Preoperative low level laser therapy in dogs undergoing tibial plateau levelling osteotomy: A blinded, prospective, randomized clinical trial

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Summary
Objectives: To evaluate the influence of pre-operative low-level laser therapy (LLLT) on therapeutic outcomes of dogs undergoing tibial plateau levelling osteotomy (TPLO).
Methods: Healthy dogs undergoing TPLO were randomly assigned to receive either a single preoperative LLLT treatment (800–900 nm dual wavelength, 6 W, 3.5 J/cm^2, 100 cm^2 area) or a sham treatment. Lameness assess-ment and response to manipulation, as well as force plate analysis, were performed pre-operatively, then again at 24 hours, two weeks, and eight weeks postoperatively. Radiographic signs of healing of the osteo-tomy were assessed at eight weeks postoper-atively.

Results: Twenty-seven dogs (27 stifles) were included and no major complications oc-curred. At eight weeks postoperatively, a sig-nificant difference in peak vertical force analy-sis was noted between the LLLT (39.6% ± 4.7%) and sham groups (28.9% ± 2.6%), (p <0.01 Time, p <0.01 L). There were no sig-nificant differences noted between groups for all other parameters. The age of dogs in the LLLT group (6.6 ± 1.6 years) was greater than that for the sham group (4.5 ± 2.0, p <0.01). Although not significant, a greater proportion of LLLT dogs (5/8) had healed at the eight-week time point than in the sham group (3/12) despite the age difference (p = 0.11)
Clinical significance: The results of this study demonstrate that improved peak vertical force could be related to the preoperative use of LLLT for dogs undergoing TPLO at eight weeks postoperatively. The use of LLLT may improve postoperative return to function following ca-nine osteotomies and its use is recommended.

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Introduction

Cranial cruciate ligament disease is the most commonly diagnosed stifle injury in dogs and is frequently surgically treated by tibial plateau levelling osteotomy (TPLO) (1, 2). Despite surgical correction, persist-ent lameness, muscle atrophy, and poor limb function may persist and current therapeutic recommendations favour early return to activity (3, 4). Low-level laser therapy (LLLT) is a laser treatment in which the energy output is low enough that the temperature of the treated tissue does not rise above the range of normal body temperature, and is thought to act via photobiomodulation to increase levels of intracellular adenosine triphosphate (ATP) (5–7). Low-level laser therapy is often rec-ommended by veterinary and human physical therapists for clinical applications, such as an earlier return to function post-operatively (8–10). It is estimated that 20% of veterinary hospitals in North America are using therapeutic lasers (8).

The preoperative use of LLLT is based on the concept of surgical preconditioning where pre-treating tissue with low levels of a stress inducing stimulus induces a protec-tive response and reduces damage caused by surgery (7, 11–15). In human studies, preconditioning with LLLT for surgery has been shown to decrease inflammation, and increase analgesia, vascularization, and tis-sue healing (7, 11–15). Increased cell sur-vival rates and decreased apoptosis have also been demonstrated (7, 11–15). Low-level laser therapy is a safe, non-mutagenic, and non-invasive adjunctive therapy (16). The exact mechanism of LLLT is not fully

understood and significant controversy remains over efficacy (17). However, many research models show benefits of LLLT in anti-inflammatory, analgesic, and bio-modulatory effects, promoting an increase in local microcirculation and increasing the speed of healing of bone and other tissues (16, 18–21). Low-level laser therapy has been shown to increase ATP production within cells *in vitro* and *in vivo* (22–23). Increased ATP content in skeletal muscle tissue suggests that more energy may be available for all metabolic processes (24).

Draper and colleagues' prospective study of 36 dogs with acute intervertebral disc herniation showed that postoperative LLLT for five days reduced the time to ambulation in dogs following hemilaminectomy from a median of 14 to 3.5 days (6). Low-level laser therapy was performed using a laser array with five 200 mW, 810 nm wavelength lasers transcutaneously delivering 25,000 mW/cm^2 to the skin once daily for five days, which delivered an approximate energy density (2 to 8 J/cm^2) to the spinal cord (6). In contrast, Kurach and colleagues' prospective study of the use of LLLT for acute wound healing in 10 healthy dogs showed no apparent benefits with the use of a dual diode laser (7.5 mW/diode) at 635 nm and a total energy density of 1.125 J/cm^2 applied three times weekly for 32 days (17).

Santiago and colleagues used LLLT both pre- and postoperatively to assess the effect of LLLT on bone repair after expansion of the midpalatal suture in dogs, and found that LLLT contributed to suture reorganization and palatal bone osteogenesis during and after expansion (16). Dogs were treated with 20 doses of laser therapy every 48 hours pre- and post-expansion for a total of 39 days, using an energy density of 90 to 120 J/cm^2 with a wavelength of 790 to 904 nm (16).

In light of the reported biostimulatory effects of LLLT, we chose to employ this modality on dogs undergoing TPLO surgery for the treatment of cranial cruciate ligament rupture. The TPLO provides a relatively standardized technique allowing the study of the efficacy of LLLT in a clinical setting.

The purpose of this study was to evaluate therapeutic outcomes of preoperative LLLT in dogs undergoing TPLO for naturally occurring cranial cruciate disease in a blinded, prospective, randomized clinical trial. Our null hypothesis was that there would be no significant difference in postoperative lameness, response to manipulation, force plate analysis, or radiographic signs of bone healing of the tibial osteotomy in dogs receiving a single preoperative dose of LLLT using a gallium-aluminium-arsenium laser (800–900 nm dual wavelength, 6 W, 3.5 J/cm^2, 100 cm^2 area), immediately prior to TPLO when compared to a sham treatment.

Material and methods

This study was designed as a randomized, blinded, prospective clinical study of client-owned dogs that were presented for surgical treatment of naturally occurring cranial cruciate disease. The research protocol used and described by this study was approved by the Institutional Animal Care and Use Committee at Oregon State University, and owner consent was obtained prior to each dog's enrolment into the study.

