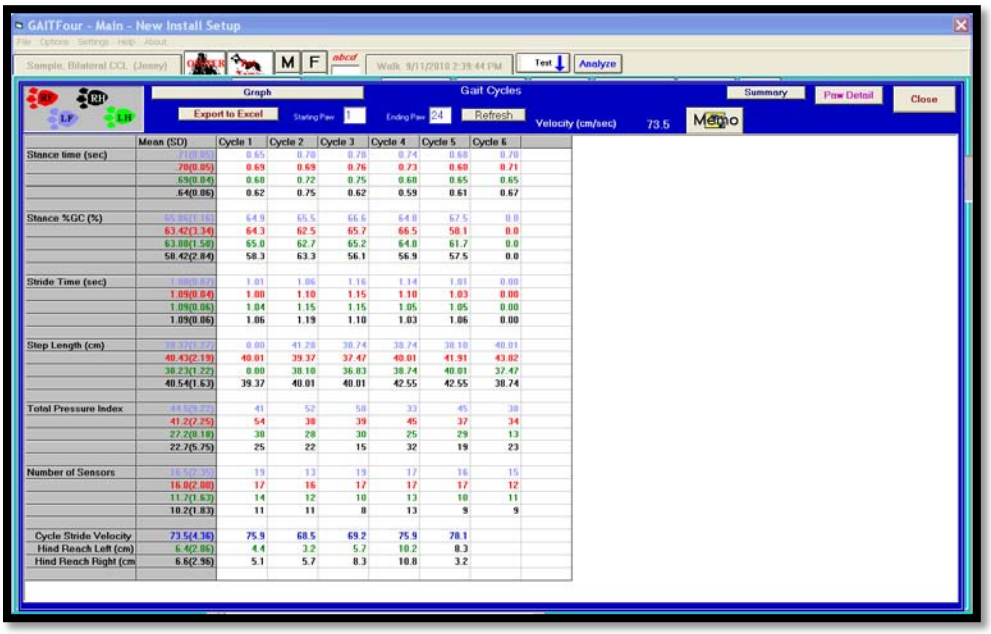


Off Loading Hind Left

This is an example of a print out from the summary page from a dog with a CCL rupture on the left hind.

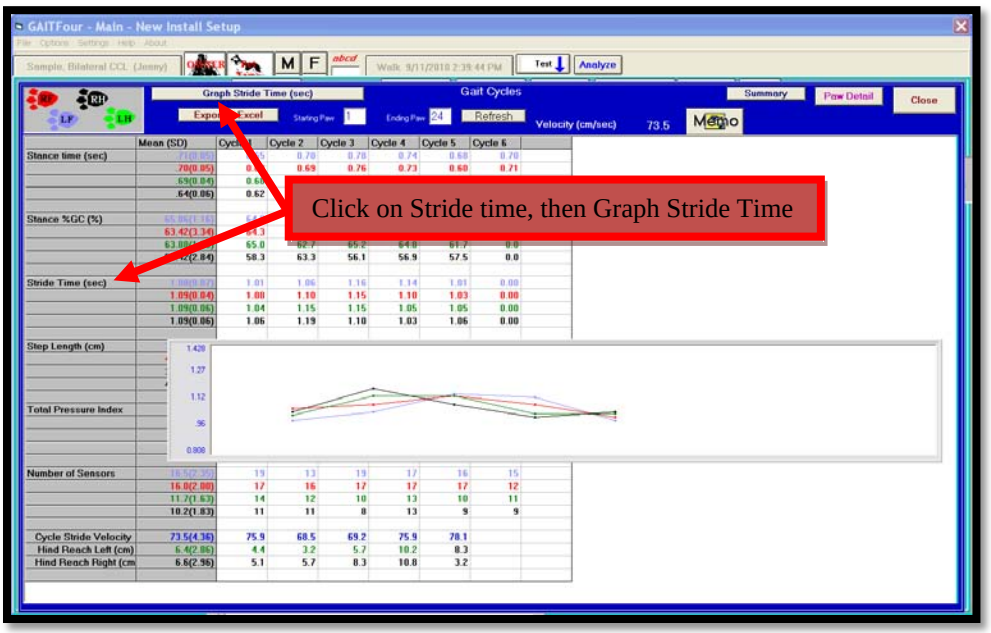
Viewing graphs of measurements

On the summary page, click on the “Close” button to view the measurements for each gait cycle.



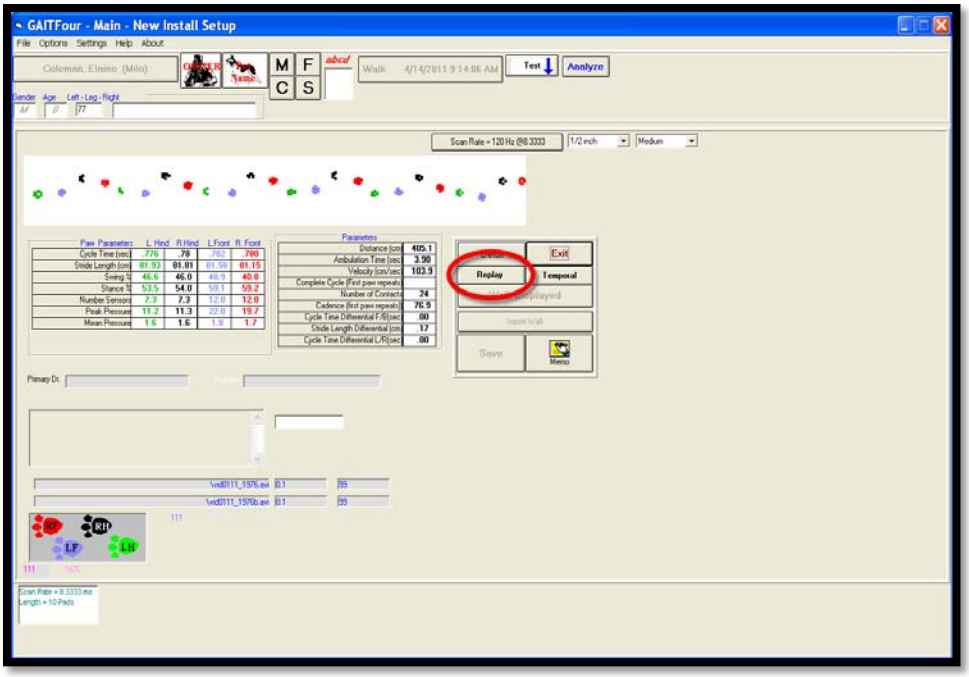
To graphically view changes between the gait cycles for a single parameter (ie-Stride time),

