Effect of tibial plateau levelling osteotomy and rehabilitation on muscle function in cruciate-deficient dogs evaluated with acoustic myography

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RESEARCH ARTICLE

Abstract

The objective of the study was to determine the function of the biceps femoris, quadriceps, gastrocnemius and semitendinosus muscles at the walk in dogs with unilateral clinical cruciate disease and palpable joint instability. To compare function before and after a tibial plateau levelling osteotomy (TPLO) procedure, and after six weeks of subsequent rehabilitation therapy. Fourteen adult client-owned dogs with cranial cruciate ligament deficiency (CCLD). Orthopaedic examination, temporospatial gait analysis and acoustic myography (AMG) recordings were made at three time points: before TPLO, and post-operatively at two and eight weeks. A rehabilitation program started 2 weeks after surgery and was either in-clinic along with in-home rehabilitation or in-home only. Statistics included: repeated measures ANOVA and paired t-tests. Significance was set at $P<0.05$. When comparing the affected versus the unaffected limb in the CCLD dogs, there were no significant differences found in AMG values between baseline and other time points for the quadriceps and semitendinosus muscles. The gastrocnemius and biceps femoris muscles had a significant change in spatial summation (S) score over time. The gastrocnemius (S) score was not significantly different to the unaffected limb by 8 weeks post TPLO. There was no significant effect of rehabilitation method on S score. Dogs with in-clinic rehabilitation regained more symmetry in thigh circumference versus in-home only. Lameness parameters improved but did not completely resolve in all dogs by week 8 post TPLO. The function of the gastrocnemius muscles in affected limbs was significantly different to normal limbs at baseline and 2 weeks post TPLO but not at 8 weeks. Thigh symmetry, but no other parameters, was improved with the addition of in-clinic rehabilitation.

Keywords: cranial cruciate ligament, TPLO, symmetry, gait, canine

1. Introduction

Cranial cruciate ligament deficiency (CCLD) in dogs is a leading cause of stifle pathology and subsequent lameness (Griffon, 2010). Research studies investigating outcomes after the tibial plateau levelling osteotomy (TPLO) stabilisation procedures have largely focused on kinetic and kinematic measures of gait (Au *et al.,* 2010; Böddeker *et al.,* 2012; Conzemius *et al.,* 2005; Nelson *et al.,* 2013; Wucherer *et al.,* 2013). Assessment of individual muscle function in cruciate deficient limbs has been limited (Adrian *et al.*, 2019; Wilke *et al.,* 2005), and it is unclear whether TPLO restores normal muscle function. Adequately functioning muscles are needed for precise muscle activation and coordination, which is necessary for controlled movement (Korvick *et al.*, 1994). Restoration of normal muscle function allows for a dynamic balance of the activation of muscles that surround the stifle joint, allowing for normal stifle kinematics (Budsberg *et al.,* 1988). Any alteration in the timing or amplitude of muscle contractions will lead to uncoordinated movement (DeCamp *et al.,* 1996), which could contribute to the worse return to athletic function seen with dogs having undergone surgical treatment for cruciate disease as compared to other injuries (Tomlinson and Manfredi, 2018).

Surface electromyography (sEMG) of the biceps femoris and semimembranosus muscles in cruciate deficient dogs during movement has been performed but the study

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had limitations (Hayes *et al.,* 2013). Examination of two muscles with similar action (flexion of the stifle joint) and exclusion of the quadriceps, a stifle extensor (opposing muscle) presented a partial picture of what happens around the joint (Hayes *et al.,* 2013). The study found that in both affected and unaffected limbs there was evidence of delay in muscle activation in both muscles as compared to normal limbs, which was speculated to contribute to progression of cranial cruciate ligament damage rather than to be active muscular compensation as a sequela of cruciate ligament insufficiency and joint instability (Hayes *et al.,* 2013). Without measuring the action of an opposing muscle, more information cannot be concluded. A separate study, using cadaver limbs, revealed that tension in the semitendinosus muscle works to reduce cranial tibial translation (Riis *et al.,* 2013). The authors also postulated that the quadriceps and gastrocnemius muscles under tension could contribute to cranial tibial translation (Riis *et al.,* 2013). It has been found however, through another cadaver study, that pre-activation of the quadriceps before ground strike could actually reduce cranial tibial translation (Valentin and Zsoldos, 2016). True muscle action in cruciate deficient limbs during gait cannot be replicated in a cadaver study. In a radiofrequency induced model of CCLR, examination of action in the quadriceps, biceps femoris and gastrocnemius identified no significant changes in muscle function at any timepoint (Adrian *et al.,* 2019). Evaluation during movement of unilaterally affected cruciate deficient dogs with natural disease and subsequent treatment should present a more complete picture.

