Systematic Review

Low-Intensity Pulsed Ultrasound Augments Tendon, Ligament, and Bone–Soft Tissue Healing in Preclinical Animal Models: A Systematic Review

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Purpose: To appraise the available animal and human studies investigating low-intensity pulsed ultrasound stimulation (LIPUS) on tendon, ligament, and bone–soft tissue (B-ST) junction healing. **Methods:** A systematic review of PUBMED, EMBASE, and the Cochrane Library was performed for animal and human studies investigating the effects of LIPUS on tendon, ligament, and B-ST junction healing. The systematic search was performed using the key term "low intensity pulsed ultrasound" and any of the following: "tendon," "ligament," "tendon-bone," and "bone-tendon." Inclusion criteria consisted of (1) randomized controlled trials assessing the effect of LIPUS on bone, tendon, and soft tissue in animals or humans and (2) English-language articles. **Results:** A total of 28 animal and 2 human studies met inclusion criteria. Animal studies utilized various models, including Achilles and patellar tendon transections, medial collateral ligament transections, and surgical repair of patellar tendon, rotator cuff tendon, and anterior cruciate ligament, to evaluate the effects of LIPUS. Animal studies demonstrated significantly improved collagen content and organization, bone formation, fibrocartilage remodeling, and mechanical strength with LIPUS treatment compared with controls. In human trials, LIPUS treatment of chronic tendinopathies did not improve clinical outcomes. **Conclusions:** In acute injury animal models, LIPUS augmented healing of acute tendon, ligament, and B-ST junction injuries through increased collagen content and organization; increased anti-inflammatory cellular signaling; and increased angiogenesis. However, in 2 human studies investigating chronic tendinopathy, LIPUS did not lead to superior outcomes compared with controls. **Clinical Relevance:** Animal models suggest that LIPUS may be a promising noninvasive treatment modality for accelerating patient recovery after acute tendon and ligament injuries, as well as after surgical repair of B-ST junction injuries, but this has not been demonstrated in human studies. Randomized clinical trials evaluating LIPUS for acute tendon and ligament injuries are warranted.

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Low-intensity pulsed ultrasound stimulation (LIPUS) transmits mechanical energy transcutaneously as high-frequency acoustical pressure waves into biological tissues in order to stimulate healing.[1] Multiple studies have demonstrated the effects of LIPUS on bone, including increased angiogenesis and osteogenesis.[1,2] LIPUS is a US Food and Drug Administration–approved treatment for the acceleration of bone healing in the setting of delayed and nonunion fractures.[3-10] LIPUS has also been shown to increase cell proliferation, cytokine production, and extracellular matrix synthesis in fibroblasts and chondrocytes in vitro and in vivo in animal models.[11-15]

Multiple animal studies have examined the effects of LIPUS on tendon, ligament, and bone–soft tissue (B-ST) junction healing. LIPUS has demonstrated potential for accelerating the healing of both acute and chronic tendon and ligament injuries in animal models, allowing for its potential application in the clinical setting.[16-18] Injured Achilles tendons treated with LIPUS in animal models have shown to exhibit significantly more mature collagen fibers, improved biomechanical properties, and increased rate of tissue regeneration compared with controls.[16-18] Studies in medial collateral ligament (MCL) transection rat models treated with LIPUS have demonstrated accelerated healing of ligaments with improved strength and stiffness just after 2 weeks.[19]

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In addition, LIPUS has shown potential benefit for B-ST junction healing in anterior cruciate ligament (ACL) reconstruction, patellar tendon repair, and rotator cuff repair in animal models.[20-24] Typically after surgical repair, the B-ST interface heals via development of a fibrovascular scar that is mechanically inferior and less effective in distributing loads compared with the native enthesis.[25-28] Thus, the use of LIPUS to improve B-ST junction healing may be a promising therapy in the postoperative setting.

Although there is a vast body of literature demonstrating the positive effects of LIPUS on bone healing, the therapeutic effects of LIPUS on tendon, ligament, and B-ST junction healing are unclear. The purpose of this systematic review was to appraise the available animal and human studies investigating LIPUS on tendon, ligament, and B-ST junction healing. We hypothesized that LIPUS treatment would induce positive effects on tendon, ligament, and B-ST junction healing through improved collagen formation and organization, resulting in improved biomechanical properties of these structures.

Methods

Search Strategy

A systematic review of the preclinical literature was conducted according to standard guidelines.[29] The PUBMED, EMBASE, and Cochrane Library databases were searched to identify studies examining the effects of LIPUS on tendon, ligament, and the B-ST junction healing. The systematic search of electronic databases was performed in September 2020, using the key terms "low intensity pulsed ultrasound," with a combination of any of the following terms: "tendon," "ligament," "tendon-bone," "bone-tendon," and "healing." Additional studies were identified through reference review of the previously queried articles. Inclusion criteria consisted of (1) animal studies with controls investigating the effects of LIPUS application on bone, tendon, and B-ST healing; (2) randomized controlled trials assessing the effect of LIPUS on bone, tendon, and soft tissue in humans; and (3) English-language articles. Exclusion criteria consisted of (1) studies examining the treatment effects of continuous, rather than pulsed, low-intensity ultrasound; (2) non-English-language studies; (3) studies discussing LIPUS effects on bone and fracture healing; (4) review papers; (5) supplements or addenda to original articles; and (6) conference abstracts. The effects of LIPUS were assessed based on significantly improved outcomes in the LIPUS-treated group compared with the control group, with improved healing defined as superior histologic (i.e., collagen content and organization) and biomechanical tissue properties in animal models.

Data Extraction

Two reviewers independently screened the titles, abstract, and full-text for study inclusion. Discrepancies between the 2 independent reviewers were resolved by discussion or a third reviewer. Two reviewers then independently extracted data from each of the included studies, including journal/article information (i.e., authors, year of publication, journal), animal model and/or disease being investigated, sample size, procedure performed, LIPUS dosage and duration, control, and treatment outcomes.[30] Fig 1 details the study selection and exclusion process performed in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines.[30]

The initial search yielded 101 unique studies. After title review, 63 studies that focused on tendon, ligament, or the B-ST junction went on to abstract review. During abstract review, studies on the following topics were excluded: periodontal cells, osteoarthritis models, conferences abstracts, and review papers. During full-text review of the remaining 31 articles, 1 non-randomized human trial was excluded. Thirty studies fulfilled the inclusion and exclusion criteria and were included in this review.

Appraisal of Bias

Risk of bias in the randomized human studies was evaluated in accordance with the methods of the Cochrane Collaboration tool. This tool includes 5 bias domains: selection, performance, detection, attrition, reporting, and other potential biases as determined by the authors.[31] For each item, each author reviewed empirical evidence and assigned risk of bias as low risk, high risk, or indeterminate. Assessment of risk of bias was performed independently by 2 authors (W.L. and B.I.), and discrepancies were resolved by discussion to reach a final decision. Animal studies were assessed using the Systematic Review Centre for Laboratory Animal Experimentation risk of bias tool,[32] a validated method adapted from the Cochrane Collaboration tool for assessing risk of bias (Appendix).

Results

Study Characteristics

Thirty studies were included in this review: 11 focused on tendon healing, 4 focused on ligament healing, and 15 focused on healing at the B-ST junction. Two human tendon studies and 28 animal studies were identified. These studies examined the effects of LIPUS on patellar tendinopathy and lateral epicondylitis. Animal tendon studies utilized profundus, Achilles, and patellar tendon models. Ligament studies focused on MCL-transection animal models. B-ST studies examined the effects of LIPUS on the patella—patellar tendon junction, infraspinatus tendon repair, extensor digitorum longus B-ST junction, and ACL reconstruction models. Sample sizes ranged from 8 to 120 animals per study. End points for the studies were measured at postprocedure day 1 up to 18 weeks.

