The evidence of low-intensity pulsed ultrasound for in vitro, animal and human fracture healing

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Background: Physical stimulation therapies are currently available to enhance fracture healing.

Sources of data: A search of PubMed, Medline, CINAHL, DH data and Embase databases was performed using the keywords ‘ultrasound’ and ‘fracture healing’.

Areas of agreement: The evidence in vitro and animal studies suggests that low-intensity pulsed ultrasound (LIPUS) produces significant osteoinductive effects, accelerating the healing process and improving the bone-bending strength.

Areas of controversy: The evidence in human trials is controversial in fresh, stress fractures and in limb lengthening. LIPUS is effective in delayed unions, in smokers and in diabetic population.

Growing points: LIPUS is an alternative, less invasive form of treatment for complicated fractures, in patients with poor bone healing and may play a role in the management of large-scale bone defects producing substantial cost savings and decreasing associated disability.

Areas timely for developing research: There is heterogeneity among in vitro, animal studies and their application to human studies. Further randomized controlled trials of high methodological quality are needed.

Keywords: low-intensity pulsed ultrasound/fracture healing/physical stimulation therapy

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Introduction

Between 5 and 10% of fractures are complicated by delayed healing. Common causes include inadequate fixation of the fracture, distraction of fracture fragments by fixation devices or traction, repeated manipulations or excessive early motion at the fracture site or excessive periosteal stripping and damage to other soft tissues during operative exposure.

Various biological, mechanical and physical interventions have been implemented to improve fracture healing. Low-energy pulsed ultrasound (LIPUS) has been used in patients with abnormal fracture healing to enhance fracture recovery and improve functional outcome. This review explores the rationale of use and potential role of LIPUS in fracture healing.

Methods

A search of PubMed, Medline, CINAHL and Embase databases was performed using the following keywords ‘low-intensity pulsed ultrasound’ and ‘fracture healing’ on 30 September 2010.

Scientific articles in vitro, animal and human studies were suitable if detailing the use of therapeutic ultrasound in fracture management. Their bibliographies were thoroughly reviewed by hand to identify further-related articles. To be included, a study had to be a prospective clinical study, a randomized controlled trial (RCT), a non-randomized clinical trial or a prospective case series. There had to be a well-described intervention in the form of application, the LIPUS, the intensity of the signal (W/cm²) and the target tissue. The outcomes had to be reported in terms of (i) molecular changes or bone formation in in vitro studies, (ii) histological changes, bone mineral density stock or biomechanical assessment in animal studies and (iii) radiographic healing time (three or four cortices bridged), or clinical healing (fracture stable, not painful to manual stress and weight bearing) in clinical studies.

We thus identified 50 studies which investigated the use of LIPUS in fracture healing (Fig. 1). Of the 50 studies, 15 were performed in vitro, 18 in animal model and 17 in humans. Tables 1–3 highlight the main findings of these studies.

Exclusion criteria

Studies in language other than English, French, Italian, Spanish, Portuguese and results published as abstracts only were excluded from the present study.
Biological effects of LIPUS