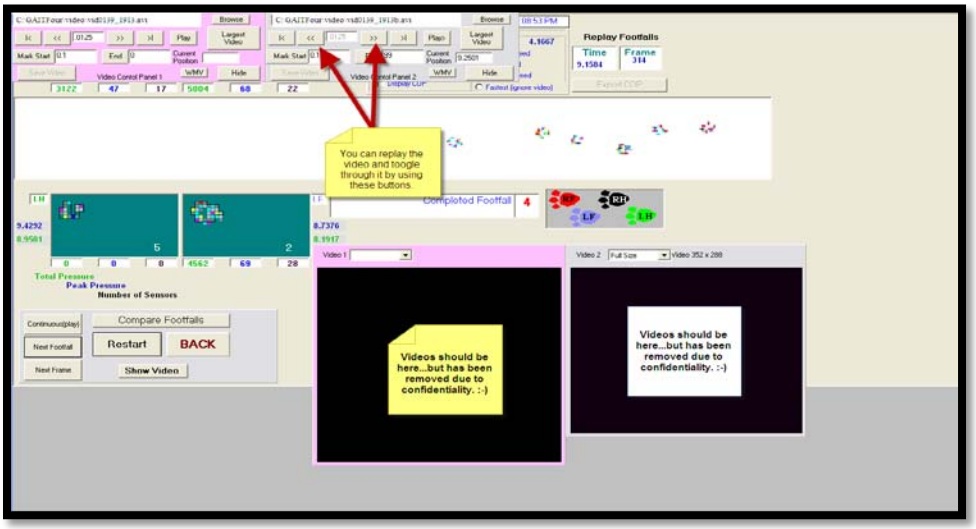
Click on the parameter, then click on graph.



Replay

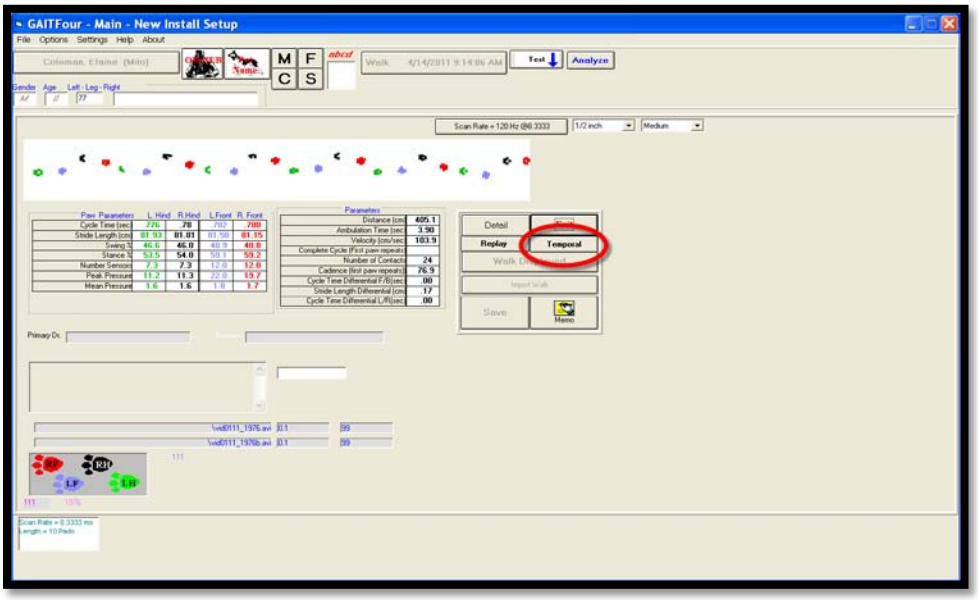
Replay allows you to watch the paws activate sensors as the dog walk across the mat. You can toggle through frame by frame ("Next Frame"), one step at a time ("Next footfall") or "continuous". You can do all this in conjunction with the video as well.

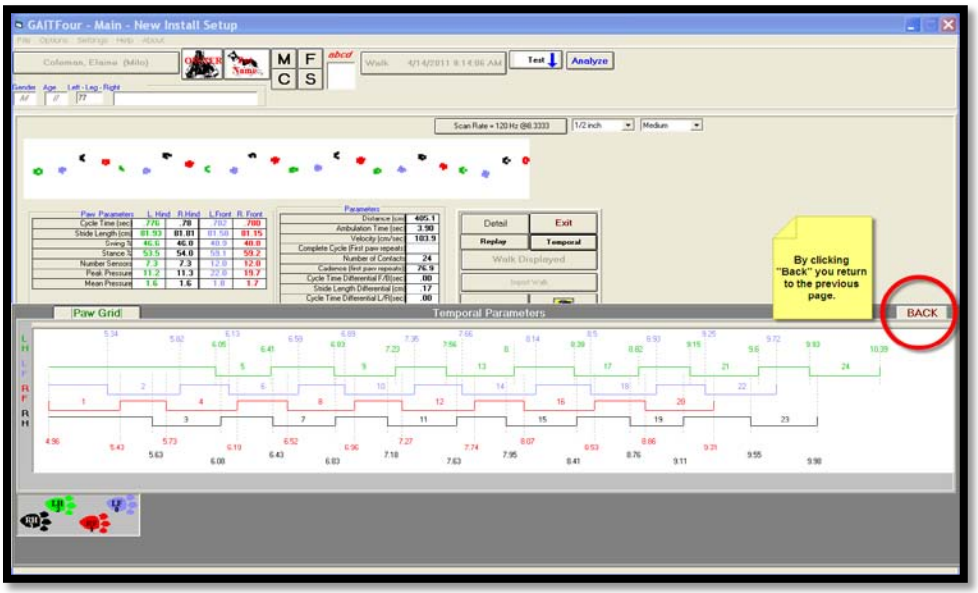




Temporal

Temporal is a color-coded according to paw linear graph that visually depicts the stance time (high plateau) and swing time (low plateau) of each paw for the entire walk.



