Received: 15 June 2015 | Revised: 1 December 2017 | Accepted: 7 February 2018
DOI: 10.1111/vsu.12798

ORIGINAL ARTICLE

Effects of low-intensity pulsed ultrasound on radiographic healing of tibial plateau leveling osteotomies in dogs: a prospective, randomized, double-blinded study

Nina R. Kieves DVM, DACVS-SA, DACVSMR, CCRT[1] | **Sherman O. Canapp DVM, MS, DACVS, DACVSMR[1]** | **Peter J. Lotsikas DVM, DACVS, DACVSMR[1]** | **Scott A. Christopher VMD, CCRP[1]** | **Christopher S. Leasure DVM[1]** | **Debra Canapp DVM, DACVSMR[1]** | **Patrick R. Gavin DVM, PhD, DACVR, DACVRO[2]**

[1] Veterinary Orthopedic and Sports Medicine Group, Annapolis Junction, Maryland
[2] MR Vets, Inc, Sagle, Idaho

Correspondence
Nina R. Kieves, Department of Veterinary Clinical Sciences, The Ohio State University, 601 Vernon L. Tharp Street, Columbus, OH 43235.
Email: nkieves@gmail.com

Abstract

Objective: To determine the influence of low-intensity pulsed ultrasound (LIPUS) on radiographic healing and limb function after uncomplicated, stable osteotomies in dogs.

Study design: In vivo, prospective, randomized, double-blinded, placebo-control study.
Sample population: Fifty client-owned dogs.

Methods: Fifty client-owned dogs with naturally occurring unilateral cranial cruciate ligament rupture were enrolled prior to tibial plateau leveling osteotomy. Dogs were assigned to an active (LIPUS) treatment group or a placebo control (SHAM) treatment group via block randomization on the basis of age, weight, and affected limb. Dogs in the LIPUS treatment group underwent LIPUS treatments for 20 minutes daily: 1.5-MHZ ultrasound wave pulsed at 1 kHZ with a 20% duty cycle at an intensity of 30 mW/cm^2 for the duration of the study (12 weeks). Radiographic evaluation was performed at 4, 8, 10, and 12 weeks postoperatively to evaluate bone healing. Limb function was assessed with temporal-spatial gait analysis preoperatively and at 4, 8, and 12 weeks postoperatively by using a pressure-sensitive walkway system.

Results: Both groups had significant improvement in radiographic score and limb use over time. However, there was no significant difference in radiographic bone healing, or limb use as measured by objective gait analysis detected between the LIPUS treatment group and SHAM treatment group at any point in the study.

Conclusion: LIPUS treatment did not improve healing in this stable osteotomy model.

Clinical significance: This study does not provide evidence to support the clinical application of LIPUS to stimulate the healing of stable, uncomplicated osteotomies to accelerate bone healing.

1 | INTRODUCTION

Traumatic fractures are seen regularly in veterinary medicine, and fresh fractures are commonly created in the form of an

Data from this study were presented at the 42nd Annual Veterinary Orthopedic Society Conference, February 28-March 7, 2015, Sun Valley, Idaho.

osteotomy for the correction of angular limb deformities and stabilization of cranial cruciate ligament (CCL) deficient stifles. Fracture healing involves a complex series of biologic events to restore adequate mechanical strength and thus allow a return to full function. Ultrasound, or the propagation of an acoustic wave, can be used as a diagnostic or therapeutic tool, depending upon the frequency, level of energy, or pressure emitted. Therapeutic ultrasound is used to stimulate tissues and induce biologic effects. Low-intensity pulsed ultrasound (LIPUS) lies between diagnostic and therapeutic ultrasound, typically being delivered at intensities below 100 mW/cm^2.[1]

LIPUS was first reported to accelerate the normal fracture repair process in humans in 1983,[2] and it was approved by the US Food and Drug Administration for the accelerated healing of fresh fractures in 1994 and for the treatment of established nonunion fractures in 2000. Sales of LIPUS in 2006 were approximately $250 million in the United States alone.[3] LIPUS has been shown to accelerate healing of fresh fractures and nonunion fractures in human and animal models and in planned osteotomies.[4-7] Although the mechanism of its effect is not completely understood, it is likely secondary to thermal and nonthermal effects at an intracellular level, with effects on cell membranes and proteins as well as molecular effects, with most effects being nonthermal.[1] LIPUS is believed to be a form of mechanical energy that produces micromechanical strain, thereby stimulating or inducing bone repair.[1]

Early return to function following traumatic fractures and surgically created osteotomies is based on the healing of the fracture or osteotomy site without clinically significant complications. Were adjunct treatment with LIPUS to accelerate healing, this could allow earlier return to function for the individual patient, potentially decreasing morbidity, and could shorten the convalescent period, allowing for an earlier return to full function. Decreasing complications associated with bone healing and allowing early active mobilization could also decrease cost of treatment. Large-scale, randomized, double-blind, placebo-controlled trials that have been reported in the human literature have provided evidence that LIPUS accelerates healing of fractures.[2,7-18] However, the use of LIPUS has not been investigated in dogs.