Ultrasound, an acoustic radiation, is a form of mechanical energy that can be transmitted into the body as high-frequency pressure waves. The acoustic energy from ultrasound is produced from a piezoelectric crystal within a transducer, which emits high-frequency acoustic pressure waves (1–12 MHz) transmitted through body tissue by molecular vibrations and collisions.\textsuperscript{11} The micromechanical strains produced by these pressure waves in body tissues can result in biochemical events at the cellular level,\textsuperscript{12} and may promote bone formation in a manner comparable with the bone responses to mechanical stress postulated by Wolff’s law.\textsuperscript{13} Ultrasound achieves its biological effects by increasing the temperature of the tissue, with intensities ranging from 0.2 to 100/cm\textsuperscript{2}.\textsuperscript{14} Ultrasonic intensities of 1–3/cm\textsuperscript{2} in conventional therapy reduce joint stiffness, pain and muscle spasm and improve muscular mobility.\textsuperscript{14} In contrast, safe intensities for diagnostic imaging...
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<td>Increase in callus mineral density US &gt; C (P = 0.001) Bending score US = C (P = 0.114)</td>
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<td>Coords et al. 28</td>
<td>Rat DM/ non-DM</td>
<td>Mid-diaphyseal femoral fracture</td>
<td>Increase in growth factor expression and cartilage formation Increase in callus neovascularization DM/non-DM DM: impaired results. However all parameters increased resembled those of non-DM</td>
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<td>McClure et al. 24</td>
<td>Horse</td>
<td>1-cm gap osteotomy 4th MTC</td>
<td>No differences: X-ray, pQCT, histology LIPUS does not affect bone formation in a fracture gap model in the horse</td>
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<td>Lu et al. 23</td>
<td>Rabbit</td>
<td>Integration grafted tendon within tibial bone tunnel</td>
<td>Interface filled with denser granulation tissue and new bone formation (6th week p.o.) Maximum tensile strength: 2 weeks: US &gt; C (P &lt; 0.05) 3 weeks: US = C (P = 0.753)</td>
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<tr>
<td>Perry et al. 31</td>
<td>Rat</td>
<td>Ulnae</td>
<td>LIPUS and load-induce periostial response &gt;80% (&lt;10% C) MAR: 2.9 LIPUS/8.6 LIPUS + load/8.7 load loading + LIPUS = LIPUS alone</td>
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<tr>
<td>Li et al. 30</td>
<td>Rat</td>
<td>Ulna stress fracture LIPUS–NSAID</td>
<td>Two groups NSAID and vehicle alone + LIPUS. C group = contralateral limb NSAID may delay tissue-level repair of stress fracture No interaction between LIPUS and NSAID</td>
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<td>Warden et al. 81</td>
<td>Rat</td>
<td>Femoral fracture</td>
<td>17% increase in bone mineral density after 40 days of treatment</td>
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<tr>
<td>Chan et al. 26</td>
<td>Rabbit</td>
<td>Osteogenesis in rabbit tibiae lengthening</td>
<td>Increase in bone mineral content + volume of distraction callus LIPUS enhanced dose-dependent endochondral formation</td>
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<tr>
<td>Hantes et al. 20</td>
<td>Sheep</td>
<td>Mid-shaft osteotomy of tibia</td>
<td>LIPUS significantly accelerates the fracture-healing process, increases the cortical bone mineral density and improves lateral-bending strength of the healing fracture in a sheep osteotomy model</td>
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<tr>
<td>Rawool et al. 75</td>
<td>Dog</td>
<td>Ulnar fractures</td>
<td>3-fold increase in blood flow around fracture site</td>
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<td>Uglow et al. 27</td>
<td>Rabbit</td>
<td>Distraction osteogenesis in rabbit tibiae</td>
<td>No differences bone mineral content, cross-sectional area, strength. No reduction of osteopenia. Histology: no differences in bone volume fraction LIPUS: fewer trabeculae of increased thickness, and fewer osteoclasts</td>
</tr>
<tr>
<td>Gebauer et al. 29</td>
<td>Rat</td>
<td>DM/non-DM</td>
<td>LIPUS did not significantly alter the proliferation in DM/C early phase Mechanical testing revealed significantly greater torque to failure and stiffness in US-treated DM vs. non-US-treated DM groups at 6 weeks post-fracture</td>
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<tr>
<td>Takikawa et al. 22</td>
<td>Rat</td>
<td>Tibial fractures</td>
<td>13.3% (3/22) of non-union healed after 2 weeks; 31% (7/22) after 4 weeks; 50% (11/22) after 6 weeks</td>
</tr>
<tr>
<td>Yang and Park 25</td>
<td>Dog</td>
<td>Ulna full defect</td>
<td>LIPUS enhanced new bone formation in small and large full defects Reduced the incidence of non-union in a large defect model</td>
</tr>
<tr>
<td>Azuma et al. 19</td>
<td>Rat</td>
<td>Femoral fractures</td>
<td>Bone mineral content and callus area did not change at day 25 Mechanical torsion greater than C</td>
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<tr>
<td>Wang et al. 18</td>
<td>Rat</td>
<td>Femoral fractures</td>
<td>LIPUS at either 0.5 or 1.5 MHz can accelerate fracture repair at 21 days</td>
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<tr>
<td>Pilla et al. 17</td>
<td>Rabbit</td>
<td>Fibular fractures</td>
<td>Biomechanical healing accelerated by a factor of nearly 1.7 than the C group</td>
</tr>
<tr>
<td>Duarte 15</td>
<td>Rabbit</td>
<td>Femoral fractures</td>
<td>Better bone healing in the treatment group as compared with C</td>
</tr>
</tbody>
</table>

MTC, metacarpal; pQCT, peripheral quantitative computed tomography; US, ultrasound; C, control; MAR, mineral apposition rate; NSAID, non-steroidal anti-inflammatory drugs.
### Table 2  In vitro studies (n = 15).