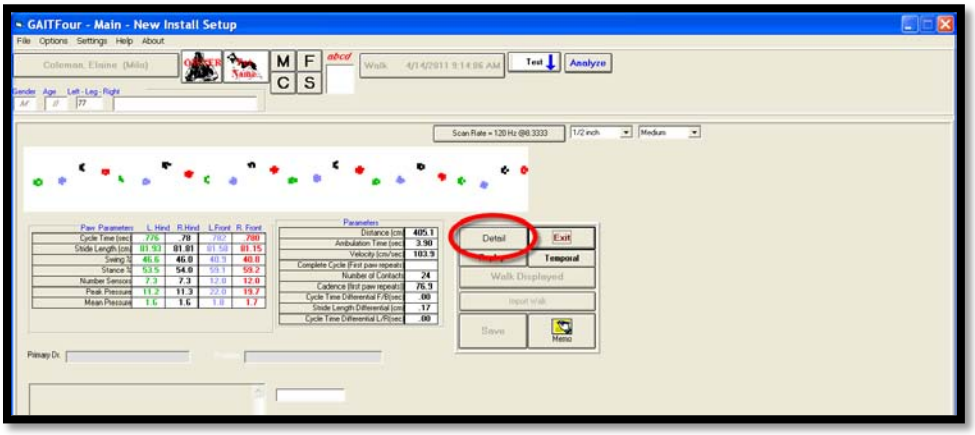


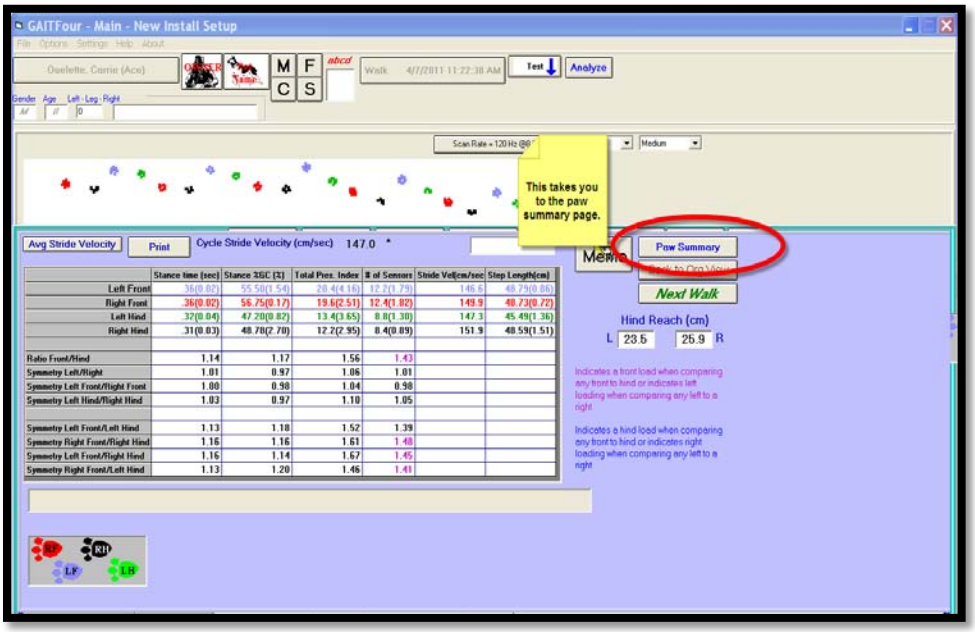
Exporting data

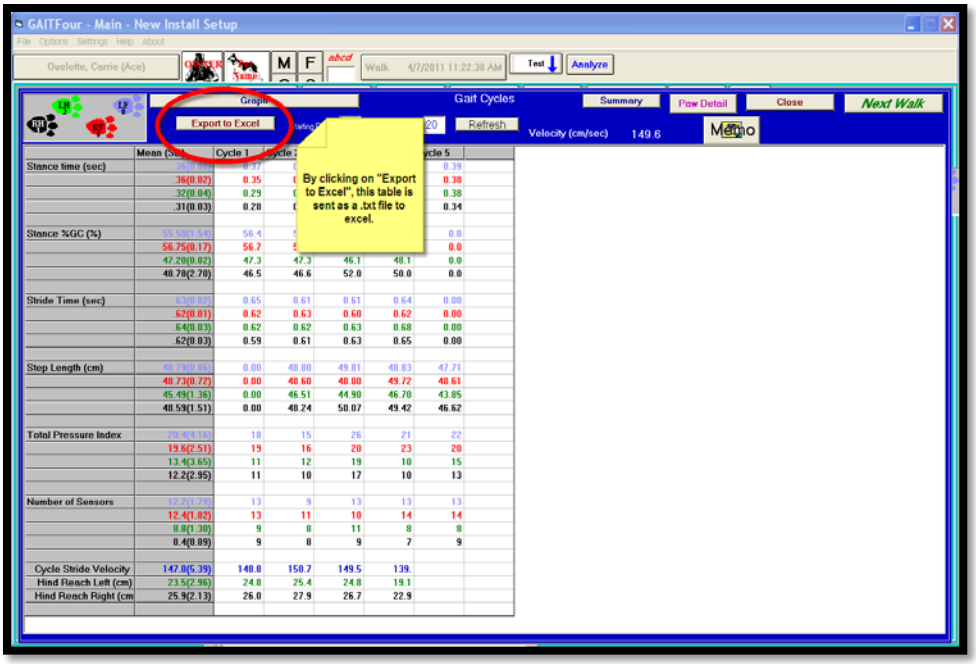
Export paw detail

You can export a detailed table of each paw strike for an individual walk. This can be done on the paw summary page.

Click on “Detail” then paw summary.