The objective of this study was to determine the influence of LIPUS on radiographic healing and limb function after tibial plateau leveling osteotomy (TPLO) in dogs. We hypothesized that application of LIPUS over the surgical site would improve radiographic healing of the osteotomy compared with a placebo-treated control group (SHAM). We also hypothesized that limb function, assessed via temporal-spatial gait analysis, would be improved in dogs treated with LIPUS compared with those in the SHAM group.

2 | MATERIALS AND METHODS

Large-breed dogs with unilateral naturally occurring CCL disease were prospectively enrolled in the study with owner consent. Age, weight, sex, limb operated on, meniscal status (intact vs torn), cruciate status (partial vs complete tear), and preoperative and postoperative tibial plateau angle (TPA) were recorded for each dog enrolled. Dogs were enrolled until 25 dogs were enrolled for each group (N = 50 dogs). The diagnosis of CCL disease was based on orthopedic examination and radiographic findings and was confirmed at the time of surgery. Dogs with concurrent orthopedic, neurological, or metabolic disease were excluded from the study, including dogs with contralateral CCL disease. All owners signed a consent form for enrollment. This study complied with the standards in the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Institutes of Health [86-23, revised 1985]).

All dogs received complete general physical, orthopedic, and neurological examinations and had routine preanesthetic blood work screening performed, consisting of a complete blood cell count and biochemistry profile. Food was withheld from each dog for a minimum of 10 hours prior to induction of anesthesia. Dogs were premedicated with a combination of hydromorphone (0.05-0.1 mg/kg IV; West-Ward Pharmaceuticals, Eatontown, New Jersey) and diazepam (0.5 mg/kg IV; Qualitest Pharmaceuticals, Huntsville, Alabama). All dogs were induced with intravenous propofol (PropoFlo; Abbott Animal Health, Abbott Park, Illinois) and maintained with isoflurane (Fluriso; VetOne, Boise, Idaho) in oxygen. A balanced crystalloid solution (Veterinary Plasma-Lyte A; Abbott Animal Health) was given for the duration of surgery (10 mL/kg IV for the first hour, 5 mL/kg thereafter). Heart rate, respiratory rate, esophageal temperature, indirect blood pressure, hemoglobin saturation, and electrocardiography were monitored during anesthesia. All patients received cefazolin (22 mg/kg IV; West-Ward Pharmaceuticals) at induction and then every 90 minutes for the duration of surgery. Postoperative pain management consisted of hydromorphone (at an average dose of 0.05 mg/kg IV every 4 hours), an oral nonsteroidal anti-inflammatory drug at the recommended dosage, and oral tramadol (tramadol hydrochloride, 2-3 mg/kg every 8-12 hours; Mylan Pharmaceuticals, Canonsburg, Pennsylvania).

Dogs underwent standard TPLO surgery performed routinely as described elsewhere,[19] with the application of a standard 3.5-mm locking TPLO plate (New Generation Devices, Glen Rock, New Jersey). All osteotomies were performed with a 24-mm radial saw blade. Joint exploration was performed through a minimedial arthrotomy, and meniscal tears were debrided; intact menisci were not released. Oral medications were first administered on the evening of surgery or the following morning, depending on the appetence level of the dog. Hydromorphone was discontinued the morning following surgery. Dogs in both groups underwent the same postoperative rehabilitation regimens, including daily home exercise programs.