<table>
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<th>Study (reference)</th>
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<th>Results</th>
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<td>Watanuki et al.</td>
<td>Mouse muscle cells</td>
<td>Accelerates maturation of ectopic bone formation on 10th day</td>
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<tr>
<td>Naruse et al.</td>
<td>Dissected rat femora</td>
<td>Increase in calcium and collagen on 10th day</td>
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<tr>
<td>Leskinen et al.</td>
<td>Osteoblast-type cells</td>
<td>Accelerate periosteal bone formation</td>
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<tr>
<td>Takayama et al.</td>
<td>Osteoblast proliferation and differentiation</td>
<td>Did not clearly promote genes involved in osteoblast differentiation</td>
</tr>
<tr>
<td>Bandow et al.</td>
<td>Osteoblasts in different stages</td>
<td>AT1 increased significantly during cell maturation</td>
</tr>
<tr>
<td>Li et al. 34</td>
<td>Rat calvarial cells</td>
<td>AT1 plays an essential role in bone metabolism as a mechanoreceptor of osteoblasts</td>
</tr>
<tr>
<td>Tang et al. 83</td>
<td>Osteoblasts</td>
<td>LIPUS increases COX-2 expression and promotes bone formation in osteoblasts via the integrin/FAK/PI3K/Akt and ERK signaling pathway</td>
</tr>
<tr>
<td>Sant’Anna et al.</td>
<td>Rat bone marrow stromal cell</td>
<td>LIPUS + BMP-2 do not lead to synergy stimuli. LIPUS or BMP-2 increases gene expression</td>
</tr>
<tr>
<td>Yang et al. 82</td>
<td>Osteoblasts and osteoclastogenesis</td>
<td>There may be a time dependence for US stimulus of osteogenic gene expression in BMSC</td>
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<tr>
<td>Mukai et al. 85</td>
<td>Chondrocytes</td>
<td>Regulatory effect on integrins, and differentiation of osteoblasts and osteoclastogenesis. These changes may contribute to the beneficial effects of US on the fracture repair</td>
</tr>
<tr>
<td>Leung et al. 43</td>
<td>Human periosteal cells</td>
<td>LIPUS did not affect the total number of viable cells</td>
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<tr>
<td>Reher et al. 36</td>
<td>Human mandibular osteoblast</td>
<td>It stimulated cell proliferation at the early phase of cell culture. Dose-dependent effect of osteogenic activities.</td>
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<tr>
<td>Parviz et al. 77</td>
<td>Rat chondrocytes</td>
<td>Increased alkaline phosphatase, osteocalcin, VEGF, calcium</td>
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<tr>
<td>Wu et al. 35</td>
<td>Cultured chondrocytes</td>
<td>Increased expression of prostaglandin E2 and nitric oxide</td>
</tr>
<tr>
<td>Ryaby et al. 37</td>
<td>Osteoblastic cells</td>
<td>Increased adenculate cyclic activity, increased expression of TGF-β</td>
</tr>
</tbody>
</table>

AT1, angiotensin II type 1 receptor; BMP-2, bone morphogenetic protein-2; BMSC, bone mineral stem cells; TGFβ-1, tissular growth factor β-1; VEGF, vascular endothelial growth factor.
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<tr>
<th>Study (reference)</th>
<th>Study design</th>
<th>Results</th>
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<tr>
<td>Lubbert et al.</td>
<td>Fresh clavicle shaft fracture</td>
<td>Do not confirm the acceleration in clinical healing</td>
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<tr>
<td>Rutten et al.</td>
<td>Delayed union fibula after HTO</td>
<td>Increased osteoblast activity: osteoid thickness, mineral apposition, bone volume in new bony callus formation</td>
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<td>Handolin</td>
<td>Lateral malleolar fractures. Prospective randomized double-blind placebo CT</td>
<td>No significant differences in visualization fracture line, external callus, percentage of bone healing or bone mineral density</td>
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<tr>
<td>El-Mowafi and Mohsen</td>
<td>Tibial distraction osteogenesis with Ilizarov</td>
<td>Increase in healing index</td>
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<tr>
<td>Gold et al.</td>
<td>Bone transport in segmental tibia defect: prospective study</td>
<td>US: 30 days/cm; C: 48 days/cm (P &lt; 0.05)</td>
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<tr>
<td>Gebauer et al.</td>
<td>Non-unions</td>
<td>57 of 67 (85%) of non-union healed</td>
</tr>
<tr>
<td>Gebauer et al.</td>
<td>Delayed and non-unions after leg lengthening in children</td>
<td>All 17 (100%) cases healed conservatively within 3–12 months</td>
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<tr>
<td>Rue et al.</td>
<td>Tibia stress fracture</td>
<td>Do not significantly reduce the healing time for tibial stress fractures</td>
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<tr>
<td>Leung et al.</td>
<td>Complex tibia fractures</td>
<td>42% reduction in healing time</td>
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<tr>
<td>Tsumaki et al.</td>
<td>Open wedge HTO</td>
<td>BMD callus LIPUS &gt; C (P = 0.02)</td>
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<tr>
<td>Lerner et al.</td>
<td>Delayed unions. High-energy fractures long bones</td>
<td>18 Fx: surgical skeletal stabilization and tissue flaps + LIPUS</td>
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<tr>
<td>Nolte et al.</td>
<td>Non-unions: tibia, femur, radius/ulna, scaphoid, humerus, MTT, clavicle</td>
<td>16 (88%) healed within 13–52 weeks</td>
</tr>
<tr>
<td>Mayr et al.</td>
<td>Delayed and non-unions (1317 cases)</td>
<td>Surgery: 21/conservative treatment: 8</td>
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<tr>
<td>Emami et al.</td>
<td>Tibial shaft fractures fixed with a reamed and statically locked intramedullary rod prospective randomized double-blind and placebo CT</td>
<td>25 (86%) healed within 22 weeks</td>
</tr>
<tr>
<td>Cook et al.</td>
<td>Tibia + distal radius Cohorts of smokers/non-smokers/C group</td>
<td>Smoking made a negative significant difference</td>
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<tr>
<td>Kristiansen et al.</td>
<td>Distal radius fractures</td>
<td>Non-significant differences in healing rate, time and fracture age. Radiographic healing rate: 88% treatment group (139 + 12.3 days) and 89% registry group (131 + 2.4 days).</td>
</tr>
<tr>
<td>Heckman et al.</td>
<td>Tibial fractures</td>
<td>Do not reduce healing time</td>
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CT, controlled trial; HTO, high tibial osteotomy; BMD, bone mineral density; MTT, metatarsal.
are much lower (0.5–50 mW/cm²) to avoid excessive heating of the tissues.¹⁴