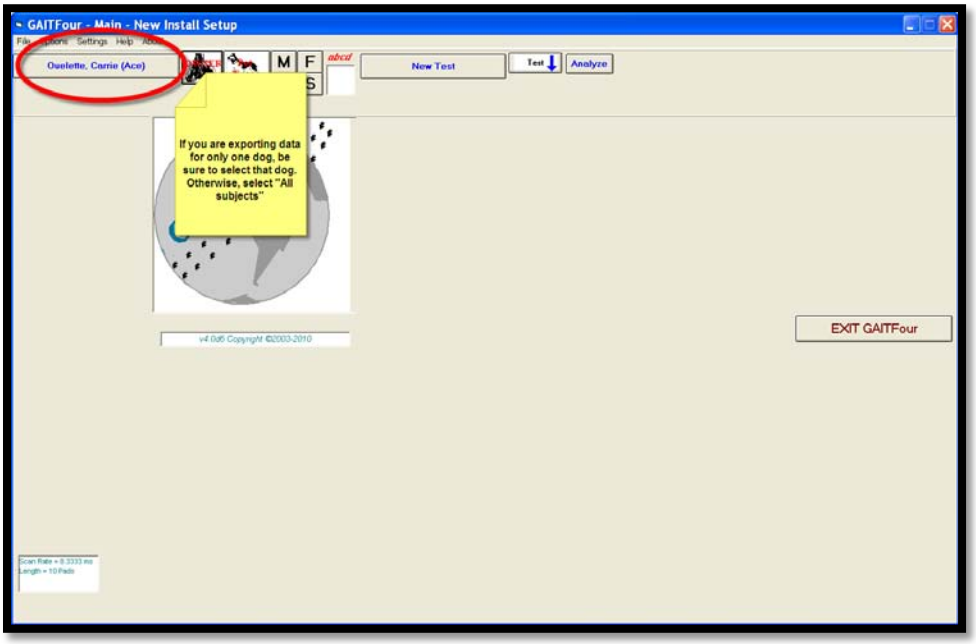




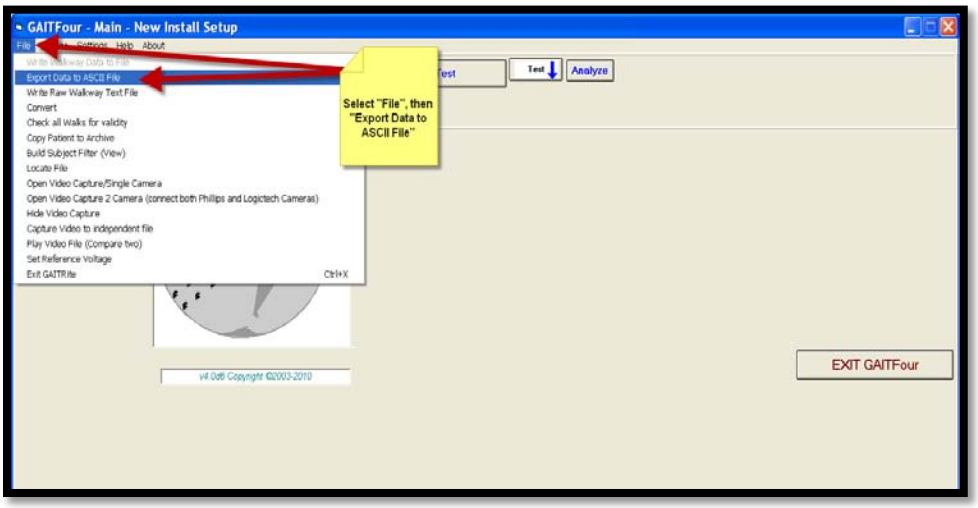


Export mass data-ASCII

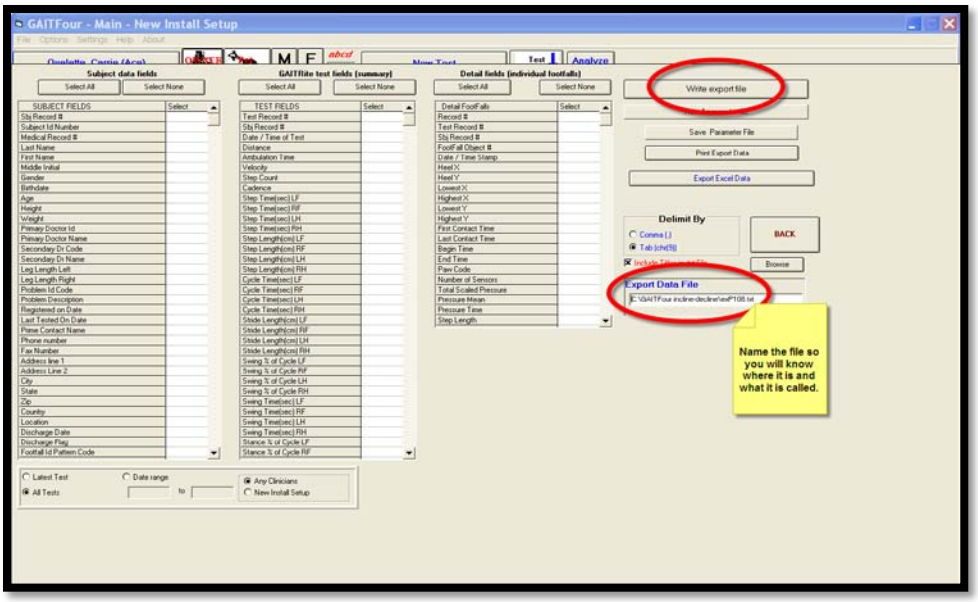
Many times it is necessary and/or beneficial to export multiple walks for a single dog or multiple walks for multiple dogs. On the main page first select your “subject” or “All subjects”.



Then go to <File> and <Export Data to ASCII File>



After you have selected your desired fields, name your file, click on <Write Export File>



In order to view this data, you will need to open a program that can import .txt files, like Excel.

Your exported .txt file should be located in the GAITFour folder on the c:\

When you are opening it you will need to select “Delimited” and then “Comma”.

In order to save any changes you make to your file, you will need to save it as an excel workbook file (.xls).

Interpretations

Gait analysis

Gait analysis provides for quantitative assessment of lameness beyond subjective analysis. It can be useful for orthopedic diagnosis and documentation of improvement during rehabilitation. The GAITFour is unique in that it measures not only temporal spatial variables, but it also measures a relative vertical component of pressure exerted by each paw. This relative vertical component is expressed as Total Pressure Index (TPI) and can be used to show weight distribution of the dog across all 4 limbs for several gait cycles. As research has shown using the force plate, kinetic data can vary among and within breeds. However, a symmetrical gait (walk, trot or pace) would be expected to yield similar symmetry ratios in healthy dogs, regardless of breed. When comparing left and right limbs, fore to fore and hind to hind, the ratio should be around 1.00 for healthy dogs. This is true not only for TPI but all of the measured variables. This deviates when you compare fore to hind in the measurements of Stance, TPI and # of sensors activated. (See Table) Research has shown that dogs carry ~60% of their weight over their forelimbs and ~40% over their hind limbs at rest and at a walk, giving a fore to hind ratio of 1.5. Dogs shift their weight forward as they increase their speed and conformation can also cause this % and ratio to vary slightly.