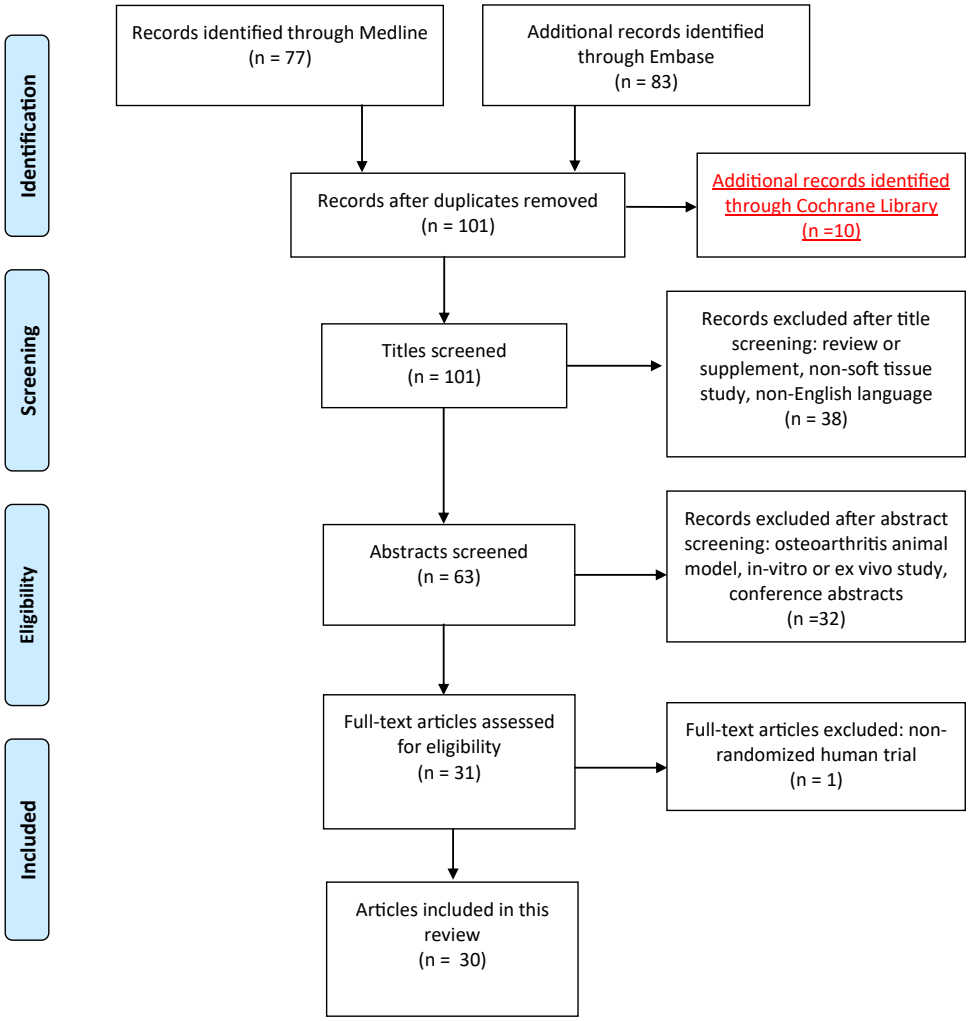

Fig 1. Preclinical studies and clinical evidence on low-intensity pulsed ultrasound for tendon, ligament, and bone-tendon junction healing.

Ultrasound frequencies used ranged from 1.0 to 3 MHz, delivering 30 to 2,300 mW/cm^2 of energy. All available B-ST junction animal studies used an intensity of 30 mW/cm^2 of energy and $\leq$1.5 mHz. Length of LIPUS treatment ranged from 5- to 20-minute intervals once or twice daily. No complications from LIPUS treatment were reported in any animal or human studies analyzed.

Appraisal of Bias

The human studies included in this review were Level I studies with low risk of selection, attrition, reporting, or other biases (Appendix). Among animal studies, we note the possibility of selection, performance, and detection bias in the included articles, as blinding of experimenters to animal treatment groups, treatment administration, and outcome analysis was not always reported.

Effects of LIPUS on Tendon Healing

Two human studies were identified in this review (Table 1). Two randomized control trials were used to assess the potential use of LIPUS to treat chronic tendinopathies. D'Vaz et al.[33] performed a randomized, placebo-controlled trial with 48 patients. LIPUS was used to treat chronic lateral epicondylitis in 25 patients, while 23 were treated with sham ultrasound. LIPUS dosage was set at 30 mW/cm^2 intensity at a 1.0-MHz signal, and patients self-administered treatment for 20 minutes per day for 12 weeks. In LIPUS-treated groups, 64% achieved at least 50% improvement in baseline elbow pain compared with 57% improvement in control groups. However, this difference was not statistically significant. Similarly, in another randomized, placebo-controlled trial, Warden et al.[34] used LIPUS to treat chronic patellar tendinopathy in 20 patients, while 17 were treated with sham ultrasound. Treatment consisted of self-administered LIPUS at 100 mW/cm^2 intensity at 1.0 MHz 20 minutes per day for 12 weeks. In this study, visual analog scales for both usual and worst tendon pain were significantly decreased in both groups ($P < .01$). This study demonstrated that LIPUS provided no additional benefit to exercise therapy for

Table 1. Human Studies Comparing LIPUS to Controls for Tendon Healing

Author and Year	Journal	No. of Subjects	Condition Treated	LIPUS Dosage	LIPUS Duration	Control	Outcome	Level of Evidence
Warden et al. (2008)[34]	*Rheumatology (Oxford)*	37	Patellar tendinopathy	1.0 MHz, 100 mW/cm^2	20 min/d, 7 d/wk for 12 weeks	Mock sonication	In patients with chronic patellar tendinopathy, visual analog scale scores improved in both the placebo and LIPUS-treated groups with no significant difference between the 2 groups. LIPUS may not provide additional benefit over placebo treatment.	I
D'Vaz et al. (2006)[33]	*Rheumatology*	48	Common extensor–supinator tendinopathy	1.5 MHz, 30 mW/cm^2	20 min/d, 12 weeks	Mock sonication	In LIPUS-treated groups, 64% achieved at least 50% improvement in baseline elbow pain score compared with 57% in control groups, but this difference was not significant.	I

LIPUS, low-intensity pulsed ultrasound.

chronic patellar tendinopathy compared with placebo control.

Nine preclinical animal studies investigating the effects of LIPUS on tendon healing were identified (Table 2). One of the first pulsed ultrasound studies by Roberts et al.[35] used a tenotomized profundus tendon in a rabbit model and ultrasound intensity of 800 mW/cm^2 at a 1.1-MHz frequency. Tendons treated with pulsed ultrasound at this intensity did not heal, whereas untreated control tendons healed, illustrating a potentially damaging effect of higher-intensity pulsed ultrasound treatment. Similarly, Larsen et al.[36] assessed LIPUS treatment in the Achilles tendon of New Zealand rabbits at varying intensities of 0 (control), 50, 100, 200, 500, 750, 1,000, and 2,000 mW/cm^2 at a 3-MHz frequency. In this study, increasing intensity of sonication decreased the stiffness and collagen content in the repaired tendons. They found that pulsed ultrasound did not improve the biomechanical properties of the healing Achilles tendon and that extensibility of tendons was higher in the group treated with 2,000 vs 50 mW/cm^2 ($P < .01$).