How post-operative rehabilitation can affect muscle function in cruciate deficient limbs is unknown. Rehabilitation used in the recovery process after TPLO and has been shown to be beneficial for improving limb function and short-term functional outcomes (Romano and Cook, 2015). Therapeutic exercise, as part of a formal rehabilitation plan is an important aspect of recovery post TPLO and can help build muscle mass and strength in dogs recovering after surgery (Kirkby Shaw *et al.,* 2019; Marsolais *et al.,* 2002; Millis and Ciuperca, 2014; Monk *et al.,* 2006). Acoustic myography (AMG) is a non-invasive way of assessing muscle function by measuring the small sound produced by a muscle contraction (Hayes *et al.,* 2013; Kanno *et al.,* 2012). Skeletal muscle emits a continuous, low frequency sound as it contracts due to vibrations generated during contraction (Ramirez *et al.,* 2015). The sound waves can be measured transdermally above the muscle of interest. Acoustic myography (AMG) is similar to surface electromyography (sEMG), as both techniques are used to measure the recruitment of muscle fibres during activity and they both relate the amplitude of the signal, whether sound (AMG) or electricity (sEMG), to motor unit activity. The piezoelectric AMG sensor is thin and only measures sound waves moving in one direction, thus minimising interference from lateral movement of the sensor on the skin which is a problem encountered with surface electromyography (Harrison, 2017).

The AMG equipment records rate of recruitment of motor units, number of active fibres recruited and fibre synchronisation. This information is translated into three scores, the E, S and T scores (Figure 1). The E score (s), referred to as the efficiency score, assesses muscle activity in relation to inactivity. For the efficiency (E) score a high score on a scale of 0-10 represents coordinated muscle contraction with less time contracting versus relaxing (Harrison, 2017). Fatigue is an exercise induced reduction in the muscle's capability to produce force (Gandevia, 2001). A fatigued muscle tends to be less coordinated (Branscheidt *et al.,* 2019; Danna Dos Santos *et al.,* 2010; Forestier and Nougier, 1998). The S-score (mV) is spatial summation and refers to the amplitude of the sound during contraction, a reflection of the number of active recruited muscle fibres. The calculated spatial summation (S) score is high when few muscle fibres are active (low amplitude) during physical activity (Harrison, 2017). AMG shows higher S scores, close to or equal to 10 on a 0-10 scale, represents optimal muscle function, such as in trained muscle (Harrison *et al.,* 2013). The T score (Hz) refers to the rate of muscle contraction during a movement (firing rate or temporal summation). A high temporal summation (T) score represents a low firing rate of active fibres, meaning that the muscle does not have to recruit fibres repeatedly to do the work (Harrison, 2017).

The aim of this study was to determine the effects of naturally occurring CCLD and TPLO procedure on muscle function and gait parameters, and to investigate whether a specifically tailored in-clinic rehabilitation regimen can improve muscle function post TPLO surgery over at-home care. Our hypothesis was that CCLD with resultant joint instability alters the function of the biceps femoris, quadriceps, gastrocnemius and semitendinosus muscles. Furthermore, we predicted that TPLO with in home rehabilitation would not fully restore function of these muscles, but that adding in-clinic rehabilitation therapy after TPLO would restore normal muscle function.

2. Materials and methods

Subjects and inclusion criteria

Fourteen client-owned skeletally mature dogs with a history of unilateral pelvic limb lameness due to CCLD and subsequent instability were recruited between December 2017 and February 2020. Participation in the study was under signed owner consent.

Study groups and overall study timeline

All affected dogs underwent TPLO surgery followed by standard post-operative instructions from the same surgeon (Diplomate of the American College of Veterinary Surgeons) for the first 2 weeks. Two weeks after the TPLO procedure,

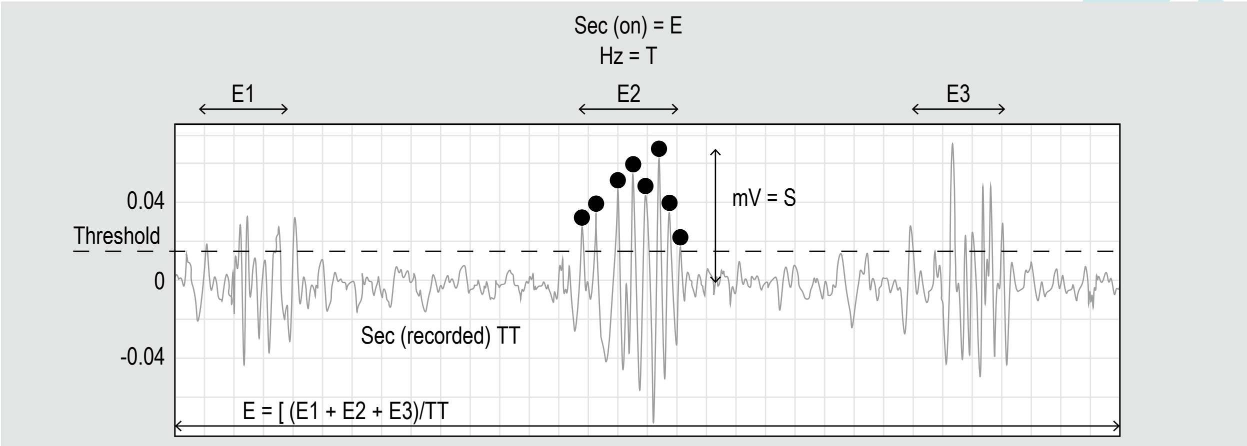

Figure 1. Graphical explanation of E, S and T scores in acoustic myography (AMG). To analyse the AMG signal, a threshold is determined (dotted line) above which individual spikes are considered derived from muscle contractions (lines marked with black circles). Adapted from Fuglsang-Damgaard *et al.* (2021).

the dogs were divided into two groups: (1) affected dogs undergoing in-clinic rehabilitation combined with in-home rehabilitation (n=7) and (2) in-home rehabilitation only (n=7). Each program was prescribed by a rehabilitation and sports medicine specialist (Diplomate of the American College of Veterinary Sports Medicine and Rehabilitation).