"Normal" symmetry ratio measurements

Symmetry ratios (mean ± SD) for healthy adult Labrador retrievers (n=56) at a walk, based on a portable electronic walkway system

	Stance time	Stance % of gait cycle	Stride time	Stride length	Total Pressure Index	# Sensors activated
Fore/Hind	1.11* † (±0.07)	1.10* † (±0.06)	1.00‡ (±0.02)	1.00‡ (±0.01)	1.62* (±0.23)	1.37* (±0.12)
Left/Right	1.00‡ (±0.03)	1.00‡ (±0.03)	1.00‡ (±0.01)	1.00‡ (±0.01)	1.01 † (±0.07)	1.00 † (±0.05)
Left Fore/ Right Fore	1.00‡ (±0.03)	1.00‡ (±0.03)	1.00‡ (±0.01)	1.00‡ (±0.01)	1.03 † (±0.09)	1.01 † (±0.06)
Left Hind / Right Hind	1.00‡ (±0.05)	1.00‡ (±0.04)	1.00‡ (±0.01)	1.00‡ (±0.01)	0.99 (±0.12)	0.99 † (±0.08)
Left Fore/ Left Hind	1.11* † (±0.08)	1.10* † (±0.07)	1.00‡ (±0.02)	1.00‡ (±0.01)	1.66* (±0.25)	1.38* (±0.16)
Right Fore/ Right Hind	1.10* † (±0.07)	1.10* † (±0.06)	1.00‡ (±0.02)	1.00‡ (±0.02)	1.60* (±0.28)	1.36* (±0.14)
Left Fore/ Right Hind	1.11* † (±0.08)	1.10* † (±0.08)	1.00‡ (±0.02)	1.00‡ (±0.02)	1.63* (±0.27)	1.37* (±0.16)
Right Fore/ Left Hind	1.11* † (±0.07)	1.10* † (±0.06)	1.00‡ (±0.02)	1.00‡ (±0.01)	1.63* (±0.25)	1.38* (±0.14)

* Forelimbs differed significantly from hind limbs, $p<0.0001$ † Repeatability Index >80% ‡ Repeatability Index >90%

Light et al. *Am J Vet Res* 2010; 71:997-1002.

Measurements and definitions

Encapsulated within the electronic walkway are sensor pads. Each sensor pad has an active area of 24 inches square (61cm square) and contains 2,304 sensors arranged in (48x48) grid pattern. The sensors are placed on .5 inch (1.27cm) centers. Multiple sensor pads are connected to form the desired length of the walkway. As the subject ambulates across the walkway, the pressure exerted by the feet onto the walkway activates the sensors. The walkway does not only sense the geometry of the activating objects but also the relative arrangement between them in a two dimensional space. In addition, the walkway senses the vertical component of the relative pressure exerted by the objects.

What makes the walkway valuable for gait analysis are the special algorithms build into the system. The algorithms isolate the objects and identify them as footprints.

Spatial parameters and definitions

The walkway does not only sense the geometry of the activating footprints but also the relative arrangement between them in a two dimensional space.

Stride length-is measured on the line of progression between the heel points of two consecutive footprints of the same foot (left to left, right to right). The unit of measure is centimeters.

Reach-is measured along the line of progression, from the heel center of the hind paw to the heel center of the previous forepaw on the same side. (For example left hind to previous left fore) Reach can be a negative value if the subject fails to bring the heel point of the hind paw forward of the previous fore paw heel point this is more likely found in a trotting gait. The unit of measure is centimeters.

Distance (Traveled)-is measured on the horizontal axis from the heel center of the first footprint to the heel center of the last footprint. The unit of measure is centimeters.

Temporal definitions

First Contact- is the time that the first sensor appears in any paw strike. It is expressed in seconds (sec).

Last Contact-is the time that the last sensor goes off in any paw strike. It is expressed in seconds (sec).

Stride Time- is the time elapsed between the first contacts of two consecutive footfalls of the same foot. It is measured in seconds (sec).

Gait Cycle Time-is the elapsed time between the first contacts of two consecutive footfalls of the same foot. It is measured in seconds (sec).

Ambulation Time-is the time elapsed between first contact of the first and the last footfalls. It is measured in seconds (sec).

Velocity-is obtained after dividing the Distance Traveled by the Ambulation time. It is expressed in centimeters per second (cm/sec).

Stride Velocity-is obtained after dividing the Stride Length by the Stride Time. It is expressed in centimeters per second (cm/sec).

Cycle Stride Velocity-is the average stride length of each gait cycle divided by the average stride time of each gait cycle. It is expressed in centimeters per second (cm/sec).

Stance Time- The stance phase is the weight bearing portion of each gait cycle. It is initiated by heel contact and ends with toe off of the same foot. It is the time elapsed between the First Contact and the Last Contact of one identified paw. It is expressed in seconds (sec).

Stance % GC- is the percentage of stance time compared to stride time or gait cycle. (stance time/stride time)

Swing Time -The swing phase is the non weight bearing portion of each gait cycle. It is initiated with toe off (Last contact) and ends with the next consecutive heel strike (First contact) of the same foot. It is expressed in seconds (sec).

Swing % GC-and it is also presented as a percent of the Gait Cycle of the same foot.

Switching levels

The GAITRite walkway's unique and patented sensor avoids false peripheral activation. Each sensor has been constructed with two flexible elements riding on a pivot point. When pressure is applied to the sensor both elements must flex around the pivot point to initiate activation, otherwise the pivot point will toggle the sensor in either side without activation. After activation, the sensor begins to change its value linearly with the vertical component of pressure exerted upon it. The walkway contains thousands of sensors, therefore calibration of each and every sensor is not impossible but cost prohibitive. Pressure values are normalized and expressed as a percent of the maximum pressure and then divided into levels. Currently there are seven switching levels, illustrated in Table 1.

The walkway does not only sense the geometry of the activating footprints but also the relative arrangement between them in a two dimensional plane and the relative vertical component of pressure exerted by each paw. The pressure is represented by a switching level.