Early animal studies showed that ultrasound treatment delayed fracture healing or even damaged healing bone.¹⁴ The intensity of ultrasound dictates its effects on healing bone. A high intensity (1.0 W/cm²) continuous wave ultrasound signal, as applied in earlier animal studies, is harmful. A low-intensity (30 mW/cm²) pulsed ultrasound signal promotes accelerated bone healing.

Evidence from animal models
Several animal studies describe the role of LIPUS in bone healing (Table 1).

Duarte¹⁵ and Xavier and Duarte¹⁶ were the first to report acceleration of healing, both radiographically and histologically, with a LIPUS device, at the site of a controlled fibular osteotomy and in a femoral drill-hole defect in a rabbit model. Pilla et al.¹⁷ investigated the effect of LIPUS on the rate of healing of fresh fractures in 139 mature New Zealand white rabbits, which had received a bilateral mid-shaft fibular osteotomy: one limb was subjected to 20 min of LIPUS stimulation a day. The ultrasonic stimulation significantly enhanced fracture healing by a factor of nearly 1.7, as measured with biomechanical testing. Wang et al.¹⁸ reported similar findings in an experiment involving 22 rats with bilateral closed femoral fractures. The fracture in one limb was treated with LIPUS, and the contralateral limb served as the untreated control. Sixteen of the 22 rats were treated with either 0.5 or 1.5 mHz of LIPUS. The remaining six received sham treatment with the ultrasound device to control for the effects of anaesthesia and handling. LIPUS at either 0.5 or 1.5 mHz enhanced fracture-healing radiographically, histologically and biomechanically. The average maximum torque and torsional stiffness were significantly greater in the LIPUS-treated limbs than in the control limbs.¹⁸,¹⁹

The application of LIPUS significantly accelerated the fracture healing process, increased cortical bone mineral density and improved the lateral-bending strength of the healing fracture in a sheep osteotomy model.²⁰ Transosseous application of low-intensity ultrasound at close proximity to the fracture site may enhance the mechanical properties of the fracture callus and reduce the time to fracture healing.²⁰

Shakouri et al.²¹ found similar results in a case–control study in 30 rabbits with mid-tibia open osteotomies, and also demonstrated that the callus mineral density in the LIPUS group was higher than in the control group at the end of the 8th week ($P = 0.001$). However, the mean recorded three-point bending test score of healed bones in the US group was not significantly different from the control group ($P = 0.114$).
Takikawa et al.\textsuperscript{22} assessed the effectiveness of LIPUS on rat tibial non-unions by placing the tibialis muscle within the fracture site, allowing callus to form at the ends of the fracture but preventing the two ends of the bone from bridging. This model probably mimics more accurately clinical non-unions than traditional animal non-union models that require segmental bone resection. After waiting 6 weeks after fracture and muscle interposition to ensure the development of a non-union, LIPUS was applied once daily for 20 min for an additional 2, 4 and 6 weeks. LIPUS-treated fractures healed 13.3\% of the time after 2 weeks of treatment, 31\% of the time after 4 weeks of treatment and 50\% of the time after 6 weeks of treatment. None of the fractures in the control group healed after 2, 4 or 6 weeks.

Lu et al.\textsuperscript{23} studied the biomechanical and histological properties of grafted tendon integration into a bone tunnel. They performed a case–control trial in 20 rabbits, with a transplant of the extensor digitorum longus into bone tunnels in both proximal tibiae. Histology revealed an interface filled with denser granulation tissue and new bone formation in the 6 and 12 week specimens. The mean maximal tensile strength was significantly higher in the US-treated group in the early stages 2 weeks postoperatively, while the results normalized after 3 weeks.

The effect of LIPUS in bone defects was studied by McClure et al.\textsuperscript{24} and Yang and Park\textsuperscript{25} in an equine model of 1-cm gap osteotomy in the 4th metacarpal and a full ulna defect in a dog (one and half the width of the diaphysis), respectively. LIPUS enhanced new bone formation in the ulna, while in the horse it did not affect bone formation.