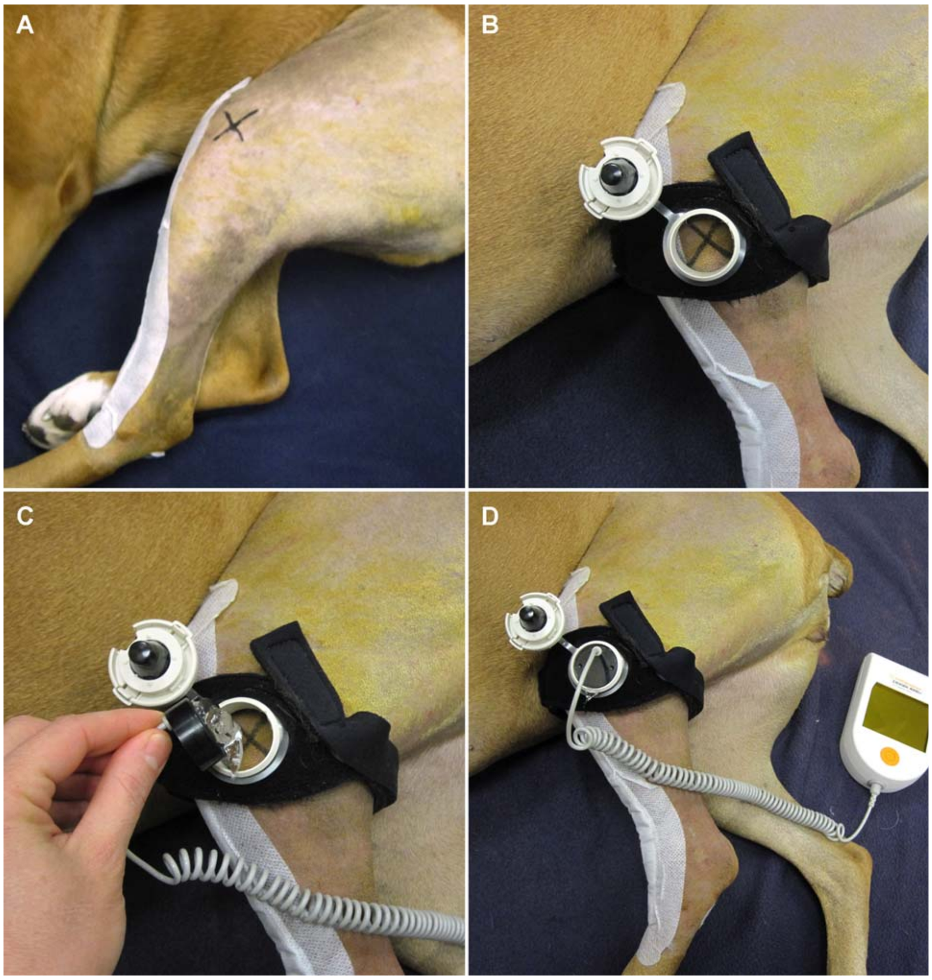

FIGURE 1 Placement of the unit on the limb. **A,** Mark on the limb (x) made over the osteotomy site as a reference point for owners. **B,** Strap in place over the mark. **C,** Transducer with coupling gel applied. **D,** Transducer in place within the strap

2.1 | LIPUS treatment

Dogs were assigned to 1 of 2 treatment groups, LIPUS (active treatment) or SHAM (placebo control treatment), via a randomized block cohort study on the basis of patient age, breed, sex, weight, and limb affected. The primary investigators and owners were blinded to the treatment group assignments. The LIPUS group underwent LIPUS treatments for 20 minutes daily. Briefly, per manufacturer's (Bioventus, Durham, North Carolina) recommendation, a Velcro strap was placed around the proximal tibia positioned over the osteotomy site, which was marked with a Sharpie marker, on the lateral aspect of the limb, with the application cap placed over the marked site (Figure 1). Coupling gel was applied to the transducer, the transducer was placed in the cap of the Velcro sleeve touching the skin, and the cap was closed to hold the transducer in place; the unit was then started. The unit produced a 1.5-MHZ ultrasound wave pulsed at 1 kHZ with a 20% duty cycle at an intensity of 30 mW/cm^2 and was set to run automatically for 20 minutes when activated. It emitted an alert when the treatment was completed. Owners were given verbal instructions, shown how to apply the unit, and given a handout with photographs showing how to apply the unit. Owners were instructed to apply the unit, for the duration of the study (12 weeks), at home once daily for 20 minutes at approximately the same time each day to the site that was marked with the Sharpie marker on their dog's skin to ensure appropriate placement of the unit. Hair was reclipped, and the site was remarked at the 2, 4, 8, and 10 week postoperative recheck examinations.

2.2 | SHAM treatment

The SHAM group underwent the exact same protocol as the LIPUS group described above; however, a sham unit was used. These units emitted the same alert noises, emitted the same light, and operated for the same amount of time as the active units; however, they did not emit any ultrasound energy. All owners were instructed in the same manner regarding how to place the unit. The application site was marked, and hair was reclipped at the 2, 4, 8, and 10 week postoperative recheck examinations.

TABLE 1 Demographics of dogs enrolled in the study

Variable	SHAM treatment group	LIPUS treatment group	*P* value
Age, months	66.6	73.1	.472
Weight, kg	35.8	34.6	.868
BCS	5.8	5.9	.77
Preoperative TPA	28.5	28.3	.87
Postoperative TPA	3.3	3.8	.23

BCS, body condition score; LIPUS, low-intensity pulsed ultrasound; SHAM, placebo-treated control; TPA, tibial plateau angle.

2.3 | Compliance evaluation

All of the units (active and sham) recorded compliance data. This included the dates of application as well as the duration of application.

2.4 | Radiographic evaluation

Routine orthogonal TPLO style stifle radiographs were taken preoperatively, immediately postoperatively, and at 4, 8, 10, and 12 weeks postoperatively. A board-certified radiologist blinded to treatment group evaluated radiographs taken at 4, 8, 10, and 12 weeks postoperatively. A digital scoring system on a scale of 0-4 (see Table 2) was used to determine the degree of healing (Figure 2).