Table 5-1 Switching level color assignment

Dark Gray	1=lowest
Light gray	2
Cyan	3
Yellow	4
Magenta	5
Red	6
Blue	7=highest

Switching level parameters

Total Pressure (P*t) for a paw, is the integrated pressure over time expressed as a percent of the overall integrated pressure over time. The overall P*t can be found by summing the P*t of each sensor, sectional P*t can be found by summing the P*t of the sensors included in the particular section also expressed as a percent of the overall P*t. (This related but not equal to impulse or Force*time)

Peak Time for a section is the first time point that one or more sensors within a section was at the maximum switching level.

Total Pressure Index (TPI)- the sum of peak pressure values recorded from each activated sensor by a paw during mat contact, represented by the switching levels and reported as a scaled pressure from 0 to 7 for each sensor. (This is related but not equal to Peak Vertical Force.)

Care of mat

The top layer of the mat is a water resistant and semi-durable material. Therefore, periodic cleaning can be done using a **damp** mop of mild cleaning/disinfectant solution.

****DO NOT ALLOW MAT TO GET OR REMAIN WET!!!!!****

When rolling up the mat, use the cardboard tube and store it flat in its case. Do not fold or otherwise crease the mat.

If you choose to leave it out, please make sure it is secure.

For example:

1. No water source nearby that could damage it.
2. Not in the middle of main traffic where unsuspecting people could trip, roll over it or damage it without realizing.
3. Do not allow high heels or x-ray machine to cross the mat.

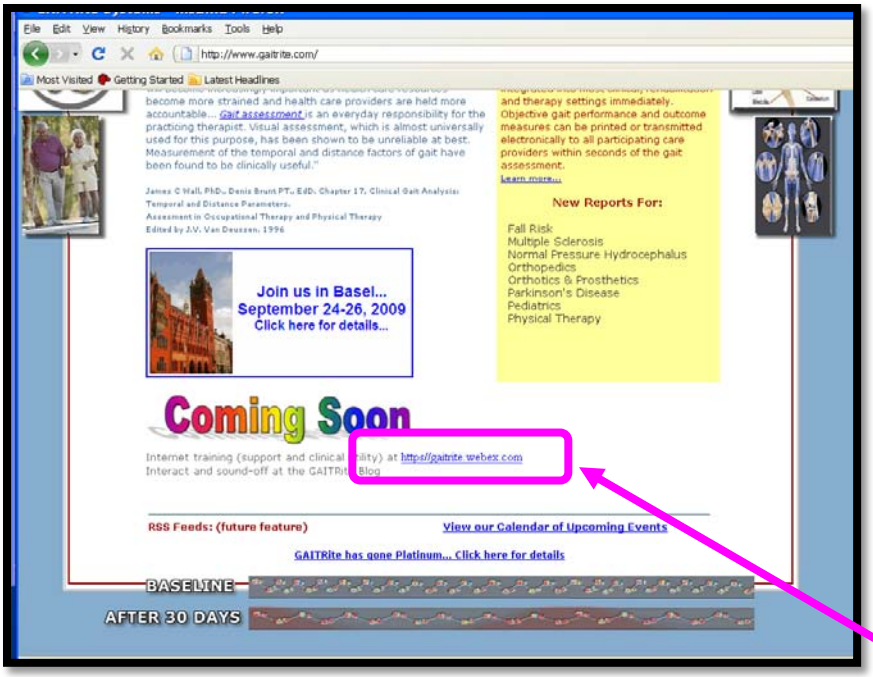
Troubleshooting

Web-Ex

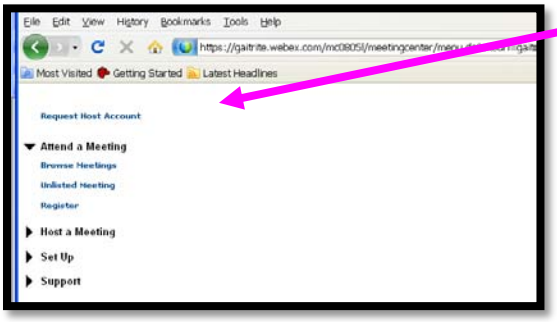
Help is available in a variety of ways. One those ways is internet conferencing via Web-Ex. These meetings can be set days or minutes in advance. But remember, You must have a computer that can access the internet and it would be best if that computer is the one you are using to collect you GAITFour data.

Once you are on the internet, (Internet Explorer is best for this program) Go to www.gaitrite.com

Scroll to the bottom of the page and find the link to GAITRite's webex link.



Once you are on this page, Click on the “Browse Meetings” link.



Search for your meeting and “join it”

Type in your email address, your name and “gaitrite” as the password.

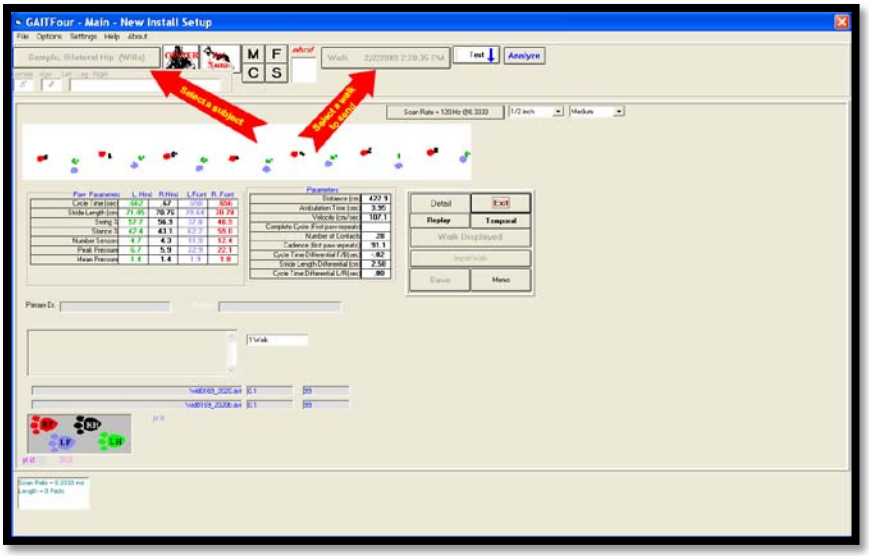
It may require you to “load” the proper software. This should only take a couple of minutes.