When bone lengthening of the tibia in rabbits is used as a model, outcomes are equally contradictory. Chan et al.\textsuperscript{26} reported an increase of bone mineral content and volume of the mineralized tissue of the distraction callus in a dose-dependent manner, while Uglow et al.\textsuperscript{27} did not find any differences in bone volume fraction, bone mineral content and strength. Both works are similar methodologically, but Uglow et al.\textsuperscript{27} discussed about the intramembranous osteogenesis that takes place in bone distractions, and the endochondral ossification that is probably modulated by the LIPUS.

To evaluate the effects of the LIPUS in diabetes mellitus (DM), Coords et al.\textsuperscript{28} and Gebauer et al.\textsuperscript{29} chose a group of DM and non-DM Wistar rats, and in both studies LIPUS was applied on middiaphyseal femoral fractures. In the DM group, healing was impaired. Even though there is an increase of growth factor expression, cartilage formation and neovascularization in the callus, the augmentation resembled but did not reach the non-DM parameters. Both authors\textsuperscript{28,29} agree that its application resulted in improved mechanical properties during the late phases of healing.
Only one study\textsuperscript{30} analysed the interaction between LIPUS and NSAID. Li \textit{et al.}\textsuperscript{30} demonstrated that the beneficial effects of LIPUS were not mediated by the cyclooxygenase-2 pathway. Therefore, the two different treatments in combination did not have interactions, and the beneficial effect of LIPUS was not impaired by the detrimental NSAID effect.

To probe similarities between LIPUS and mechanical stimulation, and understand their effects in normal bone, Perry \textit{et al.}\textsuperscript{31} experimented both responses in rat ulnae. They found an increase of mineral apposition rate higher than the controls, in 2.9 (± 0.9) for the US alone, 8.6 (± 2.4) for US and load and 8.7 microm (± 3.2) for load alone. The three treatments also induced a significant periosteal response. The changes induced by the US shared at least some features with mechanical loading.

\textbf{Evidence from in vitro studies}

\textit{In vitro} studies (Table 2) suggest that LIPUS produces significant multifunctional effects (\(P < 0.05\)) that are directly relevant to bone formation and resorption.\textsuperscript{32–35} LIPUS stimulation directly affects osteogenic cells, leading to enhanced bone mineralization,\textsuperscript{36–38} collagen production and alkaline phosphatase activity in osteoblast,\textsuperscript{39} and even an accelerated maturation of ectopic bone in rat muscles.\textsuperscript{40} However, the osteoinductive effects of LIPUS are limited to bone: it accelerated periosteal bone formation while the cartilage alone did not directly respond to LIPUS.\textsuperscript{41}

Sant’Anna \textit{et al.}\textsuperscript{42} combined the stimulation of LIPUS with BMP-2 in rat bone marrow stromal cells to determine the expression of some genes. The combination of both treatments did not lead to synergy of the two stimuli, and the gene expression was time dependent for the stimulus of osteogenic gene expression in the stromal cells. Leung \textit{et al.}\textsuperscript{43} also concluded that cell proliferation, alkaline phosphatase activity, osteocalcin secretion and vascular endothelial growth factor expression were increased in the early phases of the cell culture. They suggested that the LIPUS treatment should be started from the beginning of fracture healing.

\textbf{Human studies}

Several clinical investigations involving LIPUS\textsuperscript{44–48} have shown successful healing of acute fractures, delayed unions and non-unions, including non-unions of fractures fixed with metallic implants.

In two different trials in fresh fractures of the tibia\textsuperscript{49} and the distal radius,\textsuperscript{50} LIPUS significantly accelerated radiographic healing. Heckman \textit{et al.},\textsuperscript{49} in a multicentre, prospective, randomized, double-blind, placebo-controlled study, evaluated 33 fractures treated with
LIPUS therapy and 34 with a control device used as a placebo. At the end of the treatment, a statistically significant decrease in the time to clinical healing (86 ± 5.8 days in the active-treatment group compared with 114 ± 10.4 days in the control group) (P = 0.01) and also a significant decrease in the time to overall (clinical and radiographic) healing (96 ± 4.9 days in the active-treatment group compared with 154 ± 13.7 days in the control group) (P = 0.0001) were demonstrated.

Heckman and Sarasohn-Kahn demonstrated substantial cost savings as a result of reduced costs of workers compensation and of fewer secondary procedures when LIPUS was used in conjunction with non-operative or operative treatment of tibial diaphyseal fractures.

A multicentre, prospective, randomized, double-blind, placebo-controlled study demonstrated accelerated fracture healing in the distal radial metaphysis. This modality may be useful clinically, as its application continues to be explored for other types of fractures and for those with characteristics that lead to slow or delayed healing.

Clinical trials (Table 3) of LIPUS have shown substantial efficacy in the treatment of fractures of the upper and lower extremities. On the basis of these investigations, the USA Food and Drug Administration recently approved the marketing of these devices for use in selected patients.