2.5 | Temporal-spatial gait analysis

Dogs underwent baseline temporal-spatial gait analysis preoperatively and at 4, 8, and 12 weeks postoperatively at a walking gait (GAITFour; CIR Systems, Havertown, Pennsylvania). A 24-foot walkway system[20] was placed on a flat, solid surface in a dedicated space for gait analysis during the duration of the study. Briefly, a 3-foot-long section of inactive mat was placed before and after the active portion of the mat to provide space for the patient to achieve a constant speed for gait analysis. The mat was calibrated by the manufacturer before purchase. The walkway system interfaced with a notebook computer and software program (GAITFour software version 4.9W5) for processing and storage of raw data recorded from gait analysis. Two cameras were positioned at a height of 50 cm at opposite ends of the walkway system to record movement simultaneously in both directions. Digital video files of each pass across the walkway system were automatically linked to the data files for footfall verification.

TABLE 2 Radiographic healing scoring system

RHS	Description
0	Osteotomy with no sign of healing.
1	Widened osteotomy indicating resorption.
2	Minimal periosteal bone formation.
3	Moderate periosteal bone formation but osteotomy site still visible.
4	Healed osteotomy, ie, cannot see the osteotomy line.

RHS, radiographic healing score.

Dogs were walked on the mat until they appeared relaxed and acclimated (approximately 2 passes/dog) to the walkway system and their surroundings. Dogs were walked across the portable walkway system by 1 of 2 different handlers trained in gait analysis; 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 a minimum of 3 valid gait cycles and a maximum of 5 gait cycles. Three to 5 passes were completed across the portable walkway system by walking a dog across the mats in 1 direction. 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 and 100 cm/sec, and no overt turning of the head from midline. In addition, each gait pattern must have been a walk consisting of 3 paws on the floor at any given time.

Videos of each walk were recorded to ensure walk validity. The software program was used to distinguish the paw print of each footfall. Paw prints were identified manually as left front, right front, left hind, or right hind during the first gait cycle. From that point forward, the software program automatically replicated the gait pattern on the basis of automatically identified footprints. 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). The velocity of individual gait cycles was compared to verify that variation within each pass did not exceed 10%.

Data analysis included mean ± SD values for stride length (Str), percentage of stance phase during gait cycle (ST%), number of sensors (NS), total pressure index (TPI), mean pressure index (MPI), and GAIT4Dog Lameness Score (GLS; CIR Systems, Inc., Franklin, New Jersey). The ST% was defined as the proportion of time that the paw was in contact with the ground during 1 gait cycle compared with total gait cycle time. NS was the number of sensors activated by each paw during a single footfall. 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. MPI was defined as the sum of pressure values recorded from each activated sensor during ST divided by NS. GLS was calculated as the mean pressure of

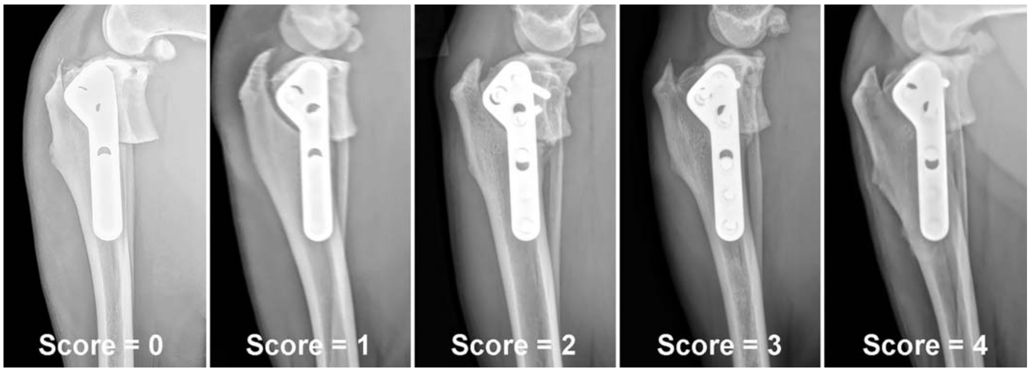

FIGURE 2 Lateral stifle radiographs taken throughout the study, with examples of radiographic healing scores 0-4, from left to right (see Table 2 for interpretation of scores)

the surgical limb compared with the expected overall weight bearing of the limb on the assumption that each hind limb should bear approximately 20% of the total weight of the animal. The mean ratio ± SD was calculated for each variable as a comparison of the surgical limb compared with the nonsurgical limb. The software program allowed for a summary for export of data for each dog to a spreadsheet for data analysis.

2.6 | Statistical analysis

Multivariable regression was used to analyze the relationship between treatment group and outcome while controlling for effects of individual and time. The mean value of each variable of each dog for each hind limb at each time point (preoperatively and at 4, 8, and 12 weeks postoperatively) was evaluated for variables including Str, ST%, MPI, TPI, hind limb reach, and GLS. Student's *t* test was performed to compare age, weight, and preoperative and postoperative TPA. All groups were evaluated to confirm normal distribution with a Shapiro-Wilk test prior to statistical evaluation. For all evaluations, $P < .05$ was considered significant (Stata 11.0; StataCorp, College Station, Texas).