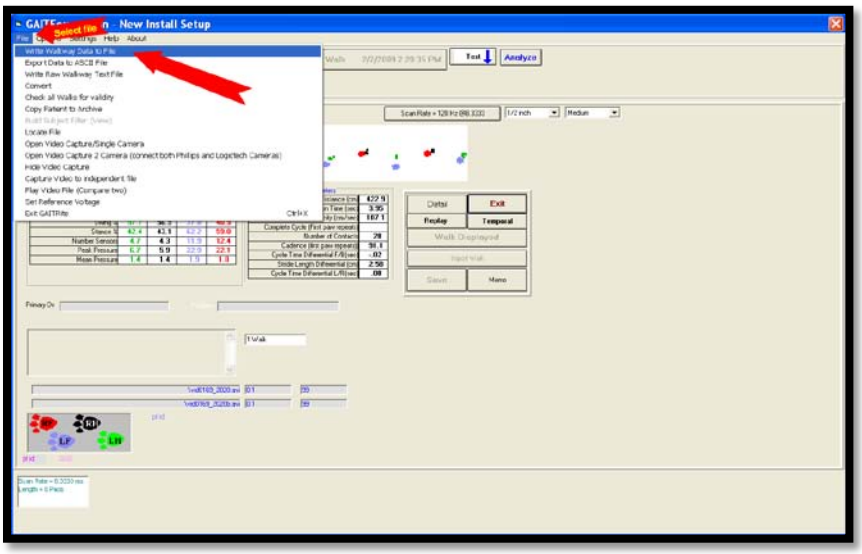
If you have a headset for your computer, you may “Use your computer” to talk to others in the conference. Otherwise, use your phone and follow the “Audio” instructions once you have joined the meeting.

Sending a single walk

If you need help with a single walk, you can email it to customer support at sales@gaitrite.com

But before you can do that you will need to prepare the data. First you will need to select the subject and the walk you are interested in sending. The figure below is a walk that has already been processed. You may do the same with an unprocessed walk.

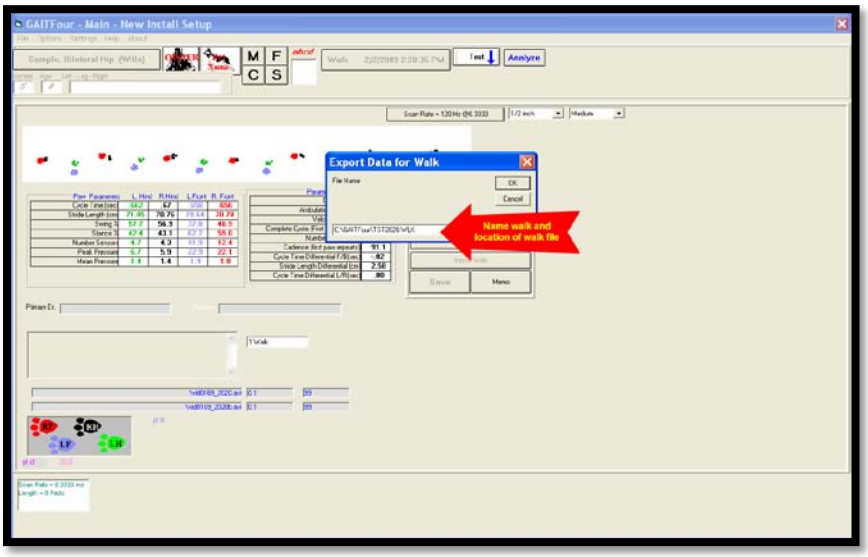




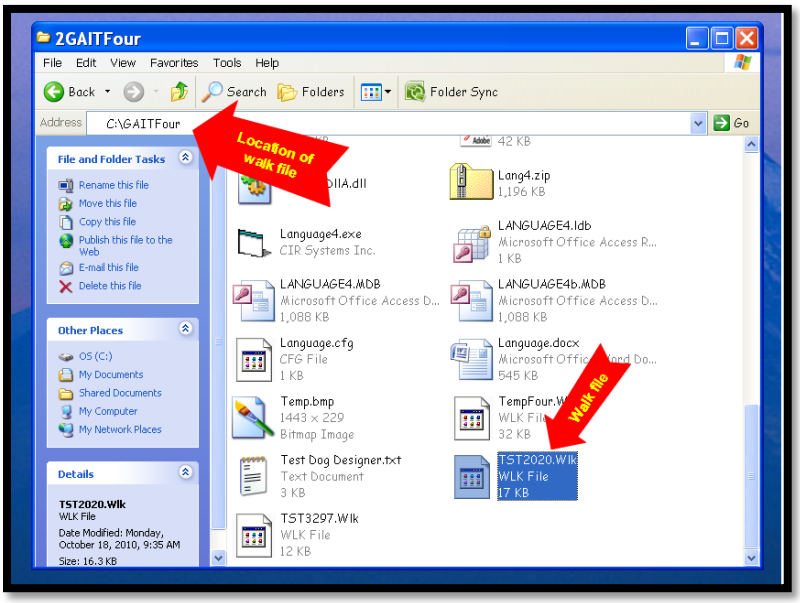
Next, you will select <File> and then <Write walkway data to file>

This will take you to a screen where you can save the file.

Typically, the walk file will be named according to the walk ID given to it by the software. You are welcome to change it so that it makes sense to you.



When you are in your email account, you can then attach the file to the email.



When you “Browse” to attach the file, it should be located in the “GAITFour” folder found on your c drive.

GAITRite P400 series specifications

Table 5-2 Dynamic force measurement test conditions

Table 2: Dynamic Force Measurement Test conditions Sensor cell, air actuated plunger, .5" diameter, with .125" rubber under sensor cell. Plunger is placed perpendicular to sensor and Digital force gauge. Digital Force Gauge, Chatillon DFI-100, .1 lbs resolution, ±5% accuracy, pre-calibrated. Data from 25 pads (randomly selected), 5 sensors per pad (randomly selected), 100 actuations per sensor.		
GAITRite Displayed Switching Level/color	GAITRite Walkway Device A/D Binary Output (Reference 100)	Digital Force Gauge Reading (Lbs)
0 / none	0-31	0.0-0.25
1 / dark gray	32-63	0.26-2.4
2 / light gray	64-95	2.5-4.8
3 / cyan	96-127	4.9-6.8
4 / yellow	128-159	6.9-8.9
5 / magenta	160-191	9.0-11.0
6 / red	192-223	11.1-13.0
7 / blue	224-256	13.1-15.0