Busse et al. performed a meta-analysis of randomized controlled trials of fracture healing. Review of the pooled data showed that the time to healing was significantly improved in the patients who received LIPUS. The study demonstrated weighted average effect size of 6.41 [95% confidence interval (CI): 1.01–11.81), which converts to a mean difference in healing time of 64 days between the treatment and control groups.

Busse et al. analysed the pooled results of the various trials, and reported a low-quality evidence of the outcomes in benefit of the LIPUS in non-operatively managed fresh fractures.

Recently, some systematic reviews of human trials questioned the reliability of the studies and the effectiveness of the LIPUS. By analysing the RCTs of the last years, they found a substantial level of inconsistency in the efficacy of the LIPUS as an adjunct to fracture healing. Although LIPUS was effective in some trials to accelerate fracture healing, no definitive statement could be made regarding its universal use for all fracture types and methods of fracture care.

In a randomized double-blind, placebo-controlled multi-centre trial in 101 adult patients with a non-operatively treated fresh clavicle shaft fracture, Lubbert et al. did not confirm that LIPUS accelerated clinical healing time. The fracture healing rate was based on clinical criteria, and the authors did not find any statistical difference between the
LIPUS and control groups (26.77 vs. 27.09 days in LIPUS and control, with a mean difference of 0.33 days, 95% CI: –5.27 to 5.92, \( P > 0.05 \)).

Rue et al.\(^5^8\) also failed to report significant beneficial effects using LIPUS for tibial stress fractures. Twenty-six midshipmen (43 tibial stress fractures) treated with LIPUS returned to active duty in a mean 55.8 days compared with 56.2 days for those receiving sham therapy (mean difference: 0.4 days, 95% CI: –13.4 to 14.2).

Leung et al.\(^4^3\) reported that the LIPUS group statistically better healed for open and/or severely comminuted tibial shaft fractures managed by intramedullary nailing or external fixation. Their results were based on measuring the radiograph callus bridging one, two or three cortices and clinical outcome measurements (6.5 vs. 9.5 weeks; 8.5 vs. 12.5 weeks; 11.5 vs. 20 weeks in LIPUS and control for first, second and third cortical bridging respectively, \( P < 0.05 \)). Not related pain at fracture site (6.1 vs. 7.9 weeks, \( P < 0.05 \)), time to start full-weight bearing (9.3 vs 15.5 weeks, \( P < 0.05 \)) and removal of external fixator (9.9 vs. 17.1 weeks, \( P < 0.05 \)) also occurred significantly earlier in the LIPUS group. Emami et al.\(^5^9\), in contrast, did not find statistical differences in healing time using LIPUS vs. non-treated controls in tibial shaft fractures treated with a reamed and statically locked intramedullary nail. The time to detection of first callus was 40 ± 3 days in the treatment group vs. 37 ± 3 days in the control group. The difference in means was 3 days, 95% CI: –16.5–76, \( P > 0.05 \). The healing rate for the third cortical bridging was 155 ± 22 vs. 125 ± 11 days in the control group. The difference in means was 30 days, \( P > 0.05 \). The time of full-weight bearing without crutches was 6.5 vs. 7.1 weeks in LIPUS and control. The difference in means was 0.6 weeks, 95% CI: –1.5 to 2.7, \( P > 0.05 \). Also, Handolin et al.\(^6^0\) did not observe benefits for lateral malleolar fractures fixed by bioabsorbable screws. They did not detect any significant differences in external callus and fracture line visualization at 2, 6, 9, 12 weeks after operation in LIPUS and control, respectively. There were no differences of bone mineral density (0.511 vs. 0.538 g/cm\(^2\) in LIPUS and control, difference in means –0.001 g/cm\(^2\), 95% CI: –0.049 to 0.046, \( P > 0.05 \)), on the ratio of endosteal healing (0.425 vs. 0.388% in LIPUS and control, difference in means 0.038%, 95% CI: –0.290 to 0.365, \( P > 0.05 \)) and Olerud–Molander clinical score (99.4 vs. 98.8 preoperation and 95.0 vs. 96.3 18 months after surgery in the LIPUS and control groups, respectively; difference in means 0.6 and 1.3, 95% CI: 98.2–99.4 and 95.0–99.4, \( P = 0.05 \)). This type of fracture usually heals rapidly with few complications; therefore, the clinical relevance of this study is unclear.\(^5^5\)