Systemically healthy client-owned dogs (n = 27) that underwent TPLO were randomly assigned to receive either LLLT with a gallium-aluminium-arsenium laser (800–900 nm dual wavelength, 6 W, 3.5 J/cm^2, 100 cm^2 area) preoperatively or a sham treatment. The LLLT or sham treatment was administered to the proximomedial region of the tibia immediately preoperatively while dogs were anaesthetized, prior to the TPLO procedure. Assessment of lameness, movement, behaviour, and response to manipulation, as well as force plate analysis (peak vertical force [PVF] and vertical impulse [VI]) were performed preoperatively, and at 24 hours, two weeks, and eight weeks postoperatively. Radiographic signs of healing of the osteotomy were assessed eight weeks postoperatively.

Animals

A preoperative diagnosis of cranial cruciate ligament disease was made based on presence of palpable stifle pathology including effusion, cranial drawer, and tibial thrust.

Once admitted, dogs were randomly assigned by coin toss to one of the following groups: LLLT (n = 12) or sham (n = 15). Both the veterinary surgeon and the owner were blinded to the treatment assignments.

Dogs were excluded from the study if preoperative abnormalities were detected in the results of a haematology or serum chemistry bioanalyses, if they had any previous surgery on the same stifle, if they had any additional orthopaedic pathology on the same limb that required treatment (such as patellar luxation), or if they were skeletally immature. Lack of osteotomy compression, or abnormal tibial conformation requiring frontal or transverse plane correction resulting in an osteotomy gap were exclusion criteria for this study as well as any concurrent or previous history of immune-mediated joint disease, patellar luxation, osteochondrosis dissecans, or fracture of the distal femur or proximal tibia in the pelvic limbs. Tibial plateau angle, sex, age, and breed were not factors for exclusion in the study. Potential confounders were assessed and these included age, sex, breed, body weight, body condition score, meniscal surgery, tibial plateau angle, radiographic osteoarthritis score, and development of contralateral cruciate ligament disease.

Laser and surgical treatment

Dogs were pre-medicated and anaesthetized following standard protocols used by the anaesthesia service at the hospital. A preoperative intravenous injection of cefazolin[a] (22 mg/kg) was administered to all animals followed by the same dose every 90 minutes during the procedure.

The dogs underwent LLLT or sham while under general anaesthesia, immediately prior to surgery. The sham group was subjected to movement of the laser in the same fashion, but with the unit in the off position. The limb was routinely clipped and the LLLT group was irradiated with a galinium-aluminium-arsenium laser[b], at continuous wavelength at 3 watts for 30 seconds, then 2 hz at 4 watts for 45

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a   Cefazolin: Apotex Corp, Weston, FL, USA
b   K-series 1200: K-Laser, Franklin, TN, USA

seconds, then at 5 hz at 4 watts for 30 seconds, then at 10 hz at 3 watts for 45 seconds, then at 500 hz at 3 watts for 30 seconds. The laser had a dual wavelength of 800 nm and 970 nm, 6 W for a unified dose of 3.5 J/cm^2 administered over a 100 cm^2 area on the medial aspect of the proximal tibia of the limb undergoing TPLO surgery (25–27).

A previously described surgical protocol for stifle arthroscopy and TPLO was performed by an ACVS diplomate or by a surgical resident under the supervision of an ACVS diplomate immediately following LLLT or sham. The stifle was examined with a 4.0 mm, 30° fore-oblique arthroscope^c as previously described (28). Probing was performed (29). Remnants of the cranial cruciate ligament arthroscopically removed. The menisci were also examined and torn tissue was removed with a combination of sharp dissection and shaver debridement using a motorized tissue shaver^d. A partial meniscectomy was performed to remove damaged tissue if present. No meniscal release or treatment was performed if the meniscus was intact. The TPLO was performed via a medial approach to the proximal tibia using previously described techniques with the aid of a jig (30). All dogs had the tendons of insertion of the gracilis, sartorius, and semitendinosus muscles elevated from the proximo-medial tibia prior to curvilinear osteotomy; then, following plateau rotation and bone plate fixation, the tendons of insertion were sutured back to their common insertion superficial to the bone plate, as previously reported (30). The curvilinear osteotomy blade was cooled by irrigation with sterile saline while in contact with the bone in all dogs. The osteotomy was secured with a 3.5 mm TPLO plate^e selected at the surgeon's discretion. Non-locking 3.5 mm plates with 3.5 mm screws^f were used in both groups.

c   Stryker Inc., Kalamazoo, MI, USA
d   Total Performance SystemTM Shaver: Stryker Inc., Kalamazoo, MI, USA
e   Selected plate was one of the following: Everost Delta TPLO plates: Everost, Sturbridge, MA, USA; or Securos TPLO plate: Securos Surgical, Fiskdale, MA, USA
f   3.5 mm cortical screws: DePuy Synthes VET, West Chester, PA, USA

The tibial plateau angle was measured for surgical planning and immediately after surgery.

Postoperative care

Pain following surgery was controlled using an injectable opioid for 12–24 hours. Administration of tramadol (3 mg/kg by mouth every 12 hours for 14 days) and carprofen (2.2 mg/kg by mouth every 12 hours for 14 days) was started the morning after surgery for all dogs in the study. Cryotherapy (5 minutes every 4 hours for 24 hours) was also performed. None of the dogs were treated with non-steroidal anti-inflammatory drugs or corticosteroid medications following the 14th postoperative day until the eight week radiographic evaluation.

Outcome assessment
Postoperative radiographic evaluation

Following the surgical procedure, caudo-cranial and mediolateral digital radiographic views centred on the stifle were performed in all animals while still under general anaesthesia. Radiographs were repeated at eight weeks after surgery under heavy sedation (butorphanol^g at 0.1 mg/kg and dexmedetomidine^h at 5 mcg/kg administered intravenously) and the same exposure settings, beam, and limb position were used for all radiographs of a single patient.