Table 5-3 GAITRite sensor cell technical specifications

Table 3: GAITRite Sensor Cell Technical Specifications	
Parameter	Sensor Specification
Sensor Cell Size	.425” x .275” x .010” (11mm x7mmx.254mm)
Accuracy	±.15 switching level
Cycle-to-Cycle Repeatability	±.1 switching level
Max. Applied Pressure	400 psi
Hysteresis	±.18 switching level
Rise Time	30usec per switching level
Projected Lifetime	>8M actuations At 79K actuations shows < ±.25 switching level change
Temperature	0° C to +70°C
Maximum Current	.7 mAmps
Creep	<1 switching level per day
Quality Control Method	Batch Testing (10% of sensor cells per pad at 20 actuations per sensor cell, randomly selected)

Table 5-4 GAITRite pad technical specifications

Table 4: GAITRite Pad Technical Specifications	
Parameter	Pad Specification
Sensor Cell Size	.425” x .275” x .010” (11mm x7mmx.254mm)
Number of cells per pad	2304 arranged in 48 x 48
Sensor Cell spacing	.5” (±.005)
Pad Dimensions	24” x 24” x .010”
Quality Control Method	Batch Testing (10% of pads, randomly selected)
Pad-to-Pad Spatial Accuracy	±.010”
Pad-to-Pad Switching Level Accuracy	±.25 switching level
Pad Spatial Accuracy Batch-to-Batch	±.020”
Pad Switching Level Accuracy Batch-to-Batch	±.5 switching level Data represented by 5 batches of pads

Table 5-5 GAITRite overall walkway technical specifications

Table 5: GAITRite Overall Walkway Technical Specifications **Specifications are for 10 pad walkway**	
Parameter	Walkway Specification
Walkway Overall Dimensions	35.25” x 276” x .250” (.375” electronics box) 90cm x 700 cm x .65 cm (.95 cm electronics box)
Active Area (for 10 pad walkway)	24” (±.015) x 240” (±.15”) 60.96cm x 609.6cm
Spatial Resolution Accuracy	±.5” ±1.27cm
Sample Rate	60, 80, 100, 120,180, 240Hz
Temporal Accuracy	±1 sample
Quality Control Method	Individual Testing Placed over existing walkway previously calibrated and compared against 3D Video System. Compare Ambulation Time and Ambulation Velocity between the two systems. Expected Ambulation time within 1 sample rate Expected Ambulation Velocity within ±2%
Walkway-to-Walkway Spatial Accuracy	±.5” ±1.27cm Data >200 walkways
Walkway-to-Walkway Temporal Accuracy	±1 sample Data >200 walkways
Walkway-to-Walkway Switching Level Accuracy	±.5 switching level Not tested here but assumed, see pad specifications, Pad Force Accuracy, Batch-to-Batch.

Chapter 6. Summary of Results and Conclusions

Cranial cruciate ligament ruptures have been one of the leading orthopedic issues in dogs and have had an enormous economic impact. Many studies have implicated multiple risk factors for cruciate rupture in the dog, including gender and gonadectomy. By our experimental design with removal of sex steroids and then supplementation of T, DHT and $E_{2,}$ we found that sex hormones influence the AR and ESR1, MMPs and in turn collagen content and collagen fiber diameter in the prepubertal male rabbit. Collagen concentrations were decreased and fiber diameters were increased in the absence of hormones, in association with ESR1 and AR protein expression. Ultimately, our study indicates that physiological changes triggered by the lack of sex hormones following prepubertal gonadectomy potentially predispose male animals to orthopedic injuries. Further research is necessary to elucidate the interaction between sex hormones and MMP activity, as well as the relative roles and/or the interaction of sex hormones and other physiological factors such as signaling pathways mediated by growth factors in the regulation of CCL development prior to attainment of puberty and/or cessation of physical development.

In order to detect gait abnormalities, such as those seen with CCLR, gait analysis is necessary. Through the evolution of gait analysis over the last 2 centuries, both kinematic data collection, such as 2D and 3D video analysis, and kinetic data collection, including force plate and pressure walkways, have emerged. Historically, in order to obtain useful data, collection and analysis protocols have been time consuming and cumbersome. Thus, objective gait analysis has not been utilized effectively in the clinical setting. Consequently subjective gait analysis, i.e.

visual analysis, has been used historically in a clinical setting but it can be unreliable and has been shown to differ within and between observations and observers. Objective measures are needed in a clinical setting in order to help diagnose subtle lameness, document recovery from surgery, analyze rehabilitation protocols and document medical treatment of abnormalities that alter gait. This might be accomplished through the use of a pressure-sensitive walkway system, a newer technology introduced to the veterinary field. A basic manual for use of the technology and a protocol for data collection and analysis were established for this walkway system. The protocol includes 1) walking the dog with a loose leash (no pulling on handler or dog), 2) a consistent velocity (i.e. steady state gait with <10% variation within the walk), and 3) no overt head turning. Take the mean of 3 valid trials at a preferred velocity, with <10% variation between walks, to assess measurements and symmetry ratios.

The pressure-sensitive walkway system utilizes temporal-spatial as well as a kinetic form of gait analysis and was used in this study to establish normative data for Labrador Retrievers. Temporal-spatial measurements were determined for ST, ST%, SrT, and SrL, as well as MPI, TPI and NS. It was determined that by using symmetry ratios for these measurements, data may be transferable between breeds and may be useful in discovering subtle lameness by identifying ratios outside 1 STDV of these symmetry ratios. Individual kinematic data not placed into ratios need to be collected as breed or body style specific.

By using the established symmetry ratios from our previous study, orthopedically normal dogs were compared to dogs recovering from TPLO surgery. TPI ratios were found to be the most reliable indicator for lameness. The trend line established in this study could be used as a basis of comparison for alternate surgical options as well as rehabilitation protocols for recovery from TPLO surgery. The walkway system could also record objective data gathered for clinical

trials or in research where lameness grade and gait assessment are outcome measures. These findings may alter clinical gait analysis by offering an alternative method of objective gait analysis. Further research using the pressure walkway system needs to be done for breed-specific kinematic measures, alternate surgical procedures for CCLR, rehabilitation protocols after TPLO surgery and alterations in gait with other pathologies, such as hip dysplasia.