3 | RESULTS

3.1 | Descriptive statistics

Both the SHAM and the LIPUS groups consisted of 25 dogs. The most commonly affected breed in both the SHAM and the LIPUS groups was the Labrador retriever, with 13 and 11 dogs affected, respectively. The SHAM group also included golden retrievers (3), German shepherd dogs (2) and 1 each of American Staffordshire terrier, Australian shepherd, boxer dog, Chesapeake Bay retriever, mixed breed, coonhound, and Rottweiler. The LIPUS group also included German shepherd dogs (2), Rottweilers (2), and 1 each of American Staffordshire terrier, Bernese mountain dog, boxer dog, cane corso, Chesapeake Bay retriever, Doberman retriever, giant schnauzer, golden retriever, Gordon setter, and Samoyed. The SHAM group had 18 (72%) females (1 intact) and 7 (28%) males (1 intact), and the LIPUS group had 15 (60%) females (1 intact) and 10 (40%) males (2 intact).

Among the 25 dogs in each group, the left limb was treated in 15 (60%) SHAM dogs and in 11 (44%) LIPUS dogs. Seventeen (68%) dogs in the SHAM group and 19 (76%) dogs in the LIPUS group had complete cruciate rupture diagnosed at the time of surgery, and 8 (32%) dogs in the SHAM group had medial meniscal injury compared with 7 (28%) in the LIPUS group. There were no lateral meniscal injuries. Age, weight, body condition score (BCS), and preoperative and postoperative TPA are described in Table 1.

3.2 | Radiographic healing

Radiographic healing scores improved at each time point throughout the study in both groups (Table 3). No difference was detected between groups at any time (Figure 3).

3.3 | Gait analysis

There were no significant differences at any time between the SHAM and the LIPUS groups regarding Str of the treated

TABLE 3 Distribution of radiographic scores of osteotomy healing across time

	4 Weeks Postoperative		8 Weeks Postoperative		12 Weeks Postoperative	
RHS	SHAM	LIPUS	SHAM	LIPUS	SHAM	LIPUS
0	6	4	0	1	0	0
1	6	14	2	1	0	0
2	10	3	3	9	2	3
3	2	3	14	12	2	4
4	0	1	3	1	16	13

RHS, radiographic healing score.

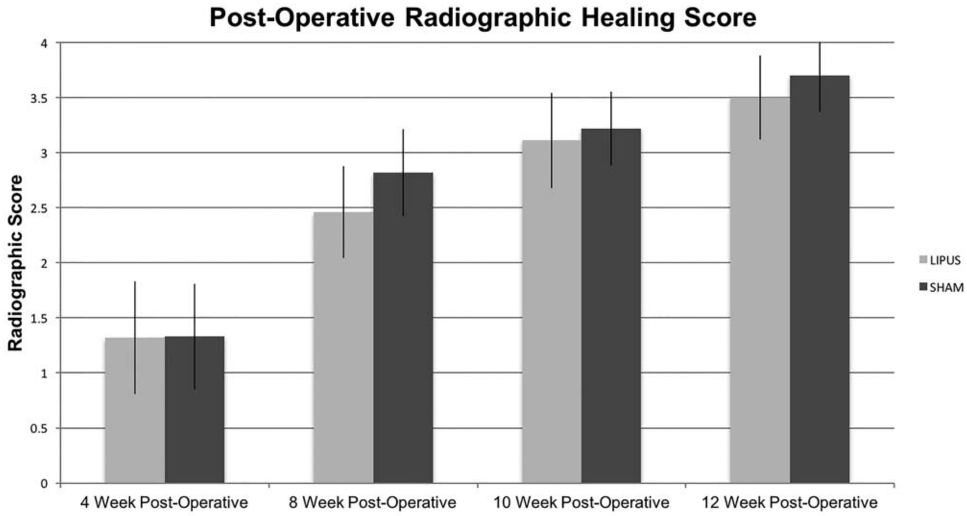

FIGURE 3 Radiographic healing score at each of the time points for the 2 groups, LIPUS and SHAM. Radiographic scores increased significantly across time for each group, but no significant difference in radiographic score was seen between groups at any time point. $P < 0.05$. LIPUS, low-intensity pulsed ultrasound; SHAM, placebo-treated control. Error bars indicate standard deviation

limb; hind limb reach of the treated limb; ST% of the affected limb; or symmetry indices for MPI, TPI, or GLS. TPI, MPI, and GLS score symmetry indices improved within each treatment group between presurgical evaluation and the 12-week time point. There was no difference within groups at any time point for hind limb reach, ST%, or Str (Tables 4, 5).

3.4 | Compliance

Overall use of units in both groups was 81.96%, with 82.19% use in the LIPUS group (20%-100%) and 81.72% use in the SHAM group (41%-200%).