In contrast, studies that used lower ultrasound intensities more consistently reported beneficial tendon-healing effects. da Cunha et al.[16] used an intensity of 500 mW/cm^2 pulsed ultrasound in a rat Achilles tenotomy model. At 15 days postoperatively, LIPUS-treated Achilles tendons showed increased synthesis and collagen fibril organization compared with mock-sonicated controls and continuous ultrasound treatment ($P = .001$). In the same rat model, Kosaka et al.[14] found that LIPUS treatment at 45 mW/cm^2 significantly increased COX-2 and EP4R in the inflammatory period and increased collagen I and III, as well as transforming groeth factor (TGF)−β1 messenger RNA expression, during the repair and reconstitution process after 2 weeks ($P < .05$). The LIPUS treatment group experienced accelerated tendon repair and increased thickness of collagen I fibrils compared with controls. Similarly, Aiyegbusi et al.[37] found that LIPUS-treated Achilles tendons of rabbits showed a higher population of tenoblasts and volume fraction of collagen fibrils compared with control tendons. Jeremias Júnior et al.[17] further examined Achilles tendon strength in 28 rats after undergoing tenotomy and tenorrhaphy. After a 4-week treatment period with LIPUS, the repaired tendons demonstrated significantly greater ultimate load ($P = .005$) and mean tensile strength ($P = .019$) compared with controls, similar to the findings of Yeung et al.[18]

With regards to temporal application, LIPUS treatment during the granulation phase of healing seems to be more effective than when it is applied during the remodeling phase. Fu et al.[38,39] discovered that rats treated with 2 weeks of LIPUS immediately after patellar tendon injury demonstrated improved collagen

Table 2. Studies Comparing LIPUS to Controls for Tendon Healing

Author and Year	Journal	No. of Animals	Animal Type	Model	LIPUS Dosage	LIPUS Duration	Control	Outcome
Aiyegbusi et al. (2012)[37]	*Connect Tissue Res*	45	Sprague Dawley rats	Achilles tendon crush injury	1 MHz, 500 mW/cm^2	5 min/d starting POD1 for 6 days with 1 day of rest for 14 days; end point POD14 or POD30	No treatment	LIPUS resulted in a significant increase in tenocyte population at POD14, and a higher population of tenoblasts at POD30 day compared with normal tendons and an increase in collagen fibers compared with controls.
Jeremias Júnior et al. (2011)[17]	*J Orthop Sports PT*	28	Wistar rats	Achilles tenotomy and repair	1 MHz, 100 mW/cm^2	5 min/d starting POD1 until POD28	Mock sonication	LIPUS treatment demonstrated significantly greater ultimate load ($P = .005$) and mean tensile strength ($P = .019$) compared with controls. No significant difference was found in cross-sectional area and energy absorption.
Kosaka et al. (2011)[14]	*W Indian Med J*	98	Sprague Dawley rats	Achilles tenotomy	1.5 MHz, 45 mW/cm^2	20 min/d, starting POD0	Contralateral nontreated limb	LIPUS treatment accelerated repair of Achilles tendon compared with untreated groups on electron microscopy. COX-2 and EP4 were overexpressed with LIPUS treatment in the inflammatory period. TGF-β1 expression was markedly induced in LIPUS-treated groups, followed by collagen I and II expression in the repair and reconstitution process.

(continued)

Table 2. Continued

Author and Year	Journal	No. of Animals	Animal Type	Model	LIPUS Dosage	LIPUS Duration	Control	Outcome
Fu et al. (2010)[38]	*J Orthop Sports PT*	78	Sprague Dawley rats	Central third patellar tendon removal	1.5 MHz, 150 mW/cm^2	20 min/d on POD4, 14, or 28	Mock sonication	LIPUS increased COL1A1 and COL3A1 mRNA in healing patellar tendons when administered on POD4 or POD14. LIPUS enhanced collagen synthesis in vivo only during the granulation phase.
Fu et al. (2008)[39]	*AJSM*	60	Sprague-Dawley rats	Central third patellar tendon removal	1 MHz, 30 mW/cm^2	20 min/d, 5 consecutive d/wk, starting POD1, 14, or 28 and treated for 2, 4, or 6 weeks	Mock sonication	LIPUS treatment improved mechanical strength and collagen alignment in healing tendons only when applied during the early stages of healing or the first 2 weeks postoperatively. Extension of treatment beyond 2 weeks did not lead to further improvement of these effects.
Yeung et al. (2006)[18]	*J Orthop Res*	48	Sprague Dawley rats	Achilles hemitenotomy	1 MHz, 500 mW/cm^2	5 min/d, 3 times/week, starting POD1 for 2 or 4 weeks	Mock sonication	LIPUS-treated tendons after 2 or 4 weeks had significantly higher ultimate tensile strength and stiffness ($P < .05$). LIPUS-treated tendons demonstrated more mature denser scar tissue and better aligned collagen fiber bundles, especially after 4 weeks.

(continued)

Table 2. Continued

Author and Year	Journal	No. of Animals	Animal Type	Model	LIPUS Dosage	LIPUS Duration	Control	Outcome
Larsen et al. (2005)[36]	*Acta Orthop*	72	New Zealand rabbits	Achilles tenotomy and repair	3 MHz; 0 (controls), 50, 100, 200, 500, 750, 1,000, and 2,000 mW	5 min/d, consecutively for 10 days	Mock sonication	Stiffness and collagen content declined with increasing intensities of ultrasound. Extensibility of the healing tendons was greater at higher intensity. There was no significant effect on the load at rupture and normalized load at rupture of ultrasound-treated healing tendons compared with controls.
da Cunha et al. (2001)[16]	*Ultrasound Med Biol*	30	Wistar rats	Achilles tenotomy	1 MHz, 500 mW/cm^2	5 min/d, starting POD1 until POD 14	Mock-sonicated tenotomized and nontenotomized tendons	LIPUS-treated tendons during the first 14 days of healing demonstrated improved arrangement, aggregation state, and molecular order of collagen fibrils compared with controls and continuous ultrasound treatment.
Roberts et al. (1982)[35]	*Hand*	14	New Zealand rabbits	Profundus tenotomy	1.1 MHz, 800 mW/cm^2	5 min/d, 5 d/wk for 6 weeks	Untreated contralateral tendons	None of the tendons treated with ultrasound healed compared with untreated controls.

AJSM, American Journal of Sports Medicine; LIPUS, low-intensity pulsed ultrasound; mRNA, messenger RNA; POD, postoperative day; TGF-β1, transforming growth factor β1.

Table 3. Animal Studies Comparing LIPUS to Controls for Ligament Healing

Author and Year	Journal	No. of Animals	Animal Type	Model	LIPUS Dosage	LIPUS Duration	Control	Outcome
Warden et al. (2006)[19]	*AJSM*	60	Sprague Dawley rats	MCL transection of bilateral knees	1.0 MHz, 100 mW/ cm^2	20 min/d, 5 d/wk for 2, 4, and 12 weeks	Mock sonication	At 2 weeks, LIPUS-treated ligaments were 34.2% stronger, were 27.0% stiffer, and had 54.4% higher energy to failure compared with controls. No significant differences were found between treated and control ligaments at 4 and 12 weeks.
Leung et al. (2005)[41]	*Ultrasound Med Biol*	36	Sprague Dawley rats	MCL transection of bilateral knees	3.0 MHz, 0, 500, 2,300 mW/cm^2	5 min/d for 1, 5, or 10 days	Mock sonication	Ultrasound treatment in high-dose application enhances ligament repair by upregulating expression of TGF-β1 after treatment for 5 and 10 days. Treatment at 10 days had higher expression of TGF-β1 than at 5 days.
Sparrow et al. (2004)[40]	*AJSM*	21	New Zealand rabbits	MCL transection of bilateral knees	1.0 MHz, 300 mW/ cm^2	10 min/d, every other day, 6 total treatments	Mock sonication	At 3 and 6 weeks postoperatively, LIPUS-treated ligaments had a higher proportion of type I collagen compared with controls. At 6 weeks, LIPUS-treated ligaments were larger, with higher ultimate load, ultimate displacement, and energy absorption compared with controls. No significant biomechanical differences were observed at 3 weeks.
Takakura et al. (2002)[12]	*J Ultrasound Med*	13	Sprague Dawley rats	MCL transection of bilateral knees	1.5 MHz, 30 mW/cm^2	20 min/d until POD12 or POD21	Untreated	At POD12, LIPUS-treated ligaments demonstrated superior mechanical properties compared with controls in ultimate load, stiffness, and energy absorption ($P < .05$). There was no difference at POD21. Mean fibril diameter was significantly larger with LIPUS treatment than on the control side ($P < .05$).