Gebauer et al.,\(^6^1\) studying the effects of LIPUS on non-unions, found that 85% (57 of 67) of non-unions healed after a 20 min of daily...
transdermal treatment for an average of 168 days. This matched the healing rates achieved after surgical intervention. In accordance with this rate, Lerner et al.\textsuperscript{62} prospectively studied the outcome of 18 delayed unions in long bones after a high-energy fracture treated with LIPUS. They found an 88\% of successful healing within 13–52 weeks. The same rate of effectiveness (86\%) of the LIPUS in non-unions was found by Nolte et al.\textsuperscript{63} in a prospective study of 29 cases. The healing rate for the established non-unions based on third cortical bridging in long bones and callus bridging of the fracture lines in the other bones was significantly higher in the LIPUS group (86\% vs. 14\% in the LIPUS and control, respectively, \( P = 0.00001 \), average healing time \( 152 \pm 15.2 \) days). Regardless of the number of cases, a high variability relating the type of fracture (tibia, femur, radius/ulna, scaphoid, humerus, metatarsal and clavicle), treatment (8 cases conservative and 21 surgical), and other risk factors (smoking habits), determine whether the results are not reliable. Mayr et al.\textsuperscript{64} also studied the effects of LIPUS therapy on delayed and non-unions in different bones. The results were compared with the registry data of the clinic. They did not find any significant differences in healing rate (88 vs. 89\%, difference in means 1\%, \( P > 0.05 \)), average healing time (139 ± 12.3 vs. 131 ± 2.4 days, difference in means 8 days, \( P > 0.05 \)), and average fracture age (482 ± 162.7, 312 ± 18.5 days, difference in means 170 days, \( P > 0.05 \)) in LIPUS and registry data respectively.

The application of LIPUS in delayed unions of the fibula after a high tibial osteotomy showed satisfactory results in an RCT.\textsuperscript{65} LIPUS significantly increased osteoid thickness by 47\% (16.9 ± 1.4 and 11.5 ± 1.9 \( \mu \)m in LIPUS and control, respectively; difference in means 5.4 \( \mu \)m, \( P = 0.04 \)), mineral apposition rate by 27\% (2.3 ± 0.2 and 1.8 ± 0.1 \( \mu \)m/day in LIPUS and control, respectively, difference in means 0.5 \( \mu \)m/day, \( P = 0.04 \)), and bone volume by 33\% (52.9 ± 3.5 and 39.8 ± 3.1\% in LIPUS and control, respectively, difference in means 13.1\%, \( P = 0.02; 70.0 \pm 2.1 \) and 58.3 ± 1.5\% in LIPUS and control, respectively, difference in means 11.7\%, \( P < 0.01 \)), with no effect of the number of blood vessels in the newly formed bony callus. This trial also explained the differences in ossification models found in both groups: in the subjects receiving LIPUS, endosteal callus formation by direct bone formation without a cartilage intermediate as well as indirect bone formation was observed, while in untreated controls only indirect bone formation was observed.

Gebauer and Correll\textsuperscript{66} also evaluated the effectiveness of LIPUS to treat delayed unions (3–7 months) and non-unions (more than 8 months) after limb lengthening with use of the Ilizarov method. Of the 112 procedures evaluated, 19 resulted in either a delayed union or non-union. Seventeen lengthening were treated with LIPUS, and all 17
healed within 3–12 months without any surgical intervention, as deter-
mined by radiographic confirmation of solid-bone consolidation. LIPUS produced an overall 30% reduction in healing time.

El-Mowafi and Mohsen\textsuperscript{67} evaluated the benefits of LIPUS on tibial distraction osteogenesis (range 5–8 cm) in 20 patients in whom the Ilizarov external fixator had been used. After completion of distraction, 10 patients received 20 min of LIPUS stimulation daily at the bone-lengthening site (Group A), while rigid fixation was maintained in the remaining patients (Group B). All patients were followed with weekly radiographs to determine the formation of an external cortex and an intramedullary canal, at which time the fixator was removed. The mean healing index in Group A was $30 \pm 2.96$ and $48 \pm 9.76$ days/cm in Group B (difference in means 18 days/cm, 95% CI: 11.7–24.3, $P < 0.001$). However, trials addressing distraction osteogenesis\textsuperscript{67,68} provide very low-quality evidence and do not report any functional outcomes or any common surrogate end point.

In relation to tibial lengthening and LIPUS, eight cases of tibial bone transport with external fixation plus LIPUS were compared with a previous control study of 12 tibia lengthening.\textsuperscript{69} The size of the bone gap ranged from 8 to 14 cm, with an average of 10.25 cm. There were significant differences between the LIPUS and non-LIPUS groups ($P = 0.033$) over the period of time that the patient was in a frame based on the size of their defect (external fixation index). The LIPUS reduced the external fixation time by 17.21\% (13.91 ± 6 and 16.71 months in LIPUS and control, respectively, difference in means 2.80 months), and the external fixation index (1.34 ± 0.44 and 2.02 months/cm in LIPUS and control, respectively, difference in means 0.68 months/cm).