Data collection (orthopaedic examination, AMG, temporo-spatial evaluation, and owner assessed Canine Brief Pain Inventory score) occurred in all dogs at baseline, 2 weeks post TPLO and 8 weeks post TPLO. All 14 dogs completed the study.

Orthopaedic evaluation, including radiography

All dogs underwent a standard orthopaedic examination by the Diplomate American College of Veterinary Surgeons (DACVS) (AJ) and the Diplomate American College of Sports Medicine and Rehabilitation (DACVSMR) (JET) investigators preoperatively. The stifle joint was examined for instability (cranial drawer and cranial tibial thrust tests). Radiographic evaluation by a board-certified radiologist Diplomate of the American College of Veterinary Radiology (DACVR), for all of the dogs in the study, imaged the coxofemoral, stifles and tarsal joints to screen for any orthopaedic abnormalities in the rear limbs, specifically for stifle intra-articular effusion and degenerative changes, such as osteophytosis of the femoral condyle surfaces, tibial condyles and patella. Dogs with evidence of orthopaedic disease other than CCLR were excluded from the study. Evaluation by the DACVSMR investigator (JET) included palpation for evidence of orthopaedic disease, discomfort, and muscle spasm and included goniometric measurement (Essner *et al.*, 2017) of passive range of motion of the stifle, tarsal and coxofemoral joints in both limbs (Jaegger *et al.*, 2002). Three consecutive goniometric measurements were made for each joint, with the mean value used in accordance with published guidelines (McCarthy, 2018). Thigh circumference, as measured by a spring weighted tape measure (Gulick II Warrenville, IL, USA) was performed on both limbs three times per limb with the mean measurement being used. The same evaluation by the DACVSMR investigator (JET) was performed at every data collection point (baseline and 2- and 8-weeks post TPLO) for the affected dogs. Body weight was recorded at each data collection point.

Temporospatial gait analysis

Dogs were evaluated at a walk, with one handler, moving over a pressure sensitive walkway (gait4dog CIR Systems Inc, Franklin, NJ, USA) while wearing a canine harness (Julius-K9 IDC® Powerharness, Tampa, FL, USA). Proprietary designated software (Gait4software®, Franklin, NJ, USA) that was made by the same company as the pressure sensitive walkway was used for acquisition and analysis of the data. If the subject became markedly more lame, as determined by a sudden decline in temporospatial parameters, then a 10 min rest was instituted before resuming data collection. Before every data collection, each dog was familiarised with the environment and walkway with a 10 min pre-measurement period to acclimate to the room followed by two slow practice walks over the walkway. Dogs were walked across the walkway in a straight line. A comfortable walking velocity was maintained and recorded by the system including analysis for any acceleration (e.g. 0.15 m/s^2). This information was used to select gait data with a consistent pace, represented as less than 10% variability in velocity (Gait4software). Three valid walks of consistent speed were analysed for each dog before placement of the AMG equipment. A valid walk was when each limb made contact with the surface of the walkway at least three times, without excessive head motion by the subject. Real time video capture of each trial enabled confirmation of straight head position and limb contact. Valid trials were obtained for both hind limbs simultaneously.

Temporospatial parameters recorded at the walk included the percent total pressure index (TPI) and the mean TPI, mean percent stance, mean stride length and mean step: stride ratio for each hind limb.

Acoustic myography

AMG sensors (MyoDynamik sensors, Copenhagen, Denmark) (50 mm diameter) were used to measure the acoustic signals of each muscle group (quadriceps, biceps femoris, gastrocnemius, semitendinosus) while walking. The sensors were placed at the same level on every dog, using bony prominences for accurate and repeatable placement (Figure 2 as similarly described by previous sEMG studies for sensor placement (McClean *et al.*, 2019). The quadriceps (vastus lateralis) sensor was placed on the craniolateral thigh at 2/3 femoral length. The biceps femoris (cranial head) sensor was placed 1/3 of the way down the femur as measured from the greater trochanter (McClean *et al.*, 2019), and the semitendinosus sensor was placed 2.5 cm distal to the ischial tuberosity. The sensor over the medial gastrocnemius muscle was placed just distal to the articular margin of the stifle joint but covered most of the muscle belly in all cases, therefore measurements of bony landmarks were not used to further pinpoint placement. Sensors were placed over the muscle by first parting the fur, (in longer haired dogs, the fur was trimmed short with clippers using a #40 blade), then a small amount of acoustic coupling gel (Ekkomarine Medico A/S, Holstebro, Denmark) was placed on the skin and the sensor, and the sensor was secured in place by an adhesive bandage (Snøgg AS, Kristiansand, Norway). The sensor was then connected with cables to the recording device (CURO unit MyoDynamik ApS, Frederiksberg C, Denmark), which had recording equipment affixed to the harness handle. The AMG signal from the muscle was transmitted remotely via an internet Wi-Fi signal from the recording device (CURO unit) to a handheld computer tablet (iPad, Apple Inc., Cupertino, CA, USA) and muscle measurements were followed in real time, viewed on the handheld computer tablet (iPad), to ensure proper transmission of recordings from the sensors. The dogs were walked by the same handler who walked them for the gait evaluation. The AMG recordings were taken with dogs walking over the pressure sensitive walkway (10-15 s) in order to ensure a consistent walking pace in a straight line without significant acceleration; AMG data was recorded three times for each muscle per session. Two separate muscles were measured simultaneously: quadriceps & biceps femoris, and gastrocnemius & semitendinosus. Frequency and amplitude were calculated from the sound waves produced. Recordings are made at a sampling rate of 1000 Hz – (1000 pps maximum recorded sound) with 10-bit resolution. The E, S, and T scores as described above were collected and analysed.