Radiographic signs of healing of the osteotomy site were assessed at eight weeks postoperatively by both a diplomate of the American College of Veterinary Surgeons (WIB) and a diplomate of the American College of Veterinary Radiologists, as either healed or not healed. All assessments were made with the assessor blinded to the treatment group assigned to each dog. Complete agreement was found between the two assessors for all dogs. A healed site was defined as a complete iso-opaque bridging bone callus connecting the two cortices (31). The osteoarthritis scale employed was modified from human and ca-

g   Torbugesic: Zoetis, Florham Park, NJ, USA
h   Dexdomitor: Zoetis, Florham Park, NJ, USA

nine osteoarthritis grading scales described previously in the literature (32–34). The scale used combined findings from the radiographs to describe the following grading scale: 0 = no evidence of joint effusion, osteophytosis, intra-articular mineralization or subchondral bone sclerosis; 1 = joint effusion present, loss or degeneration of articular cartilage; 2 = joint effusion and subchondral bone sclerosis present, minimal to mild osteophytosis; 3 = moderate to severe osteophytosis, intra-articular mineralization present or both; and 4 = appearance of subchondral cysts. Each parameter was assigned a grade between 0 and 3 (0 = normal, 1 = mild, 2 = moderate, 3 = severe). A score of 4 was not used in any of the dogs in the study since none had radiographic evidence of subchondral bone cysts. A median score was assigned to each dog calculated from the scores provided (32–34).

Lameness, movement, behaviour, and response to manipulation assessment

Dogs were assessed by a board certified veterinary surgeon or a surgical resident under the supervision of an ACVS diplomate prior to surgery, then at 24 hours, two weeks, and eight weeks postoperatively and scored according to the degree of lameness. Lameness scoring was quantified as follows: 0 = stands and walks normally; 1 = stands normally, slight lameness when walking; 2 = stands normally, obvious lameness when walking; 3 = stands abnormally, slight to obvious lameness when walking; and 4 = non-weight bearing lameness. This was based on a modified lameness scoring system developed by Cross and colleagues (35).

Behaviour and response to manipulation were also recorded on the above days. Behaviour scoring was quantified as follows: 0 = asleep or calm; 1 = mild agitation; 2 = moderate agitation; 3 = severe agitation. In addition, response to manipulation was scored as follows: 0 = no response; 1 = minimal response, moves head; 2 = minimal response, tries to move away; and 3 = strong response, tries to bite. This was based on a modified pain scale from Pibarot and colleagues (36).

Force plate gait analysis

To analyse gait, dogs were walked across a 2 m long pressure mat system[i] connected to a desktop computer[j] on the same days they were evaluated by the surgeon (the day prior to surgery, then 24 hours, 2 weeks, and 8 weeks postoperatively). Data were collected at a walk (1.5–1.8 m/s) with an acceleration of $\pm$ 0.5 m/s^2. Five foot falls each from the cranial cruciate ligament-deficient and unaffected pelvic limbs were analysed. The pressure exerted by each limb was recorded as PVF (%BW), and VI (%BW $\times$ sec) for the affected and contralateral limbs, then calculated from the measurements using software designed for the system[i], where BW was body weight in kilograms.

Statistical analysis

Statistical analyses were performed. Data were first analysed using mixed linear models to assess the effects of treatment (affected versus unaffected limb), time post-treatment, and the interaction (if any) between treatment and time. Repeated measures analysis of variance (ANOVA) followed by Tukey's honest significant difference test was used to assess between-time point changes for PVF and VI.

Data for veterinary assessment and lameness score, and a Canine Brief Pain Inventory assessment were analysed using statistical software[k] to test for normality with the D'Agostino & Pearson omnibus normality test. Data were then analysed with a repeated measures ANOVA followed by Dunnett's multiple comparison post-test to compare time points to pre-treatment data. For all statistical analyses, a calculated value of $p < 0.05$ was set as the threshold for statistical significance. All results are reported as mean $\pm$ standard deviation except for the radiographic OA scores which are reported as the median and range.

i   High Resolution Mat: Tekscan, Inc, San Diego, CA, USA
j   Gateway Pentium: Gateway Inc., Irvine, CA, USA
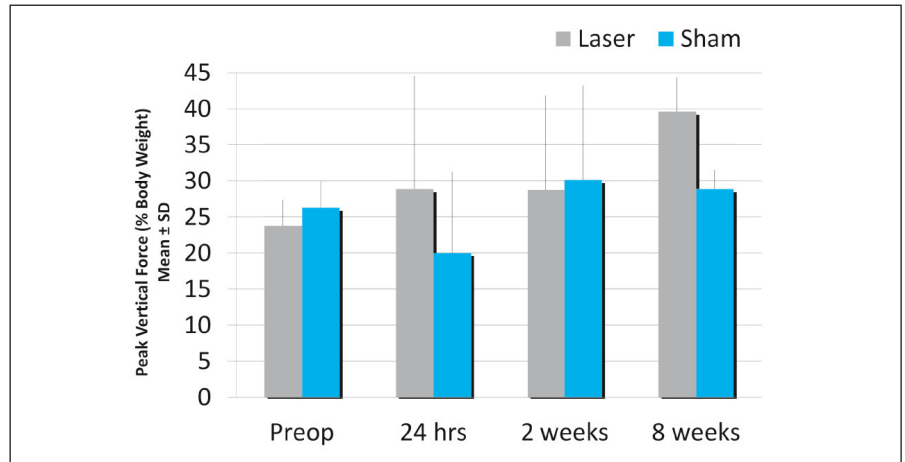k   Prism 5.0: GraphPad Software, Inc., La Jolla, Ca, USA

Figure 1   Bar graph of peak vertical force over time for dogs in each treatment group (Laser = preoperative low level laser therapy, Sham = no laser treatment). Weight bearing was assessed by the percentage of body weight transferred to the limb over time following tibial plateau levelling osteotomy surgery and was significantly improved ($p < 0.01$) in dogs that received one preoperative laser treatment over sham dogs that had surgery alone at eight weeks postoperatively, and was not significantly different at all other time points ($p > 0.05$). hrs = hours; preop = preoperative.