AJSM, American Journal of Sports Medicine; LIPUS, low-intensity pulsed ultrasound; MCL, medial collateral ligament; POD, postoperative day; TGF-β1, transforming growth factor β1.

alignment and increased strength but not when LIPUS was applied after the initial 2-week injury period.

Effects of LIPUS on Ligament Healing

Four preclinical animal studies investigating the effects of LIPUS in MCL transection models were identified (Table 3). In a rat model, Takakura et al.[12] found improved mechanical properties in LIPUS-treated MCLs compared with untreated contralateral MCLs at 12 days postoperatively. Specimens from the LIPUS-treated knees had a significantly higher mean ultimate load (21.6 ± 2.0 N) compared with untreated contralateral knees (17.8 ± 0.9 N) along with greater stiffness, mean energy absorption, and mean fibril diameter ($P < .05$). No difference was found in mechanical properties at 3 weeks postoperatively. Similarly, Warden et al.[19] showed that rats with transected MCLs treated with LIPUS for 2 weeks were 34.2% stronger, were 27.0% stiffer, and absorbed 54.4% more energy before failure than controls, but there was no difference in these properties at 4 and 12 weeks of treatment. In addition, Sparrow et al.[40] also showed that LIPUS-treated ligaments demonstrated superior ultimate load and energy absorption compared with the sham-treated controls at 6 weeks. The cross-sectional areas of ligament scars were larger, and the proportion of type I collagen was greater in LIPUS-treated ligaments.

Leung et al.[41] suggested that LIPUS could lead to ligament healing by promoting upregulation of TGF-β1 during the inflammation and proliferation phases of soft tissue healing. This study used transected MCLs in rat models at varying intensities (0, 500, and 2,300 mW/cm^2) to assess the healing ligament. Their study demonstrated that TGF-β1 was significantly elevated at 5 days and 10 days after ultrasound treatment in their high-dose group, with elevation being highest at 10 days.

Effect of LIPUS on the B-ST Junction

Fifteen preclinical animal studies investigating the effects of LIPUS on B-ST junction healing were identified (Table 4).

Cellular Effects

Several animal studies have noted increased vascularity in LIPUS-treated B-ST junctions. Lu et al.[13] demonstrated that early vascular endothelial growth factor (VEGF) upregulation plays a role in healing at the patella−patellar tendon junction in rabbits. At 4 weeks postoperatively, chondrocytes and osteoblasts in woven bone expressed significantly more VEGF on immunohistologic staining in the LIPUS group compared with controls ($P < .05$). Similarly, after ACL reconstruction in sheep, Walsh et al.[20] found increased vascularity and angiogenesis at 3, 6, and 12 weeks postoperatively at the B-ST junction in femoral and tibial bone tunnels compared with untreated controls. In an ovine rotator cuff tear model, Lovric et al.[22] further demonstrated significantly increased protein expression of VEGF ($P = .038$), RUNX2 ($P = .02$), and Smad4 ($P = .05$) in the rotator cuff B-ST junction 4 weeks postoperatively in LIPUS treatment groups.

Several studies have demonstrated that LIPUS has a role in the regulation of growth factors and cytokines at the B-ST junction. Results from this study indicated that at postoperative day (POD) 0, TGF-β1 was upregulated in a bimodal distribution (POD0 and POD14).[42] Lu et al.[43] showed that LIPUS has an anti-inflammatory role in the process of B-ST junction healing. At the inferior pole−patellar tendon junction, messenger RNA expression of TGF-β1 and interleukin (IL) 10, which has anti-inflammatory effects, was significantly higher in the LIPUS-treated groups compared with placebo-treated controls at 4, 8, and 16 weeks ($P < .05$). In contrast, expression of proinflammatory cytokines, tumor necrosis factor−α and IL-1β, were lower compared with placebo-treated specimens. Gene expression of inflammatory cytokine IL-6 in LIPUS groups was also significantly lower at week 4 ($P < .05$).

Bone Formation at the B-ST Junction

Among the reviewed studies, LIPUS promoted increased bone formation at the B-ST junction after surgery compared with controls (Table 4). Studies of the distal pole−patellar tendon junction of a rabbit patellectomy model showed that bone formation was higher in the LIPUS-treated groups compared with untreated groups at 8, 12, 16, and 18 weeks after patellectomy.[21,23,43-47] In one study by Qin et al.,[21] bone formation was 2.6 and 3.0 times greater in the LIPUS group compared with the control group at 8 and 16 weeks after patellectomy, respectively. In addition, in an ovine rotator cuff repair model, Lovric et al.[22] found that LIPUS-treated groups demonstrated a thicker region of woven bone with increased osteoblast activity at the B-ST interface in the initial phase of healing. A recent study by Chen et al.[48] found that a combination of LIPUS and adipose-derived stem cells led to greater bone formation.

Bone mineral density (BMD) is also increased with LIPUS treatment at the B-ST junction. Lovric et al.[22] demonstrated that at 4 weeks postoperatively, BMD at the footprint of the rotator cuff repair was significantly higher in the LIPUS-treated group ($P < .01$). Furthermore, in the rabbit postpatellectomy model, multiple authors have demonstrated that LIPUS enhances BMD at the B-ST junction at 6, 8, and 12 weeks postoperatively compared with controls.[21,45,47]

Last, LIPUS treatment at the B-ST junction has been associated with accelerated bone remodeling and formation of new trabecular bone.[24,38] The histologic assessment by Lu et al.[24] of LIPUS-treated patellectomy rabbit models showed formation of irregular woven bone as early as 8 weeks postpatellectomy with overall eventual progression to more mature bone compared with control groups.

Table 4. Animal Studies Comparing LIPUS to Controls for Bone–Soft Tissue Junction Healing

Author and Year	Journal	No. of Animals	Animal Type	Model	LIPUS Dosage	LIPUS Duration	Control	Outcome
Chen et al. (2019)[48]	*AJSM*	112	New Zealand rabbits	Partial patellectomy with patellar tendon repair	1.5 mHz, 30 mW/cm^2	20 min/d, starting POD3 until 8 or 16 weeks	Mock sonication	Autologous ASC transplantation stimulated with LIPUS increased bone formation, regenerated fibrocartilage, and led to higher failure load and stiffness compared with LIPUS- or ASC-treated group.
Lu et al. (2018)[46]	*Am J Phys Med Rehabil*	120	New Zealand rabbits	Partial patellectomy with patella–patellar tendon repair	1.5 MHz, 30 mW/cm^2	20-min duration with 2 groups: daily or twice daily. Starting POD7 and up to 8 or 16 weeks	Mock sonication	LIPUS treatment twice a day was more effective than once-a-day treatment on BSTJ healing, showing increased bone formation, fibrocartilage regeneration, and biomechanical properties.
Lu et al. (2016)[50]	*AJSM*	112	New Zealand rabbits	Partial patellectomy with patellar tendon repair	1.5 MHz, 30 mW/cm^2	20 min/d, starting on POD0, POD7, or POD14 until 8 and 16 weeks	Mock sonication	LIPUS-treated groups improved biomechanical properties at the BSTJ. LIPUS initiated at POD7 was more effective than initiating at POD0 or POD14, as demonstrated by increased stiffness, ultimate strength, bone volume, and bone mineral content.
Lu et al. (2016)[43]	*J Orthop Res*	36	New Zealand rabbits	Partial patellectomy with patellar tendon repair	1.5 MHz, 30 mW/cm^2	20 min/d, starting 2 weeks postoperatively until 4, 8, and 16 weeks	Mock sonication	LIPUS treatment resulted in greater mRNA expression of anti-inflammatory cytokines TGF-β1 and IL-10 compared with controls at 4, 8, and 16 weeks. Expression of proinflammatory cytokines, TNF-α and IL-1β, was lower at 4 and 8 weeks compared with controls. IL-6 mRNA expression in LIPUS groups was also lower at 4 weeks. LIPUS treatment also resulted in higher failure load, ultimate strength, and energy at failure compared with controls at 4, 8, and 16 weeks.
Lu et al. (2015)[24]	*PLoS One*	24	New Zealand rabbits	Partial patellectomy with patellar tendon repair	1.5 MHz, 30 mW/cm^2	20 min/d, starting POD7 until 8 or 16 weeks	Mock sonication	LIPUS accelerated bone formation and remodeling of new trabecular bone during the BSTJ healing process compared with controls, as evidenced by 3-dimensional visualization of new trabecular bone on micro-CT.