When analysing the AMG muscle data, the threshold was routinely set at 0.2 and adjusted as necessary, such as when scores were 0 or 10 (maximum value) (Figure 2)). Other set parameters for data analysis included a maximum frequency (max T) of 160 Hz (Fenger and Harrison, 2017) which is set to the maximum firing frequency detectable based on previous studies (Ejersted Anderson and Harrison, 2019). Muscles from both hind limbs were evaluated in all subjects.

Canine Brief Pain Inventory score

Owners filled out a canine brief pain inventory (CBPI) score form (Essner *et al.*, 2017) on the same day as, and just before each recording of temporospatial data. The same individual filled out the form each time, and it was done for all dogs.

Surgical procedure

Surgery on affected dogs was performed within 3 weeks of baseline data collection by the same surgeon (DACVS) (AJ) who did the baseline evaluations on all dogs. Arthroscopic evaluation of the affected stifle was performed, meniscal debridement performed if appropriate, and a TPLO procedure completed. Radiographs were taken immediately after surgery to verify implant placement. While in the hospital (during recovery hours), all dogs received cryotherapy. The peri-operative analgesic plan included locoregional anaesthesia with liposomal bupivacaine and non-steroidal anti-inflammatory medications (carprofen n=8, robenacoxib n=5 and deraxocib n=1). Exercise was restricted to short, leashed elimination breaks (typically of 5 min duration four to six times daily) using a sling for support.

Rehabilitation protocols

Immediate postoperative period

At home, all dogs received cryotherapy and had passive range of motion exercises on the affected stifle 2 times a day for 14 days as per the surgeon's instructions. Exercise was restricted to short, leashed elimination breaks using a sling for support. Two weeks post-operatively all the dogs were split into two groups: those that received in-home rehabilitation only (n=7), and those that had in clinic and at home rehabilitation (n=7).

In-home rehabilitation protocol (n=14)

Beginning after the 2-week data collection (2 weeks post TPLO), in-home rehabilitation exercises were prescribed for all dogs. All dogs completed the in-home rehabilitation

Figure 2. Dog wearing acoustic myography equipment.

as prescribed by the surgeon. Seven dogs continued with in-home rehabilitation exercises for six more weeks after the initial two weeks post TPLO, and seven dogs were enrolled in an in-clinic rehabilitation protocol (see the next section below). The in-home rehabilitation consisted of passive range of motion exercises and gentle postural perturbations (2 times a day, 7 days a week, for four weeks), cryotherapy (2 times a day, 7 days a week, for 2 weeks), walking over cavaletti poles (once a day, 7 days a week, for 3 weeks), and progressive walks starting at 5 minutes twice daily after two weeks and lengthening by 5 min a week. At week 4, the sidestep exercise was introduced (1 time daily, 7 days a week for 4 weeks). At week 5 the sit to stand transition exercise was introduced (5 brief sits, 1 time daily, 7 days a week for 3 weeks). At week 6 (week eight post op) walks were increased to 30 min. The sit repetition was increased to 10 reps, side stepping was increased to 10 steps each way. Owners were instructed to contact the rehabilitation clinic if there was an issue with the in-home exercises (patient compliance, patient ability to perform).

In-clinic rehabilitation protocol (n=7)

These dogs received six weeks of in-clinic rehabilitation twice weekly starting at 2-weeks post TPLO procedure in addition to the in-home rehabilitation program described above with the exception that owners were asked in person how the in-home rehabilitation was progressing, and those protocols would be adjusted if deemed necessary (n=0). Low level laser therapy (810 nm 500 mW, contact probe) was applied to the operated stifle at each session for all dogs. The progression of in-clinic rehabilitation exercises was dependent on patient comfort and apparent limb use. In-clinic therapeutic exercises included hydrotherapy walking in an underwater treadmill building from 5 to 20 min (n=7), balance and coordination exercises for 5 min (n=7) and stretching for 1 min (n=7) for all the in-clinic rehabilitation group dogs.