Results

Twenty-seven stifles from 27 dogs were included in the study (LLLT group n = 12, sham group n = 15). Three dogs in the laser group were lost to follow-up and did not return at eight weeks postoperatively. A tear of the cranial cruciate ligament was confirmed during surgery in all dogs. There were no major complications during the study that required additional surgery and none of the dogs developed a contralateral cranial cruciate ligament tear in the eight weeks following surgery. All osteotomies healed uneventfully. The body weight (mean $\pm$ SD) of the LLLT group (35.0 $\pm$ 10.9 kg) and the sham group (39.3 $\pm$ 13.5 kg) were not significantly different (p = 0.44). Body condition scores were also similar in the LLLT group (6.25 $\pm$ 0.8,) and the sham group (6.34 $\pm$ 1.1) (p = 0.96). In the LLLT group, five dogs were spayed females and seven were neutered males. In the sham group, four dogs were spayed females, two dogs were intact females, and nine of the sham dogs were neutered males. There were no significant differences in the distribution of sexes between the groups (p = 0.42). Breeds in the LLLT group included three Labrador Retrievers, a Golden Re-

Figure 2
Bar graph of vertical impulse (VI) (percentage body weight $\times$ seconds) over time for dogs in each treatment group. Vertical impulse was not significantly ($p > 0.05$) different between groups, however both groups increased over time ($p < 0.05$). hrs = hours; preop = preoperative.
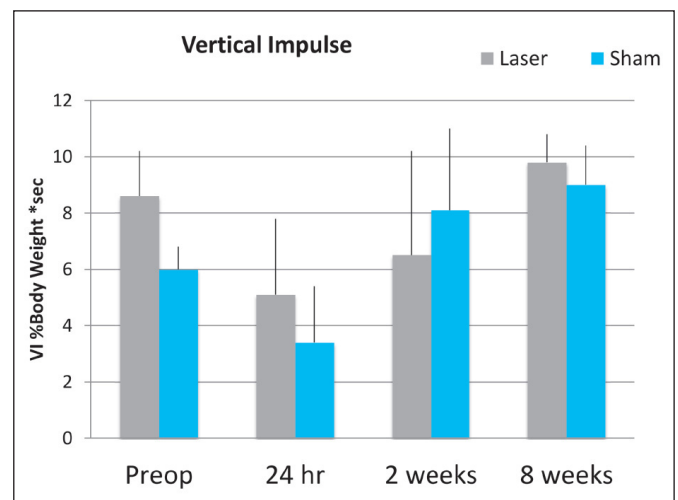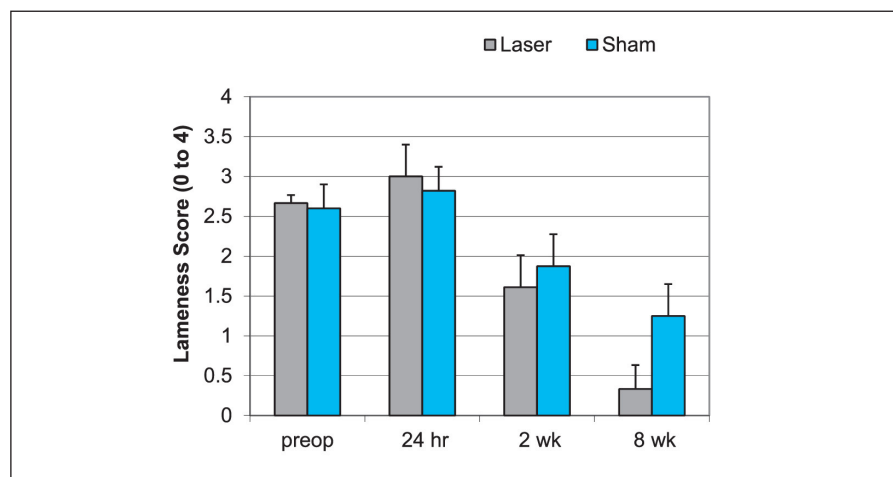
Figure 3 Lameness scores prior to and following tibial plateau levelling osteotomy surgery in each treatment group: Laser = preoperative low level laser therapy, Sham = no laser treatment. There was a trend toward decreasing lameness in dogs that received preoperative Laser, however it did not reach significance (p >0.05). Lameness scoring was quantified as follows: 0 = stands and walks normally; 1 = stands normally, slight lameness when walking; 2 = stands normally, obvious lameness when walking; 3 = stands abnormally, slight to obvious lameness when walking; 4 = non-weight bearing lameness. This was based on a modified lameness scoring system developed by Cross and colleagues (35). hr = hour; preop = preoperative; wk = week.

triever, a Rottweiler, a Staffordshire Bull Terrier, a Pembroke Welsh Corgi, and five mixed breed dogs. Breeds present in the sham group included three Labrador Retrievers, an Australian Shepherd, a Golden Retriever, a German Wirehaired Terrier, an Akbash, a Pitbull, a Weimaraner, a Boxer, a Rottweiler, and four mixed breed dogs. Age of the dogs at the time of surgery in the LLLT group (6.6 ± 1.6 years) was significantly older than in the sham group (4.5 ± 2.0 years) (p <0.01).

The number of stifle joints with meniscal pathology, defined as damage to the lat-eral or medial meniscus requiring a partial or complete meniscectomy on arthroscopic evaluation in the LLLT group (n = 8) and the sham group (n = 9), did not differ significantly between groups (p = 0.19). The preoperative tibial plateau angle preoperatively was not significantly different between the LLLT (27.6 ± 3.02) and sham (23.5 ± 4.1). Both groups had a significant (p <0.0001) reduction in the tibial plateau angle immediately (LLLT 7.3 ± 3.3 and sham 8.0 ± 3.9) and at eight weeks postoperatively (LLLT 10.6 ± 5.5 and sham 9.1 ± 3.9), however the two groups had a similar tibial plateau angle at each time point. The osteoarthritis score was similar between groups and over time with no significant differences identified (p = 0.28, two-way ANOVA). For the LLLT group, the amount of osteoarthritis present on radiographs was scored as 1 (range: 0–3 prior to surgery) and 1.25 (range: 1–3) at eight weeks following surgery. The preoperative score for the sham group (median, range) was 2 (range: 1–3) and at eight weeks postoperatively was 1.5 (range: 1–2.5).