(continued)

Table 4. Continued

Author and Year	Journal	No. of Animals	Animal Type	Model	LIPUS Dosage	LIPUS Duration	Control	Outcome
Hu et al. (2013)[45]	*J Orthop Res*	20	New Zealand rabbits	Partial patellectomy with patellar tendon repair	1.5 MHz, 30 mW/cm^2	LIPUS: 20 min/d, starting POD3 until 6 weeks. FES: 30 min/d, 5 times/wk for 6 weeks, starting 6 weeks postoperatively	Untreated	LIPUS treatment alone, late FES, or LIPUS + late FES demonstrated increased area and bone mineral content of new bone, improved remodeling of newly formed bone/fibrocartilage zone, and improved failure load and ultimate strength of the BSTJ compared with untreated controls. LIPUS alone improved ultimate strength (LIPUS: 7.24 ± 1.58 MPa vs control: 4.20 ± 0.69 MPa, $P < .05$).
Lovric et al. (2013)[22]	*Knee Surg Sports Traumatol Arthrosc*	8	Wethers	Infraspinatous tendon complex injury with tendon repair	1.5 MHz, 30 mW/cm^2	20 min/d, 5 d/wk, until 4 weeks	Untreated	LIPUS upregulates growth factor expression (Smad 4, VEGF, and RUNX2), improves bone-tendon integration, and increases BMD at the rotator cuff repair footprint compared with untreated controls.
Lu et al. (2009)[49]	*Orthopedics*	20	New Zealand rabbits	Extensor digitorum longus transplantation to proximal tibial bone tunnel	1.0 MHz, 48 mW/cm^2	20 min/d until 1, 3, 6, or 12 weeks	Mock sonication	LIPUS-treated BSTJ demonstrated denser granulation tissue and increased tensile strength during the early stages of healing. LIPUS treatment also exhibited new bone formation compared with mock-treated controls.
Lu et al. (2009)[47]	*Conf Proc IEEE Eng Med Biol Soc*	24	New Zealand rabbits	Partial patellectomy with patellar tendon repair	1.5 mHz, 30 mW/cm^2	20 min/d, starting POD3, until 6, 12, or 18 weeks	Untreated	LIPUS treatment showed higher BMD at week 6 and increased new bone and stiffness of tissues at the BSTJ compared with controls at week 18.
Papatheodorou et al. (2009)[42]	*Ultrasound Med Biol*	52	New Zealand rabbits	ACL excision and reconstruction with long digital extensor tendon autograft	1 MHz, 30 mW/cm^2	20 min/d, starting POD1 until POD1, 2, 5, 7, 9, 12, 14, and 21	Untreated	Transosseous LIPUS treatment led to faster healing rate of the BSTJ and stable graft incorporation within the tibial bone tunnel compared with the control group. LIPUS induced upregulation of biglycan and collagen I gene, while TGF-β1 expression exhibited a bimodal profile.

(continued)

Table 4. Continued

Author and Year	Journal	No. of Animals	Animal Type	Model	LIPUS Dosage	LIPUS Duration	Control	Outcome
Lu et al. (2008)[13]	*Ultrasound Med Biol*	64	New Zealand rabbits	Partial patellectomy with patellar tendon repair	1.5 MHz, 30 mW/cm^2	5 d/wk, 20 min/d, starting POD3 until 2, 4, 8, and 16 weeks	Untreated	LIPUS treatment accelerated early angiogenesis by upregulating VEGF, leading to subsequent chondrogenesis during BSTJ healing. LIPUS treatment led to thicker fibrocartilage zone and larger cartilaginous metaplasia at BSTJ.
Walsh et al. (2007)[20]	*Arthroscopy*	89	Wethers	ACL excision and reconstruction with long digital extensor tendon autograft	1.5 mHz, 30 mW/cm^2	20 min/d, treated daily until 3, 6, and 12 weeks	Untreated	LIPUS appears to improve healing at the BSTJ for soft tissue grafts as evidenced by greater peak load, increased bone organization, vascularity and angiogenesis, and a more mature BSTJ compared with untreated controls.
Lu et al. (2006)[44]	*AJSM*	48	New Zealand rabbits	Partial patellectomy with patellar tendon repair	1.5 MHz, 30 mW/cm^2	20 min/d, starting POD3 until 2, 4, 8, or 16 weeks	Mock sonication	LIPUS treatment accelerated repair at the BSTJ via increased new bone formation, advanced bone remodeling, and fibrocartilage regeneration. LIPUS treatment resulted in 35.1% higher failure load and 35.0% higher ultimate strength compared with controls at 16 weeks.
Qin et al. (2006)[23]	*Clin Biomech (Bristol, Avon)*	16	New Zealand rabbits	Partial patellectomy with patellar tendon repair	1.5 mHz, 30 mW/cm^2	20 min/d, starting POD3, up to 8 or 16 weeks	Untreated	LIPUS treatment resulted in 3 times greater bone formation and 20% increase in matrix hardness of new bone compared with untreated controls at week 16.
Qin et al. (2006)[21]	*Ultrasound Med Biol*	32	New Zealand rabbits	Partial patellectomy with patellar tendon repair	1.5 mHz, 30 mW/cm^2	20 min/d, 5 d/wk, starting POD3 until 8 and 16 weeks	Untreated	LIPUS treatment resulted in 2.6 and 3.0 times greater size of new bone compared with controls at weeks 8 and 16, respectively. LIPUS group showed higher BMD at week 8 than controls.

ACL, anterior cruciate ligament; *AJSM, American Journal of Sports Medicine*; ASC, adipose-derived stromal cell; BMD, bone mineral density; BSTJ, bone—soft tissue junction; CT, computer tomography; FES, functional electrical stimulation; LIPUS, low-intensity pulsed ultrasound; mRNA, messenger RNA; POD, postoperative day; TGF-β1, transforming growth factor β1; TNF-α, tumor necrosis factor—α; VEGF, vascular endothelial growth factor.

Collagen Organization at the B-ST Junction

Several studies reported superior collagen organization with LIPUS treatment at the B-ST junction. Qin et al.[21] and Lu et al.[43] both demonstrated improved collagen alignment and cross-sectional area of the B-ST junction within the LIPUS treatment group after patellectomy. Lovric et al.[22] and Walsh et al.[20] also both found that LIPUS-treated ovine rotator cuff repairs and intra-articular ACL reconstructions in sheep produced earlier Sharpey fibers and a more mature continuous B-ST interface compared with untreated controls. The control group demonstrated a discontinuous B-ST interface and some discrete Sharpey fibers much later than the LIPUS-treated group at week 26 postoperatively.[20] In addition, Lu et al.[49] demonstrated increased collagen fibers as early as 3 weeks after initiation of LIPUS treatment at the extensor digitorum longus tendon—tibial interface in a rabbit model.