Statistical analysis

Normality was evaluated with a Shapiro-Wilks Test. Comparisons between time points and groups were made with repeated measures ANOVAs and Student's t tests (significant at $P<0.05$) using a dedicated statistical program (Prism 8, Graph Pad Software, San Diego, CA, USA).

3. Results

Subjects

Affected dogs were mixed breed (n=5), Weimaraner (n=1), Golden Retriever (n=2), Labrador Retriever (n=2), Boxer (n=1), American Staffordshire Terrier (n=1), Goldendoodle (n=2). There was one intact male, four neutered males, and nine female spayed dogs. Ages ranged from 1 year to

13 years of age, 1 year (n=1), 2 years (n=1), 3 years (n=1), 4 years (n=1), 6 years (n=5), 8 years (n=1), 9 years (n=2), 10 years (n=1), 13 years (n=1). All dogs were skeletally mature. Mean ± standard deviation (SD) for age was 6.4±3.3 years and for body weight was 63.9±12.9 pounds. The right stifle was affected in two dogs and the left stifle was affected in twelve dogs. The mean duration of lameness prior to presentation was 7.7±7.3 weeks.

Orthopaedic evaluation

Range of motion in extension

There was a significant difference in maximum range of motion in extension at baseline between the affected and unaffected ($P<0.005$) limbs. The affected limb had significantly less range of motion in extension. Two weeks post TPLO procedure the range of motion in extension values of the affected limb were no longer significantly different to the unaffected limb.

Thigh circumference

Overall, thigh circumference decreased in both the affected limb and unaffected limbs ($P<0.001$) at weeks 2 and 8 post TPLO as compared to baseline (Figure 3). There was a significant effect of in-clinic rehabilitation on thigh circumference at 8 weeks post TPLO, with Group 1 (in-clinic rehabilitation with at-home rehabilitation) having more symmetric thigh circumference at 8 weeks post TPLO as compared to Group 2 (in-home rehabilitation only) ($P=0.02$). Nine dogs had improved thigh circumference by week 8 post TPLO, six of these were in Group 1 and three in Group 2. Two dogs developed a greater difference in thigh circumference versus the unaffected limb by week 8 post TPLO, both dogs were in Group 2. One dog in Group 2 maintained the same difference in thigh circumference (Figure 4). The mean thigh circumference for the affected limb in all dogs was 39.2±3.5 cm and for the unaffected limb in all dogs was 41.9±3.8 cm.

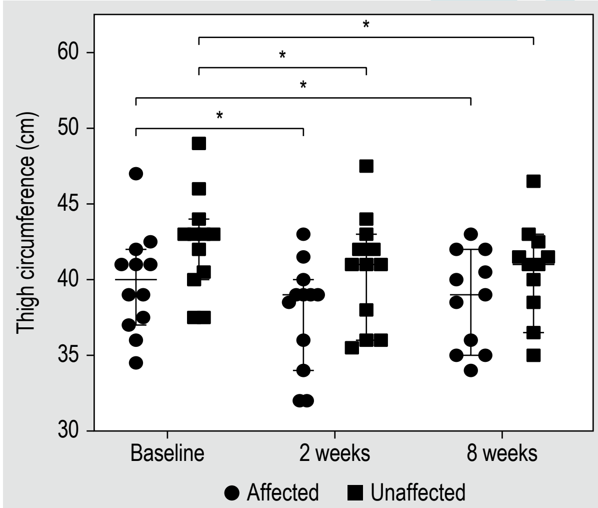

Figure 3. Thigh circumference (cm) of the affected and unaffected limbs at baseline, 2, and 8 weeks post tibial plateau levelling osteotomy for 8 adult dogs. * Indicates a significant difference (*P*<0.05).

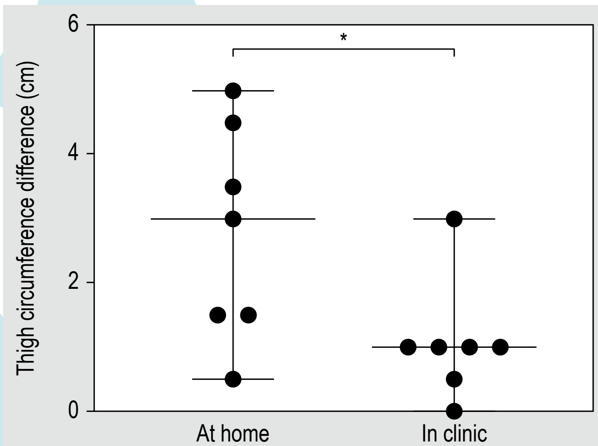

Figure 4. Thigh circumference difference (cm) between affected and unaffected limbs for dogs receiving in home rehabilitation in addition to in clinic rehabilitation (n=7) versus in home rehabilitation only (n=7). * Indicates a significant difference (*P*<0.05).

Objective gait data

Total pressure index

The TPI of the affected limbs were significantly improved with time ($P<0.001$). However, at week 8 post TPLO, the mean TPI of the affected limb was still significantly different to the unaffected limb ($P<0.001$). The mean TPI was not significant at 8 weeks post TPLO between Group 1 and 2. There was a significant interaction between mean TPI and time ($P<0.001$). There was a significant difference between the affected and unaffected limbs at each time point at baseline ($P<0.0001$) and 2 weeks post TPLO ($P<0.0001$), and at 8 weeks post TPLO ($P<0.001$).