Gait analysis

Preoperative PVF (%BW) and VI (%BW × sec) analysis results were not significantly different between the two groups. See ►Figure 1 and the ►Appendix Table (Available online at www.vcot-online.com).

At twenty-four hours and two weeks postoperatively, the PVF and VI were not significantly different between groups. The eight week postoperative PVF was significantly different between the LLLT (39.6% ± 4.7%) and the sham (28.9% ± 2.6%) groups (p <0.01 Time, p <0.01 laser treatment or sham) (►Figure 1). Eight weeks postoperatively, there was no significant difference for VI between the groups (p <0.05 Time, p >0.05 laser treatment or sham). See ►Figure 2 and the ►Appendix Table (Available online at www.vcot-online.com).

Figure 4 Assessment of behaviour was characterized by the frequency of movement of the dogs in each group (Laser versus Sham). Movement scoring was quantified as follows: 0 = asleep or calm; 1 = regular change of position; 2 = uncomfortable with constant movement and unrest. No significant difference was identified between the two treatment groups at each of the time points (p >0.05). (Taken from y-axis labelling: 0 = calm; 2 = uncomfortable, continuous). Laser = low level laser therapy preoperatively, Sham = no laser treatment preoperatively. hr = hour; preop = preoperative; wk = week.

Bone healing

At eight weeks postoperatively, the proportion of stifles with radiographic signs of osteotomy healing in the LLLT (5/8) and the sham group (3/12) was not significantly
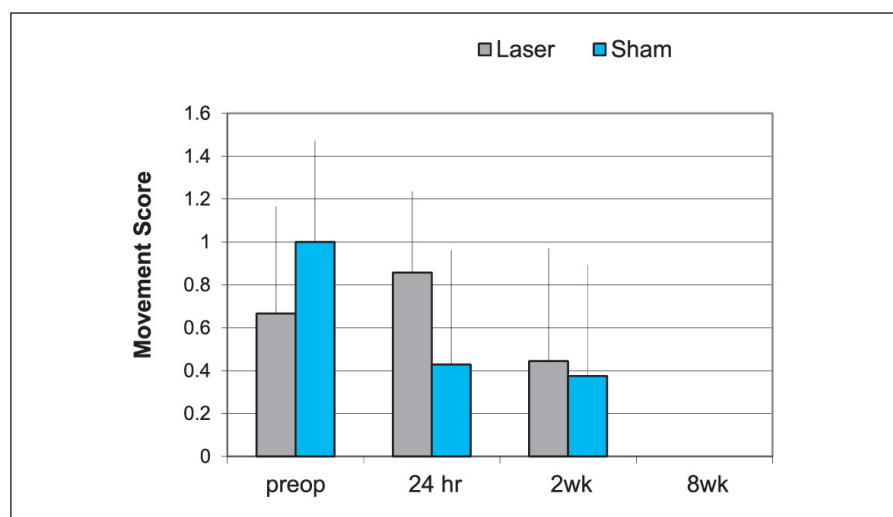
different (p = 0.11) (►Appendix Table, available online at www.vcot-online.com).
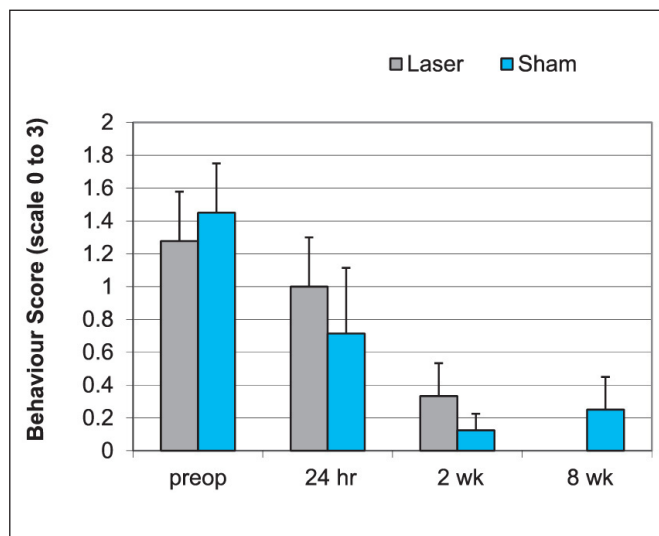
Lameness, movement, behaviour, and manipulation scores

There was no significant difference in lameness scores between the LLLT and sham groups preoperatively or at eight weeks postoperatively (p >0.05, ►Figure 3). The differences beect of LLLT preoperatively administered to clinical canine patients undergoing TPLO surgery. A single dose of preoperative LLLT was associated with a significant improvement in PVF for dogs undergoing TPLO on the operated limb eight weeks postoperatively. Thus, we were able to reject our null hypothesis that a single preoperative dose of LLLT using a gallium-aluminium-arsenium laser, 4 J/cm^2, does not produce significant effects on the gait and radiographic signs of bone healing in dogs following TPLO. Ground reaction forces are considered the gold standard for objective assessment of limb function in dogs (37). Measurement of PVF (maximum load placed on the ground during the stance phase) and the VI (the area under the curve if the entire stance phase) has been shown to have good correlation to limb function (38–41). Peak vertical force has been shown to be the single most accurate ground reaction force for kinetic pelvic limb lameness evaluation in dogs (37, 42, 43).