Fibrocartilage Remodeling at the B-ST Junction

At the cartilaginous zone, LIPUS treatment advanced remodeling at the distal pole—patellar tendon junction in partial patellectomy rabbit models.[13,44,46,50] Lu et al.[13] found regeneration of the fibrocartilage layer and a larger field of cartilaginous metaplasia at the patella—patellar tendon healing junction at 8 and 16 weeks after patellectomy in LIPUS treatment groups. By week 8, LIPUS-treated samples showed cartilage-like tissue at the patella—patellar tendon osteotomy site with larger, more mature cartilage cells compared with the untreated controls.[43] In addition, other authors have found increased Vickers hardness of new fibrocartilage tissue and improved remodeling of the fibrocartilage zone after patellectomy in LIPUS-treated partial-patellectomy rabbits.[23,45]

Mechanical Properties at the B-ST Junction

In rabbit patellectomy model studies, Lu et al.[43,44] and Hu et al.[45] showed that failure load, ultimate strength, and energy at failure of the patella—patellar tendon junction were significantly higher in LIPUS groups than control groups ($P < .05$). For example, Lu et al.[44] demonstrated a 35.1% higher failure load and 35.0% higher ultimate strength in LIPUS-treated groups at 16 weeks. In addition, in a ovine rotator cuff tear model, Walsh et al.[20] demonstrated that LIPUS treatment resulted in a greater peak load and stiffness compared with controls. Lu et al.[49] found similar results with LIPUS treatment, resulting in higher maximal tensile strength at 2 weeks at the tendon-tibial interface using transplanted extensor digitorum tendons in a rabbit model.

Discussion

In acute injury animal models, LIPUS augmented healing of acute tendon, ligament, and B-ST junction injuries, while LIPUS demonstrated no benefit for chronic tendinopathies in humans in 2 studies. In acute tendon and ligament injury animal models, LIPUS markedly increased collagen content and organization, correlating with improved biomechanical properties. At the B-ST junction in animals, LIPUS treatment demonstrated increased TGF-β1 and anti-inflammatory cellular signaling, angiogenesis, bone formation, and improved fibrocartilage remodeling, resulting in improved failure loads at the interface.

The body of literature from animal models suggests that LIPUS treatment improves tendon, ligament, and B-ST junction healing through anabolic and angiogenic growth factor signaling, which is similar to the effects observed in healing bone. In vitro studies demonstrate that LIPUS treatment increases levels of bone morphogenetic protein-2, -4, and -7 and VEGF production as well as phosphorylation of Smad1,[51-53] and these effects occur in vivo as well.[13,20,22] Furthermore, LIPUS may promote an environment for healing by increasing vascularity and the expression of anti-inflammatory cytokines and down-regulating proinflammatory cytokines.[43] The molecular mechanisms by which LIPUS accelerates Achilles tendon repair are not well understood but are thought to be related to COX-2 and EP4 expression in the inflammatory period and increased collagen expression via TGF-β1 signaling. EP4 seems to be an important receptor not only for mediating inflammation but also for repairing tissues.[14]

Review of the available studies demonstrated that the effects of LIPUS on healing ligaments and tendons differ based on timing and dosage.[24,48,50] Lu et al.[50] found that ultimate strength, stiffness, and bone formation were superior when LIPUS was started on POD7 compared with POD0 in a rabbit patellectomy model. LIPUS may modulate growth factor and cytokine expression at the B-ST junction. Maintaining the initial native inflammatory phase during acute tendon and ligament injury is important to allow for secretion of cytokines and growth factors to initiate the healing process, and application of LIPUS, which has anti-inflammatory effects, may be better suited during the reparative and remodeling stages. These patterns are also seen in animal studies that demonstrate that early administration of nonsteroidal anti-inflammatory drugs inhibits B-ST junction healing.[54,55] Given the variability in healing noted across postoperative time periods, these studies stress the need to elucidate an ideal treatment window for application of LIPUS. Lu et al.[46] also demonstrated that effects of LIPUS on bone formation are frequency dependent, with twice-daily dosing resulting in increased bone formation. Bone formation has been shown to be an essential step in B-ST junction healing, and the increased density of the trabecular network allows for greater invasion of the tendon fibrous tissue and improved mechanical strength of the B-ST interface.[56-58] Further, restoration

of the fibrocartilage transition zone between bone and tendon/ligament is thought to be important in reducing B-ST junction failure.[56]

There was significant heterogeneity with regards to the LIPUS dosage and tendon injury model among the animal studies reviewed. As a result, the evidence for LIPUS treatment for tendinous injuries remains weak. This study search found only 2 randomized control trials investigating LIPUS treatment in humans. LIPUS-treated and control groups for chronic tendinopathies demonstrated similar improvement in pain scores from baseline, with no significant differences between groups, which may be due to the chronic nature of tendinopathies in comparison to the acute nature of tendon rupture models used in the animal studies.[33,34] It is important to note that in these studies, LIPUS treatment was administered for chronic tendinopathies or after first-line treatments had failed.[33,59] For instance, treatment with LIPUS for chronic lateral epicondylitis was initiated only after patients had at least 6 weeks of symptoms and failed anti-inflammatory medications or corticosteroid injections.[33] In contrast, the animal studies collected in this review investigated the effects of LIPUS in acute surgical injury models, likely representing a different clinical scenario. LIPUS dosage and duration will likely differ depending on the pathology, thickness, and properties of the tissue, as well as the species being treated. Animal models do not always correlate with human conditions. In addition to the discrepancies between chronic tendinopathies in humans and surgical acute injury models in animal models as aforementioned, rodents have robust healing potential, reduced healing time, and ability to produce vitamin C to facilitate collagen synthesis compared with humans.[60] One of the main deficiencies of the many translational animal models utilized to investigate therapies is that they use an acute intervention to study tissues typically affected by a chronic, degenerative process.[61] Because LIPUS has demonstrated anti-inflammatory cellular effects, it may have limited benefit for chronic, degenerative tendinopathies for which inflammation is not generally present or present at low levels.[12,38,40] However, given the existing evidence of histologic and biomechanical strength improvement in Achilles tendon animal models, the refractory nature of chronic tendinopathy, and relative safety of LIPUS, further investigation of the effects of LIPUS on tendinopathy and tendon healing is warranted. Furthermore, this systematic review can help guide future human studies on the clinical application of LIPUS, such as treatment for acute tendon and ligament injuries (e.g., acute MCL knee injuries) or after surgical repair.

Limitations

There are several limitations of this review. First, the definition of LIPUS is not consistent in the current literature, with some considering low-dose intensity to be <1 W/cm^2.[62] The intensity and dosage also differed across the various studies, limiting potential for generalization across multiple studies. Furthermore, there was a paucity of human clinical evidence, as there were only 2 randomized controlled trials to draw from for chronic tendinopathy. Finally, negative results are not often published, and therefore, there may be inherent publication bias in the studies examined in this review.

Conclusions

In acute injury animal models, LIPUS augmented healing of acute tendon, ligament, and B-ST junction injuries through increased collagen content and organization, increased anti-inflammatory cellular signaling, and increased angiogenesis. However, in 2 human studies investigating chronic tendinopathy, LIPUS did not lead to superior outcomes compared with controls.