Percent stance

The percent stance on the affected limb was significantly improved with time ($P=0.02$). However, at all-time points the percent stance was significantly lower in the affected limb versus the unaffected limb ($P<0.001$). The percent stance was not significant ($P=0.9$) at 8 weeks post TPLO between Group 1 and 2 (Figure 5).

Mean stride length

There was no reportable significant data for stride length.

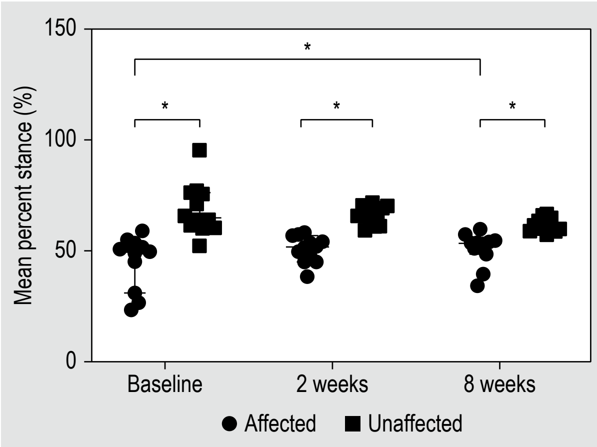

Figure 5. The mean percent stance (%) in affected limb compared to unaffected limb in dogs (n=14) with cranial cruciate ligament disease post tibial plateau levelling osteotomy procedure. * Indicates a significant difference (*P*<0.05).

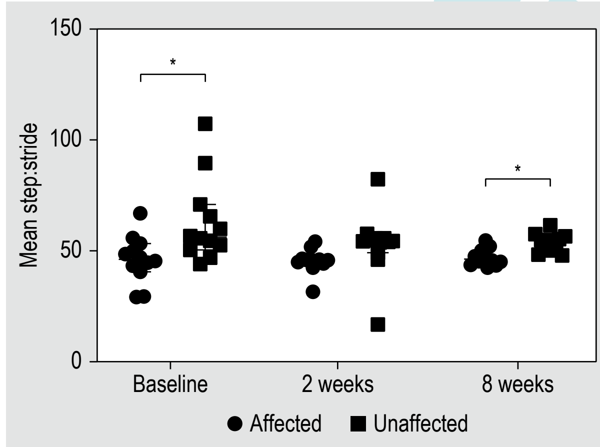

Figure 6. The mean step:stride in affected limb compared to unaffected limb in dogs (n=14) with cranial cruciate ligament disease post tibial plateau levelling osteotomy procedure. * Indicates a significant difference (*P*<0.05).

Mean step: stride ratio

There was a significant difference between the affected and unaffected limbs at baseline and at 8 weeks post TPLO with the affected dogs having a lower step stride ratio in the affected limb at baseline (P=0.04) and at 8 weeks post TPLO (P=0.001) but not at 2 weeks post TPLO (P=0.26) (Figure 6).

Acoustic myography data

Gastrocnemius (medial head) muscle

The efficiency (E) score and the temporal summation (T) score were not significantly different between the affected and unaffected limbs at any time point in the study (P=0.42 and 0.89, respectively). There was a significant difference in spatial summation (S) score for the gastrocnemius muscle, with the affected limbs having a higher S score than the unaffected (P=0.03). The spatial summation (S) score in all limbs accounted for 6.8% of the total variance. In-clinic rehabilitation did not have a significant effect on the gastrocnemius S scores (P=0.44) over the duration of the study.

Biceps femoris (cranial head) muscle

The efficiency (E) score (P=0.99) and the temporal summation (T) score (P=0.07) were not significantly different between the affected and unaffected limbs at any time point in the study. The spatial summation (S) score for the biceps femoris muscle of the affected versus the unaffected limb accounts for 8.5% of the total variance and was significant (P=0.02) with S scores being higher in affected versus unaffected legs at all time points. In-clinic rehabilitation had no significant effect on the biceps femoris muscle on S parameters (P=0.04).

Quadriceps (vastus lateralis) muscle

The efficiency (E) score (P=0.88), spatial summation (S) score (P=0.59) and the temporal summation (T) score (P=0.82) were not significantly different between the affected and unaffected limbs throughout the study period.

Semitendinosus muscle

The efficiency (E) score (P=0.08), spatial summation (S) score (P=0.33) and the temporal summation (T) score (P=0.77) were not significantly different between the affected and unaffected limbs throughout the study period.

Canine Brief Pain Inventory score

CBPI scores at baseline were reported as fair (n=8), reported as good (n=5), and excellent (N=1). The CBPI score were reported as fair (n=4), as good (n=9) and as very good (n=1) at 2 weeks post TPLO. The CBPI scores were reported as fair (n=1), good (n=5), very good (n=5) and excellent (n=3) at 8 weeks post TPLO. By week 8 more dogs had very good/excellent pain scores versus at baseline (Chi square statistic = 17.1 (df is 3, n=14, P=0.001). There was no difference between in clinic rehabilitation versus at home rehabilitation in at any time point. (baseline, P=0.27; week 2, P=0.3; week 8, P=0.29).