The results of this study support LLLT as a means to improve postoperative limb use. Early return of dynamic stability has shown significant benefit in promoting a more complete return to function, reduction in ongoing pain, and prevention of re-injury without a compromise in stability (44, 45). However, further studies are necessary to determine the ideal LLLT protocol and to elucidate the exact mechanism of action of LLLT, potentially via decreased pain and inflammation, or by expediting tissue healing. Reducing pain and swelling following anterior cruciate ligament surgery in people leads to an improved range of motion and quadriceps function, and reduces the risk of limited range of motion and contracture, which could cause gait abnormalities and delay healing; all of which are also goals in ca-

nine cranial cruciate ligament surgery (46).

There were not any significant differences between the groups in lameness, behaviour and response to manipulation scores, however these are subjective measurements. Studies have shown that subjective scoring scales do not replace force plate gait analysis and that agreement between force plate and subjective scales is low unless lameness is severe (45).

Investigations of the effects of LLLT in the context of bone repair have been increasingly reported within the literature. Studies have shown positive influences of LLLT on osteoblast proliferation and bone formation (16, 47). The lack of significant effect on osteotomy healing was probably due to the small numbers of animals in each group and the age difference between the two groups. The younger population of dogs in the sham group would have been expected to have a greater proportion of osteotomies healed at eight weeks compared to the older LLLT group of dogs. Our definition of a healed tibial osteotomy site as a complete iso-opaque bridging bone callus connecting the two cortices, is a scale that is not specific or validated for TPLO (31). The use of a five- or 10-point radiographic scoring system developed to evalu-
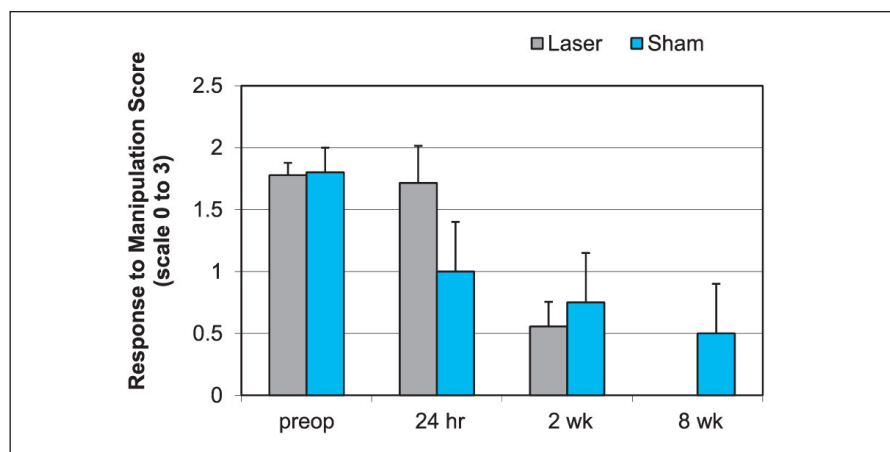
Figure 5
Behaviour scores of the two treatment groups over time. Dogs' behaviour scores were assigned as the following: 0 = asleep or calm, 1 = mild agitation, 2 = moderate agitation, 3 = severe agitation. Scores for both groups decreased over time, however no significant difference was identified between the groups (p >0.05). hr = hour; preop = preoperatively; wk = week.

Figure 6 Response of the dogs to manipulation of the affected stifle joint was assessed at each time point and was scored as follows: 0 = no response; 1 = minimal response, moves head; 2 = minimal response, tries to move away; 3 = strong response, tries to bite. No significant difference was found between the treatment groups, p >0.05. hr = hour; preop = preoperatively; wk = week.

ate bone healing specifically following TPLO surgery as described by Kieves and colleagues may have been more sensitive to differences in radiographic healing (48). In addition, a larger study with greater numbers of dogs in the same age range is indicated to determine the effect of LLLT on tibial bone healing. Wavelength is an important factor, which relates to penetration of laser light through biological tissue (49). The greatest stimulatory effects of LLLT would be a wavelength that penetrates to the desired level of tissue to stimulate increased ATP synthesis. Discrepancies in protocols using LLLT can profoundly affect the results, as seen with significant improvement in dogs treated by LLLT after spinal surgery, as compared to no improvement in wound healing in dogs in another study (6, 17). Additional studies attempting to standardize dose and wavelength may be beneficial.

The protocol used was the one recommended by the laser manufacturer and has been shown in previous studies of pre-injury LLLT to have an effect on tissue response to injury (2–5 J/cm^2) (25–27). It is possible that a different protocol for the administration of LLLT in the treatment group, such as a greater number of treatments could result in greater beneficial effects. A longer follow-up period may have yielded additional significant differences between groups. The use of LLLT in the early stages of tissue healing versus LLLT over a certain period could have different amounts of effectiveness. The dual wavelength and alternating frequency used in this study may have allowed for greater biostimulation of osteoblasts and fibroblasts resulting in accelerated healing, but further research is needed before claims such as this can be made.

Low-level laser therapy has been shown to reduce pain and affect all three basic phases of wound healing (inflammation, proliferation, and maturation), resulting in increased wound tensile strength and improved microcirculation (50–55). Low-level laser therapy may selectively target nerve fibres conducting at slower velocities, such as nociceptors (54). Clinically, LLLT may be of benefit for control of postoperative pain, however this was not assessed and is a limitation of the study. Healing of

tissues over the course of eight weeks including completion of tissue remodelling may have accounted for the lack of differences in PVF at two weeks and the significantly improved PVF found at eight weeks.

Limitations of the study include variables such as age diet, and activity level that were not controlled. Data regarding laxity, limb circumference, and range of motion postoperatively were lacking. The small sample size was a major limitation to this study. An additional limitation was the inability to control for confounding factors that may influence bone healing such as home activity level, and diet, leading to a change in load bearing of the treated limb. Histopathology and other imaging modalities were not performed to compare groups since this was a clinical study. A single preoperative LLLT treatment may not have been enough to stimulate osteogenic healing and our findings may reflect a Type II statistical error.

Conclusion

In conclusion, we reject our null hypothesis. A single dose of preoperative LLLT prior to TPLO resulted in a significant improvement in PVF on the affected limb eight weeks postoperatively. However, a single preoperative dose of LLLT was not associated with significant effects on radiographic signs of healing at eight weeks postoperatively. The exact mechanism of action of LLLT in this scenario is not known, but the findings reported here support further research on the clinical use of LLLT for the treatment of common conditions in dogs.