References

1. Rubin C, Bolander M, Ryaby JP, Hadjiargyrou M. The use of low-intensity ultrasound to accelerate the healing of fractures. *J Bone Joint Surg Am* 2001;83:259-270.
2. Leung KS, Cheung WH, Zhang C, Lee KM, Lo HK. Low intensity pulsed ultrasound stimulates osteogenic activity of human periosteal cells. *Clin Orthop Relat Res* 2004: 253-259.
3. 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.
4. Heckman JD, Ryaby JP, McCabe J, Frey JJ, Kilcoyne RF. Acceleration of tibial fracture-healing by non-invasive, low-intensity pulsed ultrasound. *J Bone Joint Surg Am* 1994;76:26-34.
5. El-Mowafi H, Mohsen M. The effect of low-intensity pulsed ultrasound on callus maturation in tibial distraction osteogenesis. *Int Orthop* 2005;29:121-124.
6. Azuma Y, Ito M, Harada Y, Takagi H, Ohta T, Jingushi S. Low-intensity pulsed ultrasound accelerates rat femoral fracture healing by acting on the various cellular reactions in the fracture callus. *J Bone Miner Res* 2001;16:671-680.
7. Freeman TA, Patel P, Parvizi J, Antoci V, Shapiro IM. Micro-CT analysis with multiple thresholds allows detection of bone formation and resorption during ultrasound-treated fracture healing. *J Orthop Res* 2009;27:673-679.
8. Wang SJ, Lewallen DG, Bolander ME, Chao EY, Ilstrup DM, Greenleaf JF. Low intensity ultrasound treatment increases strength in a rat femoral fracture model. *J Orthop Res* 1994;12:40-47.
9. Gebauer D, Mayr E, Orthner E, Ryaby JP. Low-intensity pulsed ultrasound: Effects on nonunions. *Ultrasound Med Biol* 2005;31:1391-1402.
10. Dijkman BG, Sprague S, Bhandari M. Low-intensity pulsed ultrasound: Nonunions. *Indian J Orthop* 2009;43: 141-148.
11. Cook SD, Salkeld SL, Popich-Patron LS, Ryaby JP, Jones DG, Barrack RL. Improved cartilage repair after treatment with low-intensity pulsed ultrasound. *Clin Orthop Relat Res* 2001:S231-S243.

12. Takakura Y, Matsui N, Yoshiya S, et al. Low-intensity pulsed ultrasound enhances early healing of medial collateral ligament injuries in rats. *J Ultrasound Med* 2002;21:283-288.
13. Lu H, Qin L, Cheung W, Lee K, Wong W, Leung K. Low-intensity pulsed ultrasound accelerated bone-tendon junction healing through regulation of vascular endothelial growth factor expression and cartilage formation. *Ultrasound Med Biol* 2008;34:1248-1260.
14. Kosaka T, Masaoka T, Yamamoto K. Possible molecular mechanism of promotion of repair of acute Achilles tendon rupture by low intensity-pulsed ultrasound treatment in a rat model. *West Indian Med J* 2011;60:263-268.
15. Zhang Z, Huckle J, Francomano CA, Spencer RG. The influence of pulsed low-intensity ultrasound on matrix production of chondrocytes at different stages of differentiation: an explant study. *Ultrasound Med Biol* 2002;28:1547-1553.
16. da Cunha A, Parizotto NA, Vidal BeC. The effect of therapeutic ultrasound on repair of the achilles tendon (tendo calcaneus) of the rat. *Ultrasound Med Biol* 2001;27:1691-1696.
17. Jeremias Júnior SL, Camanho GL, Bassit ACF, Forgas A, Ingham SJM, Abdalla RJ. Low-intensity pulsed ultrasound accelerates healing in rat calcaneus tendon injuries. *J Orthop Sports Phys Ther* 2011;41:526-531.
18. Yeung CK, Guo X, Ng YF. Pulsed ultrasound treatment accelerates the repair of Achilles tendon rupture in rats. *J Orthop Res* 2006;24:193-201.
19. Warden SJ, Avin KG, Beck EM, DeWolf ME, Hagemeier MA, Martin KM. Low-intensity pulsed ultrasound accelerates and a nonsteroidal anti-inflammatory drug delays knee ligament healing. *Am J Sports Med* 2006;34:1094-1102.
20. Walsh WR, Stephens P, Vizesi F, Bruce W, Huckle J, Yu Y. Effects of low-intensity pulsed ultrasound on tendon-bone healing in an intra-articular sheep knee model. *Arthroscopy* 2007;23:197-204.
21. Qin L, Lu H, Fok P, et al. Low-intensity pulsed ultrasound accelerates osteogenesis at bone-tendon healing junction. *Ultrasound Med Biol* 2006;32:1905-1911.
22. Lovric V, Ledger M, Goldberg J, et al. The effects of low-intensity pulsed ultrasound on tendon-bone healing in a transosseous-equivalent sheep rotator cuff model. *Knee Surg Sports Traumatol Arthrosc* 2013;21:466-475.
23. Qin L, Fok P, Lu H, Shi S, Leng Y, Leung K. Low intensity pulsed ultrasound increases the matrix hardness of the healing tissues at bone-tendon insertion: A partial patellectomy model in rabbits. *Clin Biomech (Bristol, Avon)* 2006;21:387-394.
24. Lu H, Zheng C, Wang Z, Chen C, Chen H, Hu J. Effects of low-intensity pulsed ultrasound on new trabecular bone during bone-tendon junction healing in a rabbit model: A synchrotron radiation micro-CT study. *PLoS One* 2015;10:e0124724.
25. Palesy PD. Tendon and ligament insertions—a possible source of musculoskeletal pain. *Cranio* 1997;15:194-202.
26. Wong MW, Qin L, Tai JK, Lee SK, Leung KS, Chan KM. Engineered allogeneic chondrocyte pellet for reconstruction of fibrocartilage zone at bone-tendon junction—a preliminary histological observation. *J Biomed Mater Res B Appl Biomater* 2004;70:362-367.
27. Qin L, Leung KS, Chan CW, Fu LK, Rosier R. Enlargement of remaining patella after partial patellectomy in rabbits. *Med Sci Sports Exerc* 1999;31:502-506.
28. Kinneberg KR, Galloway MT, Butler DL, Shearn JT. The native cell population does not contribute to central-third graft healing at 6, 12, or 26 weeks in the rabbit patellar tendon. *J Orthop Res* 2013;31:638-644.
29. Harris JD, Quatman CE, Manring MM, Siston RA, Flanigan DC. How to write a systematic review. *Am J Sports Med* 2014;42:2761-2768.
30. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med* 2009;6:e1000097.
31. Higgins JPT, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011;343:d5928.
32. Hooijmans CR, Rovers MM, de Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol* 2014;14:43.
33. D'Vaz AP, Ostor AJ, Speed CA, et al. Pulsed low-intensity ultrasound therapy for chronic lateral epicondylitis: A randomized controlled trial. *Rheumatology (Oxford)* 2006;45:566-570.
34. Warden SJ, Metcalf BR, Kiss ZS, et al. Low-intensity pulsed ultrasound for chronic patellar tendinopathy: A randomized, double-blind, placebo-controlled trial. *Rheumatology (Oxford)* 2008;47:467-471.
35. Roberts M, Rutherford JH, Harris D. The effect of ultrasound on flexor tendon repairs in the rabbit. *Hand* 1982;14:17-20.
36. Larsen A, Kristensen G, Thorlacius-Ussing O, Oxlund H. The influence of ultrasound on the mechanical properties of healing tendons in rabbits. *Acta Orthop* 2005;76:225-230.
37. Aiyegbusi AI, Duru FI, Akinbo SR. The morphology of the healing tendon: A comparison of the effects of intrasound therapy and therapeutic pulsed ultrasound. *Connect Tissue Res* 2012;53:478-484.
38. Fu SC, Hung LK, Shum WT, et al. In vivo low-intensity pulsed ultrasound (LIPUS) following tendon injury promotes repair during granulation but suppresses decorin and biglycan expression during remodeling. *J Orthop Sports Phys Ther* 2010;40:422-429.
39. Fu SC, Shum WT, Hung LK, Wong MW, Qin L, Chan KM. Low-intensity pulsed ultrasound on tendon healing: A study of the effect of treatment duration and treatment initiation. *Am J Sports Med* 2008;36:1742-1749.
40. Sparrow KJ, Finucane SD, Owen JR, Wayne JS. The effects of low-intensity ultrasound on medial collateral ligament healing in the rabbit model. *Am J Sports Med* 2005;33:1048-1056.
41. Leung MC, Ng GY, Yip KK. Therapeutic ultrasound enhances medial collateral ligament repair in rats. *Ultrasound Med Biol* 2006;32:449-452.
42. Papatheodorou LK, Malizos KN, Poultsides LA, et al. Effect of transosseous application of low-intensity ultrasound at the tendon graft-bone interface healing: Gene