4. Discussion

We hypothesised that cruciate ligament insufficiency resulting in palpable joint instability alters the function of the biceps femoris, quadriceps, gastrocnemius and semitendinosus muscles. Findings in this study confirm alteration in function of the biceps femoris and gastrocnemius muscles but did not identify alterations in the quadriceps

Table 1. Mean ± standard deviation scores for muscles of both hind limbs at each time point (n=14).

Muscle	Baseline			2 weeks post TPLO[1]			8 weeks post TPLO		
	E	S	T	E	S	T	E	S	T
Gastrocnemius affected	6.7± 2.2	5.9±1.5	7.6±1.8	6.2±2.5	6.1±2.0	7.6±1.8	6.8±3.2	6.5±2.0	8.0±1.8
Gastrocnemius unaffected	5.7±1.9	4.9±1.6	8.1±1.5	6.4±2.1	5.0±2.4	7.8±1.4	6.7±2.0	6.0±1.7	8.0±1.1
Biceps femoris affected	7.6±2.2	7.1±1.8	7.7±1.4	6.6±2.3	5.7±1.3	7.4±1.9	7.2±2.7	6.9±1.8	7.9±1.6
Biceps femoris unaffected	6.9±2.1	5.8±1.6	8.0±1.5	6.2±1.5	4.7±2.0	6.7±1.9	7.1±2.2	6.1±2.2	8.0±2.0
Quadriceps affected	7.1±2.1	6.2±1.5	7.9±1.3	6.3±2.3	5.6±1.7	7.1±1.6	7.0±2.6	6.1±1.7	8.0±1.7
Quadriceps unaffected	6.7±2.7	5.6±1.6	7.8±1.8	6.7±1.8	5.4±1.4	7.3±1.5	6.8±2.6	5.5±1.6	7.6±1.7
Semitendinosus affected	7.9±1.9	6.2±2.5	7.5±2.6	6.0±3.4	6.3±1.7	7.8±2.2	7.2±1.9	6.4±1.6	7.6±2.1
Semitendinosus unaffected	6.9±2.3	6.4±1.5	8.1±1.4	7.0±2.0	6.0±2.4	7.9±1.3	8.0±2.0	6.0±2.3	7.3±2.5

[1] TPLO = tibial plateau levelling osteotomy.

and semitendinosus muscles. There was no immediate effect of the TPLO stabilising procedure on the function of the gastrocnemius and biceps femoris muscles as expected per our hypothesis. This was despite an improvement in gait and range of motion at 2 weeks post TPLO over baseline. There was no effect of type of rehabilitation (in clinic with at home versus in home only) on AMG parameters, both groups restored gastrocnemius function to equal that of the unaffected limb by 8 weeks but did not fully restore biceps femoris function.

AMG analysis directly measures muscle contraction. Results showed that there was a difference in spatial summation (S) score in the gastrocnemius muscle of the affected limb versus the unaffected, with the affected muscle having a lower amplitude signal (higher S score) during contraction. It has been previously hypothesised that a lower amplitude of sound from fibres contracting during the recording period can be interpreted as recruitment of less fibres, a smaller size of individual muscle fibres due to atrophy with resultant reduction in contraction noise, or a combination of both (Claudel *et al.*, 2018). For the biceps femoris muscle the spatial summation (S) score remained higher in the affected versus the unaffected limb at all time points with the muscle producing a lower amplitude signal during contraction versus the unaffected limb. The fact that surgery, and rehabilitation did not normalise function in the biceps femoris muscle may point to a deficiency in the rehabilitation plan or may be a function of time needed for recovery of muscle size, as correlated via thigh circumference. If data had been collected over a longer period of time it may have provided more insight. There was a trend toward some effect of time on the temporal summation (T) score of the biceps femoris (P=0.07). We may have missed changes in AMG readings from the muscles studied due to the fact that we only collected AMG data at two points post TPLO and did not follow the dogs out beyond 8 weeks, even though it is reported that full healing from the procedure can take 12 weeks (Kirkby Shaw *et al.*, 2019).

Atrophy of thigh muscles was verified via thigh circumference measurements which included the semitendinosus, biceps femoris and quadriceps muscles, though differentiation of each muscle size was not possible using the measurement. A discrepancy in thigh circumference between affected and unaffected limbs was identified in all cases, and that discrepancy was larger at 2 weeks post-surgery than at baseline, which should have further changed spatial summation score in the biceps femoris if a lower amplitude signal was due to atrophy. Muscle atrophy has been shown to continue after TPLO surgery as measured at 5 weeks post TPLO procedure (without rehabilitation) (Moeller *et al.*, 2010). However, despite thigh muscle atrophy there was no difference in spatial summation (S) score in the quadriceps and semitendinosus over time, even as thigh circumference changed. Circumference of the crus at the level of the gastrocnemius but was not recorded due to poor repeatability in a pilot study during evaluation of normal dogs. In a study of muscle size measured via DEXA after CCLR, the gastrocnemius was found to be increased in mass relative to unaffected limbs, and atrophy mostly limited to the quadriceps muscle (McCarthy *et al.*, 2018).