Conflict of interest

There are no conflicts of interest to declare.

References

1. Witsberger TH, Villamil JA, Schultz LG. Prevalence of and risk factors for hip dysplasia and cranial cruciate ligament deficiency in dogs. J Am Vet Med Assoc 2008; 232: 1818-1824.
2. Kowaleski MP, Boudrieau RJ, Pozzi A . In: Tobias KM , Johnston SA , editors. Veterinary Surgery Small Animal. Vol 1. St. Louis (MO): Elsevier; 2012. pg. 906–998.
3. Vasseur PB, Berry CR. Progression of stifle osteoarthrosis following reconstruction of the cranial cruciate ligament in 21 dogs. J Am Anim Hosp Assoc 1992; 28: 129–136.
4. Priddy NH II, Tomlinson JL, Dodam JR, et al. Complications with and owner assessment of the outcome of tibial plateau leveling osteotomy for treatment of cranial cruciate ligament rupture in dogs: 193 cases (1997–2001). J Am Vet Med Assoc 2003; 222: 1726–1732.
5. Firat ET, Dağ A, Günay A, et al. The effect of low-level laser therapy on the healing of hard palate mucosa and the oxidative stress status of rats. J Oral Pathol Med 2014; 43: 103-110.
6. Draper WE, Schubert TA, Clemmons RM, et al. Low-level laser therapy reduces time to ambulation in dogs after hemilaminectomy: a preliminary study. J Small Anim Pract 2012; 53: 465-469.
7. Agrawal T, Gupta GK, Rai V, et al. Pre-conditioning with low-level laser (light) therapy: light before the storm. Dose Response 2014; 12: 619-649.
8. Pryor B, Millis DL. Therapeutic laser in veterinary medicine. Vet Clin North Am Small Anim Pract 2015; 45: 45–56.
9. Shumway R. Rehabilitation in the first 48 hours after surgery. Clin Tech Small Anim Pract 2007; 22: 166-170.
10. Leal-Junior EC, Vanin AA, Miranda EF, et al. Effect of phototherapy (low-level laser therapy and light-emitting diode therapy) on exercise performance and markers of exercise recovery: a systematic review with meta-analysis. Lasers Med Sci 2015; 30: 925-939.
11. Barretto SR, De Melo GC, Dos Santos JC, et al. Evaluation of anti-nociceptive and anti-inflammatory activity of low-level laser therapy on temporomandibular joint inflammation in rodents. J Photochem Photobiol B 2013; 129: 135-142.
12. Meneguzzo DT, Lopes LA, Pallota R, et al. Prevention and treatment of mice paw edema by near-infrared low-level laser therapy on lymph nodes. Lasers Med Sci 2013; 28: 973-980.
13. Yang X, Cohen MV, Downey JM. Mechanism of cardioprotection by early ischemic preconditioning. Cardiovasc Drugs Ther 2010; 24: 225-234.
14. Hagiwara S, Iwasaka H, Hasegawa A, et al. Pre-Irradiation of blood by gallium aluminum arsenide (830 nm) low-level laser enhances peripheral endogenous opioid analgesia in rats. Anesth Analg 2008; 107: 1058-1063.
15. Zhang J, Bian HJ, Li XX, et al. ERK-MAPK signaling opposes rho-kinase to reduce cardiomyocyte

apoptosis in heart ischemic preconditioning. Mol Med 2010; 16: 307-315.

16. Santiago VC, Piram A, Fuziy A. Effect of soft laser in bone repair after expansion of the midpalatal suture in dogs. Am J Orthod Dentofacial Orthop 2012; 142: 615-624.

17. Kurach LM, Stanley BJ, Gazzola KM, et al. The effect of low-level laser therapy on the healing of open wounds in dogs. Vet Surg 2015; 44: 988–996.

18. Liu X, Lyon R, Meier HT, et al. Effect of lower-level laser therapy on rabbit tibial fracture. Photomed Laser Surg 2007; 25: 487–494.

19. Fabre HS, Navarro RL, Oltramari-Navarro PV, et al. Anti-inflammatory and analgesic effects of low-level laser therapy on the postoperative healing process. J Phys Ther Sci 2015; 27: 1645-1648.

20. Sousa LR, Cavalcanti BN, Marques MM. Effect of laser phototherapy on the release of TNF-alpha and MMP-1 by endodontic sealer-stimulated macrophages. Photomed Laser Surg 2009; 27: 37–42.

21. Fekrazad R, Sadeghi-Ghuchani M, Eslaminejad MB, et al. The effects of combined low level laser therapy and mesenchymal stem cells on bone regeneration in rabbit calvarial defects. J Photochem Photobiol B 2015; 151: 180–185.

22. Karu T, Pyatibrat L, Kalendo G. Irradiation with He-Ne laser increases ATP level in cells cultivated in vitro. J Photchem Photobio B 1995; 27: 219–223.

23. Tafur J, Mills PJ. Low-intensity light therapy: Exploring the role of redox mechanisms. Photomed Laser Surg 2008; 26: 323–328.

24. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. Physiol Rev 2008; 88: 287–332.

25. Alves AN, Ribeiro BG, Fernandes KP, et al. Comparative effects of low-level laser therapy pre- and post-injury on mRNA expression of MyoD, myogenin, and IL-6 during the skeletal muscle repair. Lasers Med Sci 2016; 31: 679–685.

26. Leal-Junior EC, Lopes-Martins RA, Baroni BM, et al. Effect of 830 nm low-level laser therapy applied before high-intensity exercises on skeletal muscle recovery in athletes. Lasers Med Sci 2009; 24: 857-863.

27. De Almeida P, Lopes-Martins RÁ, Tomazoni SS, et al. Low-level laser therapy improves skeletal muscle performance, decreases skeletal muscle damage and modulates mRNA expression of COX-1 and COX-2 in a dose-dependent manner. Photochem Photobiol 2011; 87: 1159-1163.