expression and histological analysis in rabbits. *Ultrasound Med Biol* 2009;35:576-584.
43. Lu H, Liu F, Chen H, et al. The effect of low-intensity pulsed ultrasound on bone-tendon junction healing: Initiating after inflammation stage. *J Orthop Res* 2016;34:1697-1706.
44. Lu H, Qin L, Fok P, et al. Low-intensity pulsed ultrasound accelerates bone-tendon junction healing: A partial patellectomy model in rabbits. *Am J Sports Med* 2006;34:1287-1296.
45. Hu J, Qu J, Xu D, Zhang T, Qin L, Lu H. Combined application of low-intensity pulsed ultrasound and functional electrical stimulation accelerates bone-tendon junction healing in a rabbit model. *J Orthop Res* 2014;32:204-209.
46. Lu H, Liu F, Chen C, et al. Low-intensity pulsed ultrasound stimulation for tendon-bone healing: A dose-dependent study. *Am J Phys Med Rehabil* 2018;97:270-277.
47. Lu MH, Zheng YP, Huang QH, Lu HB, Qin L. Low intensity pulsed ultrasound increases the mechanical properties of the healing tissues at bone-tendon junction. *Conf Proc IEEE Eng Med Biol Soc* 2009;2009:2141-2144.
48. Chen C, Zhang T, Liu F, et al. Effect of low-intensity pulsed ultrasound after autologous adipose-derived stromal cell transplantation for bone-tendon healing in a rabbit model. *Am J Sports Med* 2019;47:942-953.
49. Lu CC, Liu YC, Cheng YM, Chih TT, Tien YC. Augmentation of tendon-bone interface healing with low-intensity pulsed ultrasound. *Orthopedics* 2009;32:173.
50. Lu H, Chen C, Qu J, et al. Initiation timing of low-intensity pulsed ultrasound stimulation for tendon-bone healing in a rabbit model. *Am J Sports Med* 2016;44:2706-2715.
51. Suzuki A, Takayama T, Suzuki N, et al. Daily low-intensity pulsed ultrasound stimulates production of bone morphogenetic protein in ROS 17/2.8 cells. *J Oral Sci* 2009;51:29-36.
52. Doan N, Reher P, Meghji S, Harris M. In vitro effects of therapeutic ultrasound on cell proliferation, protein synthesis, and cytokine production by human fibroblasts, osteoblasts, and monocytes. *J Oral Maxillofac Surg* 1999;57:409-420.
53. Sun JS, Hong RC, Chang WH, Chen LT, Lin FH, Liu HC. In vitro effects of low-intensity ultrasound stimulation on the bone cells. *J Biomed Mater Res* 2001;57:449-456.
54. Cohen DB, Kawamura S, Ehteshami JR, Rodeo SA. Indomethacin and celecoxib impair rotator cuff tendon-to-bone healing. *Am J Sports Med* 2006;34:362-369.
55. Ferry ST, Dahners LE, Afshari HM, Weinhold PS. The effects of common anti-inflammatory drugs on the healing rat patellar tendon. *Am J Sports Med* 2007;35:1326-1333.
56. Wong MW, Qin L, Lee KM, Leung KS. Articular cartilage increases transition zone regeneration in bone-tendon junction healing. *Clin Orthop Relat Res* 2009;467:1092-1100.
57. Rodeo SA, Arnoczky SP, Torzilli PA, Hidaka C, Warren RF. Tendon-healing in a bone tunnel: A biomechanical and histological study in the dog. *J Bone Joint Surg Am* 1993;75:1795-1803.
58. Mihelic R, Pecina M, Jelic M, et al. Bone morphogenetic protein-7 (osteogenic protein-1) promotes tendon graft integration in anterior cruciate ligament reconstruction in sheep. *Am J Sports Med* 2004;32:1619-1625.
59. Hsu AR, Holmes GB. Preliminary treatment of achilles tendinopathy using low-intensity pulsed ultrasound. *Foot Ankle Spec* 2016;9:52-57.
60. Weber B, Lackner I, Haffner-Luntzer M, et al. Modeling trauma in rats: Similarities to humans and potential pitfalls to consider. *J Transl Med* 2019;17:305.
61. Rodeo SA. Translational animal models in orthopaedic research. *Am J Sports Med* 2017;45:1487-1489.
62. Xin Z, Lin G, Lei H, Lue TF, Guo Y. Clinical applications of low-intensity pulsed ultrasound and its potential role in urology. *Transl Androl Urol* 2016;5:255-266.

Appendix: Risk of Bias for Human and Animal Studies

Appendix Table 1. Risk of Bias for Human Studies

	Selection		Performance	Detection	Attrition	Reporting	Other
Risk of Bias	Random Sequence Generation	Allocation Concealment	Blinding of Participants and Personnel	Blinding of Outcome Assessment	Incomplete Outcome Data	Free of Selective Reporting	Other Bias
Warden et al. (2008)[34]	Low	Low	Low	Low	Low	Low	Low
D'Vaz et al. (2006)[33]	Low	Low	Low	Low	Low	Low	Low

Appendix Table 2. Risk of Bias for Tendon Studies

	Selection			Performance		Detection		Attrition	Reporting	Other
Risk of Bias	Sequence Generation	Baseline Characteristics	Allocation Concealment	Random Housing	Caregiver Blinding	Random Outcome Assessment	Blinding	Incomplete Outcome Data	Free of Selective Reporting	Other Bias
Aiyegbusi et al. (2012)[37]	Low	Low	Low	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Jeremias Júnior et al. (2011)[19]	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Kosaka et al. (2011)[14]	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Fu et al. (2010)[38]	Low	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Fu et al. (2008)[39]	Low	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Yeung et al. (2006)[18]	Low	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Larsen et al. (2005)[36]	Low	Unclear	Unclear	Low	Unclear	Low	Unclear	Low	Low	Low
da Cunha et al. (2001)[16]	Low	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Roberts et al. (1982)[35]	Low	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low

Appendix Table 3. Risk of Bias for Ligament Studies

	Selection			Performance		Detection		Attrition	Reporting	Other
Study	Sequence Generation	Baseline Characteristics	Allocation Concealment	Random Housing	Caregiver Blinding	Random Outcome Assessment	Blinding	Incomplete Outcome Data	Free of Selective Reporting	Other Bias
Warden et al. (2006)[19]	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Leung et al. (2005)[41]	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Sparrow et al. (2004)[40]	Low	Low	Unclear	Low	Unclear	Low	Unclear	Low	Low	Low
Takakura et al. (2002)[12]	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low

Appendix Table 4. Risk of Bias for Bone–Soft Tissue Studies

	Selection			Performance		Detection		Attrition	Reporting	Other
Study	Sequence Generation	Baseline Characteristics	Allocation Concealment	Random Housing	Caregiver Blinding	Random Outcome Assessment	Blinding	Incomplete Outcome Data	Free of Selective Reporting	Other Bias
Chen et al. (2019)[48]	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Lu et al. (2018)[46]	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Lu et al. (2016)[50]	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Lu et al. (2016)[43]	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Lu et al. (2015)[24]	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Hu et al. (2013)[45]	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Lovric et al. (2013)[22]	Low	Unclear	Unclear	Low	Unclear	Low	Unclear	Low	Low	Low
Lu et al. (2009)[49]	Low	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Lu et al. (2009)[47]	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Papatheodorou et al. (2009)[42]	Low	Low	Unclear	Low	Unclear	Low	Low	Low	Low	Low
Lu et al. (2008)[13]	Low	Low	Unclear	Low	Unclear	Low	Unclear	Low	Low	Low
Walsh et al. (2007)[20]	Low	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Lu et al. (2006)[44]	Low	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Qin et al. (2006)[23]	Low	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low
Qin et al. (2006)[21]	Low	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	Low	Low