Thigh circumference did not appear to correlate with the AMG measurements. In dogs that received both in-clinic and in-home rehabilitation, there was no significant effect on muscle function as seen by AMG parameters, despite a positive effect on thigh circumference.

If the change in spatial summation was not due to atrophy (Adrian *et al.*, 2013; Limbird *et al.*, 1988), then it may have been due to the biceps femoris and gastrocnemius muscles working harder and so the muscles becoming trained. A trained muscle recruits less fibres to do the same work as an untrained muscle and has been found in human AMG studies to have higher spatial summation (S) secondary to less active muscle fibres (Claudel *et al.*, 2018; Harrison, 2017). Increased activation of the biceps femoris and medial head of the gastrocnemius muscles has been noted in humans with cruciate injury and may reduce anterior translation in people (Limbird *et al.*, 1988). This certainly fits with the reported relative hypertrophy of the gastrocnemius muscle in cruciate deficient dogs (Mostafa *et al.*, 2010). After TPLO procedure, the need for extra work in the gastrocnemius and biceps femoris muscles would have declined in the absence of excess tibial translation and normalised by 8 weeks in the gastrocnemius muscle.

There were no statistical differences found for AMG parameters between baseline and other time points for either the quadriceps (vastus lateralis) or semitendinosus muscles. In human medicine, one of the main focuses of rehabilitation post-surgery for ACL tears is restoration of quadriceps function (Buckthorpe *et al.*, 2019). It appears from our results, that targeting an alteration in quadriceps contractile function (via neuromuscular electrical stimulation) may not be as important as rebuilding muscle size and strength. Induced CCLR has been shown to result in shorter EMG active time in the vastus lateralis (Adrian *et al.*, 2013), it was speculated that this was due to reduced use of the quadriceps muscle because of increased stifle range of motion in flexion during walk. We did not see this change in active time (E score) as measured via AMG in our study. The semitendinosus muscle was evaluated in the present study because the muscle functions as an agonist of the cranial cruciate ligament (Almuth *et al.*, 2018), therefore, it should be working more as it acts to reduce cranial tibial thrust in a cruciate deficient limb. A study using needle EMG on induced CCLR showed change in activity in semitendinosus, correlated with histologic evidence of atrophy (Almuth *et al.*, 2018). The lack of significant findings in our study is puzzling.

The fact that there were no significant changes in temporal summation (T) score, or efficiency (E) score may mean these parameters are not useful measurements of change in muscle function due to pathology, although there was a trend toward a change in T score for the biceps femoris. Changes in these AMG parameters have been seen in other studies when assessing muscle function in dogs when transitioning from a walk to a trot in dogs (Fenger and Harrison, 2017).

Limitations

Potential issues with AMG recordings include loss of contact of sensor with skin and therefore altered transmission of signals. In this study, hair was clipped short to ensure adequate contact. Unlike EMG, the AMG recording apparatus used in this study (CURO unit MyoDynamik ApS) measures unidirectional sound waves transmitted through muscle and skin to the sensor and does not record sound from sensor movement.

A walking pace was used to analyse gait and muscle function since the affected dogs presented with various degrees of lameness and not all were able to trot without their gait worsening. The walk is a slow-paced gait in which there is less excitation in the extrinsic muscles. As the dog transitions from a walk to a trot, there is an increase in muscle excitation (Deban *et al.*, 2012). and more changes in AMG recordings at the different points may have been noted if the trotting pace was used.

Many dogs with CCLR have bilateral disease, with one limb being subclinical. We tried to rule out any evidence of contralateral limb cruciate disease by DACVS and DACVSMR orthopaedic exam and palpation in addition to radiographs of bilateral rear limbs, evaluated by DACVR. Even in the absence of subclinical pathology in the contralateral limb changes in muscle activation may occur, making the contralateral limb a problematic control. In a radiofrequency induced model of CCLR, though there were no statistical differences for any EMG values, a delay in muscle activation in the quadriceps and biceps femoris along with a shorter active time was noted in both affected and unaffected limbs (Adrian *et al.*, 2019).

There was no significant difference in muscle function between rehabilitation groups, and no comparable group who did not receive rehabilitation. This limitation was due to using client owned dogs with natural disease. Rehabilitation is best practice after CCLR surgery and so was performed for all dogs. Though both groups had the same instructions for home rehabilitation, it was unclear whether adequate home therapies were performed due to the variability of client compliance which could have affected results in either group.

5. Conclusions and clinical relevance

The function of the gastrocnemius muscles in the affected limbs was significantly different to normal limbs at baseline and 2 weeks post TPLO but not at 8 weeks. The gastrocnemius (medial head) and biceps femoris (cranial head) muscles appear to be the most affected by CCLD, TPLO surgery and rehabilitation. The affected dogs had to recruit less muscle fibres in the affected limbs, which is likely an appropriate adaptation to workload.

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Conflict of interest

The authors declare no conflict of interest.

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