4 | DISCUSSION

The use of LIPUS for bone healing in dogs had not been evaluated previously. In this study, we did not detect a difference in radiographic bone healing between the LIPUS and SHAM groups at any time point. Radiographic assessment of cortical bridging is one of the main factors influencing a clinician's recommendation to return dogs to normal activity after fracture repair or osteotomy. Therefore, radiographic healing was chosen as the outcome measure for this study because of its clinical availability and practicality for client-owned animals. The healing process is a continuum, and any scoring system has some potential for error. Previously reported radiographic scoring systems similar to the one selected in this study frequently use scales that are not specific to TPLO.[21-23] Radiographic evaluation of healing in a stable osteotomy may not be sufficiently sensitive to identify subtle changes in bone healing. Computed tomography or dual x-ray absorptiometry have been used in previous studies of bone healing[24-26] and may have identified changes indicative of accelerated healing that were not noticeable by radiographic evaluation. Our inability to perform histopathology in these clinical cases precludes forming any conclusions regarding microscopic influence of LIPUS.[7]

TPLO was chosen for evaluating the effects of LIPUS on canine bone healing in a clinical setting because of the prevalence of the procedure. In addition, failure of an elective procedure is associated with high patient morbidity and economic impact for the owner and surgeon. A stable osteotomy was also selected for this study on the basis of previous evidence of enhanced bone healing with LIPUS in such models, including tibial osteotomies stabilized with external

TABLE 4 Temporospatial gait values[a]

Ratio	SHAM, Preop	LIPUS, Preop	SHAM, 4 wk	LIPUS, 4 wk	SHAM, 8 wk	LIPUS, 8 wk	SHAM, 12 wk	LIPUS, 12 wk
MPI	0.88	0.84	0.84	0.86	0.89	0.91	0.95	0.93
TPI	0.66	0.57	0.67	0.63	0.72	0.75	0.77	0.81
GLS	0.69	0.57	0.69	0.63	0.80	0.75	0.87	0.81

GLS, Gait4Dog Lameness Score; LIPUS, low-intensity pulsed ultrasound; MPI, mean pressure index; Preop, preoperative; SHAM, placebo-treated control; TPI, total pressure index.

[a]Mean values of symmetry indices of temporal-spatial gait analysis values are compared between groups preoperatively and at 4, 8, and 12 weeks postsurgery.

TABLE 5 Comparisons of temporospatial values between groups[a]

Time point	Str symmetry ratio	TPI symmetry	GLS symmetry	MPI symmetry	Hind limb reach	Affected limb ST%
Preop	0.561	0.688	0.464	0.614	0.579	0.577
4 weeks Postop	0.014	0.297	0.109	0.651	0.754	0.035
8 weeks Postop	0.301	0.477	0.634	0.27	0.756	0.549
12 weeks Postop	0.057	0.585	0.194	0.403	0.488	0.993

GLS, GAIT4Dog Lameness Score; MPI, mean pressure index; Preop, preoperatively; Postop, postoperatively; ST%, stance percentage; Str, stride length; TPI, total pressure index;

[a]Mean values of temporal-spatial gait analysis values are compared between SHAM and LIPUS treatment groups prior to surgical treatment and at 4, 8, and 12 weeks following surgery. There were no significant differences between these groups.

skeletal fixation.[10,14,16,18] Urita et al[27] reported improved radiographic bone healing in forearm shortening procedures stabilized with a bone plate and screws. In addition to studies evaluating healing with stabilized osteotomies, others have reported improvement in bone healing with LIPUS treatment for stable fractures.[10,28]

Limb function was assessed with a pressure-sensitive walkway to provide objective measures of gait analysis.[20,29] TPI and GLS improved within groups from presurgical analysis to 12-week follow-up, as expected in dogs recovering from TPLO. However, no difference in limb function was detected between groups at any time. The lack of a validated owner assessment questionnaire such as the Canine Brief Pain Inventory (CBPI)[30] is a limitation because an instrument such as this could have detected differences in function at home. Indeed, improvement in CBPI scores does not necessarily correlate with objective force plate data.[31]

Few studies have evaluated the dosage effects of LIPUS on bone healing.[32] The dosage in this study followed the manufacturer's recommendations for the unit. This dosage cannot be changed on the unit and has been used in most studies that have been reported in the human literature.[1] The LIPUS unit was applied at home by owners, which is consistent with its use in man, but could have led to errors in application. To obtain optimal results, dogs should have been treated by a trained professional to ensure compliance; daily treatments of 20 minutes each for a 12-week period require commitments from owners. The compliance data retrieved from the units indicated that average compliance was 82% in both groups, which probably eliminates differences in compliance as a confounding factor. These data must, however, be interpreted with caution because the unit recorded only the amount of time it was turned on per day, not whether it was actually placed on the dog or whether it was placed in the appropriate location. The appropriate location was marked on the limb with a Sharpie marker several times throughout the study, but owners were not directly supervised beyond the training session at the time of discharge. Finally, we cannot eliminate the possibility that 100% compliance would have improved the effect of the treatment.