28. Whitney WO. Arthroscopically assisted surgery of the stifle joint. In: Beale BS, Hulse DA, Schulz KS, et al, editors. Small Animal Arthroscopy. USA: Elsevier Science; 2003. pp 117–156.

29. Pozzi A, Hildreth BE, Rajala-Schultz PJ. Comparison of arthroscopy and arthrotomy for diagnosis

of medial meniscal pathology: an ex vivo study. Vet Surg 2008; 37: 749-755.

30. Slocum B, Slocum TD. Tibial plateau leveling osteotomy for repair of cranial cruciate ligament rupture in the canine. Vet Clin North Am Small Anim Pract 1993; 23: 777–795.

31. Whelan DB, Bhandari M, Mckee MD, et al. Interobserver and intraobserver variation in the assessment of the healing of tibial fractures after intramedullary fixation. J Bone Joint Surg Br 2002; 84: 15-18.

32. Chan WP, Lang P, Stevens MP, et al. Osteoarthritis of the knee: comparison of radiography, CT, and MR imaging to assess extent and severity. AJR Am J Roentgenol 1991; 157: 799–806.

33. Kyrkos MJ, Papavasiliou KA, Kenanidis E, et al. Calcitonin delays the progress of early-stage mechanically induced osteoarthritis. In vivo, prospective study. Osteoarthritis Cartilage 2013; 21: 973–980.

34. Innes JF, Costello M, Barr FJ, et al. Radiographic Progression of Osteoarthritis of the Canine Stifle Joint: A Prospective StudyVet Radiol Ultrasound 2004; 45: 143–148.

35. Cross AR, Budsberg SC, Keefe TJ. Kinetic gait analysis assessment of meloxicam efficacy in a sodium urate-induced synovitis model in dogs. Am J Vet Res 1997; 58: 217–224.

36. Pibarot P, Dupuis J, Grisneaux E, et al. Comparison of ketoprofen, oxymorphone hydrochloride, and butorphanol in the treatment of postoperative pain in dogs. J Am Vet Med Assoc 1997; 211: 438–444.

37. Fanchon L, Grandjean D. Accuracy of asymmetry indices of ground reaction forces for diagnosis of hind limb lameness in dogs. Am J Vet Res 2007; 68: 1089-1094.

38. McLaughlin RM. Kinetic and kinematic gait analysis in dogs. Vet Clin North Am Small Anim Pract 2001; 31: 193–201.

39. Budsberg SC, Verstraete MC, Soutas-Little RW. Force plate analysis of the walking gait in healthy dogs. Am J Vet Res 1987; 48: 915–918.

40. Budsberg SC, Verstraete MC, Soutas-Little RW, et al. Force plate analyses before and after stabilization of canine stifles for cruciate injury. Am J Vet Res 1988; 49: 1522–1524.

41. Stejskal M, Torres BT, Sandberg GS, et al. Variability of vertical ground reaction forces collected with one and two force plates in healthy dogs. Vet Comp Orthop Traumatol 2015; 28: 318-322.

42. Au KK, Gordon-Evans WJ, Dunning D, et al. Comparison of short- and long-term function and radiographic osteoarthrosis in dogs after postoperative physical rehabilitation and tibial plateau le-

veling osteotomy or lateral fabellar suture stabilization. Vet Surg 2010; 39: 173-180.

43. Quinn MM, Keuler NS, Lu Y, et al. Evaluation of agreement between numerical rating scales, visual analogue scoring scales, and force plate gait analysis in dogs. Vet Surg 2007; 36: 360–367.

44. Monk ML, Preston CA, Mcgowan CM. Effects of early intensive postoperative physiotherapy on limb function after tibial plateau leveling osteotomy in dogs with deficiency of the cranial cruciate ligament. Am J Vet Res. 2006; 67: 529-536.

45. Romano LS, Cook JL. Safety and functional outcomes associated with short-term rehabilitation therapy in the post-operative management of tibial plateau leveling osteotomy. Can Vet J 2015; 56: 942-946.

46. Yabroudi MA, Irrgang JJ. Rehabilitation and return to play after anatomic anterior cruciate ligament reconstruction. Clin Sports Med 2013; 32: 165-175.

47. Dörtbudak O, Haas R, Mallath-Pokorny G. Biostimulation of bone marrow cells with a diode soft laser. Clin Oral Implants Res 2000; 11: 540-545.

48. Kieves NR, Mackay CS, Adducci K, et al. High energy focused shock wave therapy accelerates bone healing. A blinded, prospective, randomized canine clinical trial. Vet Comp Orthop Traumatol 2015; 28: 425-432.

49. Medrado AR, Pugliese LS, Reis SR, et al. Influence of low level laser therapy on wound healing and its biological action upon myofibroblasts. Lasers Surg Med. 2003; 32: 239-244.

50. Iijima K, Shimovama N, Shimovama M, et al. Effect of repeated irradiation of low-power He-Ne laser in pain relief from postherpetic neuralgia. Clin J Pain 1989; 5: 271–274.

51. Toida M, Watanabe F, Goto K, et al. Usefulness of low-level laser for control of painful stomatitis in patients with hand, foot-and-mouth disease. J Clin Laser Med Surg 2003; 21: 363–367.

52. Ferraresi C, De Sousa MV, Huang YY, et al. Time response of increases in ATP and muscle resistance to fatigue after low-level laser (light) therapy (LLLT) in mice. Lasers Med Sci 2015; 30: 1259-1267.

53. Lacjaková K, Bobrov N, Poláková M, et al. Effects of equal daily doses delivered by different power densities of low-level laser therapy at 670nm on open skin wound healing in normal and corticosteroid-treated rats: a brief report. Lasers Med Sci 2010; 25: 761–766.

54. Stadler I, Lanzafame RJ, Evans R, et al. 830-nm irradiation increases the wound tensile strength in a diabetic murine model. Lasers Surg Med 2001; 28: 220–226.