Our results contrast with several reports of improved radiographic healing of fractures as well as clinical limb use after LIPUS treatment in man. LIPUS has been found to accelerate the healing of acute fractures[2,9] as well as that of delayed and nonunion fractures in humans.[15-17] However, such positive results have not been consistently reported. Meta-analyses and reviews in the human literature have found that bone healing is most accelerated when LIPUS is used to manage fresh fractures in older patients, with a comminuted configuration.[4,7] The most recent meta-analysis by Lascelles et al[28] did not find any effect of LIPUS on pain reduction, days to full weight bearing, time to return to work, or number of subsequent surgeries in man. This study focused on clinically relevant outcome measures that would affect patient quality of life and economic impact.

Other limitations of our study include the small number of cases enrolled in each group, which may have led to a type II error. The lack of previous studies of LIPUS in dogs precluded power and sample size calculations during study design. In addition, all dogs in this study received an oral nonsteroidal anti-inflammatory agent in the immediate postoperative period, which inhibits the cyclooxygenase (COX) pathway. The COX-2 pathway is proposed to play a role in the mechanism of LIPUS stimulation on bone healing,[1] so this treatment may have affected the results of our study. However, both groups received the same postoperative treatment for the same amount of time, mitigating the influence of this factor on our comparisons.

Although this study did not detect any positive influence of LIPUS on the healing of an acute stable osteotomy (TPLO), the protocol used here seemed safe and well tolerated by all dogs. Future studies in delayed and nonunion fractures may produce results that support the use of LIPUS in dogs in specific cases. However, the results of this study do not provide evidence to support the routine use of LIPUS after TPLO or other stable osteotomies in dogs.

CONFLICT OF INTEREST

The LIPUS units used were on loan from the manufacturer for the study. None of the authors have proprietary interest in this technology. The authors declare no conflicts of interest related to this report.

REFERENCES

[1] Padilla F, Puts R, Vico L, Raum K. Stimulation of bone repair with ultrasound: a review of the possible mechanic effects. *Ultrasonics.* 2014;54:1125-1145.

[2] Duarte LR. The stimulation of bone growth by ultrasound. *Arch Orthop Trauma Surg.* 1983;101:153-159.

[3] Malizos KN, Hantes ME, Protopappas V, Papachristos A. Low-intensity pulsed ultrasound for bone healing: an overview. *Injury.* 2006;37(Suppl 1):S56-S62.

[4] Bashardoust Tajali S, Houghton P, MacDermid JC, Grewal R. Effects of low-intensity pulsed ultrasound therapy on fracture healing: a systematic review and meta-analysis. *Am J Phys Med Rehabil.* 2012;91(4):349-367.

[5] Wantanabe Y, Matsushita T, Bhandari M, Zdero R, Schemitsch EH. Ultrasound for fracture healing: current evidence. *J Orthop Trauma.* 2010;24:S56-S61.

[6] Zacheral M, Gruber G, Radl R, Rehak PH, Windhager R. No midterm benefit from low intensity pulsed ultrasound after chevron osteotomy for hallux valgus. *Ultrasound Med Biol.* 2009;35(8):1290-1297.

[7] Pilla AA, Mont MA, Nasser PR, et al. Noninvasive low-intensity pulsed ultrasound accelerates bone healing in the rabbit. *J Orthop Trauma.* 1990;4(3):246-253.

[8] Heckman JD, Ryaby JP, McCabe J, Frey JJ, Kilcoyne RF. Acceleration of tibial fracture-healing by noninvasive, low-intensity pulsed ultrasound. *J Bone Joint Surg Am.* 1994;76(1):26-34.

[9] Kristiansen TK, Ryaby JP, McCabe J, Frey JJ, Roe LR. Accelerated healing of distal radial fractures with the use of specific, low-intensity ultrasound. A multicenter, prospective, randomized, double-blind, placebo-controlled study. *J Bone Joint Surg Am.* 1997;79:961-973.

[10] Tis JE, Meffert CR, Inoue N, et al. The effect of low intensity pulsed ultrasound applied to rabbit tibiae during the consolidation phase of distraction osteogenesis. *J Orthop Res.* 2002;20(4):793-800.

[11] El-Mowafi H, Mohsen M. The effect of low-intensity pulsed ultrasound on callus maturation in tibial distraction osteogenesis. *Int Orthop.* 2005;29(2):121-124.

[12] Mayr E, Laule A, Suger G, Rüter A, Claes L. Radiographic results of callus distraction aided by pulsed low-intensity ultrasound. *J Orthop Trauma.* 2001;15(6):407-414.

[13] Sakurakichi K, Tsuchiya H, Uehara K, Yamashiro T, Tomita K, Azuma Y. Effects of timing of low-intensity pulsed ultrasound on distraction osteogenesis. *J Orthop Res.* 2004;22(2):395-403.

[14] Hantes ME, Mavrodontidis AN, Zalavras CG, Karantanas AH, Karachalios T, Malizos KN. Low-intensity trans-osseous ultrasound accelerates osteotomy healing in a sheep fracture model. *J Bone Joint Surg.* 2004;86A(10):2275-2282.

[15] Yang KH, Park SJ. Stimulation of fracture healing in a canine ulna full-defect model by low-intensity pulsed ultrasound. *Yonsei Med J.* 2001;42(5):503-508.

[16] Tsumaki N, Kakiuchi M, Sasaki J, Ochi T, Yoshikawa H. Low-intensity pulsed ultrasound accelerates maturation of callus in patients treated with opening-wedge high tibial osteotomy by hemicallotasis. *J Bone Joint Surg Am.* 2004;86-A(11):2399-2405.

[17] Rutten S, Nolte PA, Korstjens CM, van Duin MA, Klein-Nulend J. Low-intensity pulsed ultrasound increases bone volume, osteoid thickness and mineral apposition rate in the area of fracture healing in patients with a delayed union of the osteotomized fibula. *Bone.* 2008;43(2):348-354.

[18] Aonuma H, Miyakoshi N, Kasukawa Y, et al. Effects of combined therapy of alendronate and low-intensity pulsed ultrasound on metaphyseal bone repair after osteotomy in the proximal tibia of aged rats. *J Bone Miner Metab.* 2014;32:232-239.

[19] 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.

[20] Light VA, Steiss JE, Montgomery RD, Rumph PF, Wright JC. Temporal-spatial gait analysis by use of a portable walkway system in healthy Labrador retrievers at a walk. *Am J Vet Res.* 2010;71(9):997-1002.

[21] Duerr FM, Duncan CG, Savicky RS, Park RD, Egger EL, Palmer RH. Comparison of surgical treatment options for cranial cruciate ligament disease in large-breed dogs with excessive tibial plateau angle. *Vet Surg.* 2008;37:49-62.

[22] Krotscheck U, Thompson MS, Ryan KK, Mohammed HO. Comparison of TPA, bone healing, and intra-articular screw placement using conventional nonlocked application of surgeon-contoured versus locked application of precontoured TPLO plates in dogs. *Vet Surg.* 2012;41:931-937.

[23] Hoffer MJ, Griffon DJ, Schaeffer DJ, Johnson AL, Thomas MW. Clinical applications of demineralized bone matrix: a retrospective and case-matched study of seventy-five dogs. *Vet Surg.* 2008;37:639-647.

[24] Ostlere SJ, Gold RH. Osteoporosis and bone density measurement methods. *Clin Orthop Relat Res.* 1991:149-163.

[25] Souza TF, Andrade AL, Ferreira GT, et al. Healing and expression of growth factors (TGF-β and PDGF) in canine radial ostectomy gap containing platelet-rich plasma. *Vet Comp Orthop Traumatol.* 2012;25:445-452.

[26] Barnes K, Lanz O, Were S, Werre S, Clapp K, Gilley R. Comparison of autogenous and cancellous bone grafting and extracorporeal shock wave therapy on osteotomy healing in the tibial tuberosity advancement procedure in dogs. Radiographic densinometric evaluation. *Vet Comp Orthop Traumatol.* 2015;28(3):207-214.

[27] Urita A, Iwasaki N, Kondo M, Nishio Y, Kamishima T, Minami A. Effect of low intensity pulsed ultrasound on bone healing at osteotomy sites after forearm bone shortening. *J Hand Surg Am.* 2013;38(3):498-503.

[28] Rutten S, van den Bekerom MP, Sierevelt IN, Nolte PA. Enhancement of bone-healing by low-intensity pulsed ultrasound: a systematic review. *JBJS Rev.* 2016;4(3):e6.

[29] Lascelles BD, Roe SC, Smith E, et al. Evaluation of a pressure walkway system for measurement of vertical limb forces in clinically normal dogs. *Am J Vet Res.* 2006;67:277-006.

[30] Brown DC, Boston RC, Coyne JC, Farrar JT. Ability of the Canine Brief Pain Inventory to detect response to treatment in dogs with osteoarthritis. *J Am Vet Med Assoc.* 2008;233(8): 1278-1283.

[31] Brown DC, Boston RC, Farrar JT. Comparison of force plate gait analysis and owner assessment of pain using the Canine Brief Pain Inventory in dogs with osteoarthritis. *J Vet Intern Med.* 2013;27(1):22-30.

[32] Chan CW, Qin L, Lee KM, Cheung WH, Cheng JC, Leung KS. Dose-dependent effect of low-intensity pulsed ultrasound on callus formation during rapid distraction osteogenesis. *J Orthop Res.* 2006;24:2072-2079.

How to cite this article: Kieves NR, Canapp SO, Lotsikas PJ, et al. Effects of low-intensity pulsed ultrasound on radiographic healing of tibial plateau leveling osteotomies in dogs: a prospective, randomized, double-blinded study. *Veterinary Surgery*. 2018;00:1-9. https://doi.org/10.1111/vsu.12798