The association of LIPUS and other risk factors has also been studied in humans. Cigarette smoking has a profound effect on all types of wound healing. Individually and in combination, the particulate and gaseous compounds of cigarette smoke undermine the conditions required for expeditious wound repair.\textsuperscript{70} The effects of nicotine, carbon monoxide and hydrogen cyanide combine to cause tissue anoxia, cellular hypoxia, prevention of the proliferation of cells, vaso-
constriction and a decrease in the oxygen-carrying capacity of blood. A cohort study\textsuperscript{71} of tibia and distal radius fractures in smokers and non-
smokers showed that LIPUS reduced significantly the healing time in all groups treated with the active ultrasound device. There were no statistical differences in time to healing between smokers and non-smokers treated with the active ultrasound device, but radiographic outcome and clinical fracture healing were better when applying LIPUS in both smokers (41 and 51\% in radius and tibial fractures, respectively, $P < 0.03$) and non-smokers (26 and 34\% in radius and tibia fractures, respectively, $P < 0.0001$), indicating that LIPUS had a positive effect.
on fracture healing in smokers to significantly decrease the time to healing.

**Mechanism of action**

The mechanisms by which ultrasound accelerates bone healing are unknown. In terms of physical mechanism, ultrasounds impart mechanical forces at the cellular level. Mechanical force modulates bone formation both *in vivo* and *in vitro*. Low-intensity ultrasound produces increased blood flow in an animal fracture model. New blood vessels formed during the inflammatory stage of repair, and *in vitro* cell studies have demonstrated that exposure to LIPUS increases nitric oxide production and activation of hypoxia-inducible factor-1α, thus leading to increased expression of vascular endothelial growth factor-A levels in osteoblasts. This may stimulate angiogenesis, which is crucial in early bone repair and a necessary precursor to endochondral ossification. LIPUS also increases the production of cytokines, such as vascular endothelial growth factor, fibroblast growth factor and interleukin-8 in osteoblasts and periosteal cells, all of which are necessary for angiogenesis. Rawool *et al.* used Doppler sonography to study blood flow around mid-shaft fractures of the ulna in dogs, and found a 3-fold increase in blood flow after 1 week of 20 min daily LIPUS treatment. This finding is consistent with the previously mentioned *in vitro* studies.

A multifaceted biological mechanism is possible. Wang *et al.* suggested that the primary effect of LIPUS is on the chondrocyte population in the healing fracture, as LIPUS increases soft-callus formation, advances endochondral ossification of the callus and increases stiffness and strength of the fracture site. Chondrocytes exhibit an increase in aggrecan gene expression and proteoglycan synthesis after LIPUS exposure. Further, Parvizi *et al.* showed that this stimulation is a result of an increase of LIPUS-induced intracellular Ca²⁺ that occurs within seconds after LIPUS stimulation. The link between LIPUS and intracellular Ca²⁺ does not seem to be unique to chondrocytes. Li *et al.* demonstrated an increase in osteoblast proliferation after LIPUS exposure. The mechanism for this proliferation was related to an increase in cytosolic calcium.

In animal and *in vitro* studies, LIPUS modifies cell processes such as proteoglycan and transforming growth factor-β synthesis (TGF-β), type-II collagen content, messenger-RNA (mRNA) aggrecan production, calcium uptake and reduced parathyroid-hormone response. All these observations are directly related to bone formation or resorption.
LIPUS induces acoustic streaming and cavitation, both of which may result in fluid flow through the extracellular matrix and impart shear stresses and strains to osteoblasts. Osteoblasts respond to applied shear forces from fluid flow and substrate strain similarly to the way in which they respond to LIPUS. This suggests that shear forces and LIPUS may share common mechanisms to influence cellular behaviour.

**Conclusion**

Delayed fracture healing can result in significant disability and lead to substantial socioeconomic costs. Both in animal and *in vitro* studies, LIPUS improves the healing response of the bone. However, the quality of the randomized clinical trials testing the effects of LIPUS in humans is poor, and well-planned investigations should be performed on a wide array of clinically relevant fractures. As more information is available on the effect of LIPUS on osteoprogenitor cells, and with the advances in biomaterials, it may be possible to engineer bone-graft substitutes that are uniquely designed to exploit the beneficial attributes of LIPUS to more rapid bone repair.

It is difficult to prove the results of LIPUS on bone healing through the reviewed studies, given the lack of unification of clinical criteria and measurements. Moreover, we did not find enough evidence about the application of LIPUS in patients at risk of fracture healing complications.

There is evidence from randomized trials that LIPUS treatment may significantly reduce the time-to-fracture healing for fractures treated non-operatively. We were not able to locate this evidence in favour of LIPUS in mid-shaft clavicle fractures. The structure of that study is of moderate quality, but the management method, which included passive support with collar and cuff, was variable, and probably affected the final outcomes. The results of the LIPUS on tibial stress fractures also provided weak evidence. The authors did not clarify the type of fixation, and the results were based only on pain on physical examination and return to duty. There does not appear to be any additional benefit to LIPUS treatment following reamed intramedullary nailing. Evidence to support the use of low-intensity pulsed ultrasongraphy in operatively managed fresh fractures is inconsistent. Although the process of fracture healing is well characterized, there is always variability related to the type of fracture, bone location, type of management and soft tissue compromise. All these variables may influence the effectiveness of LIPUS.

Simple fractures managed with proper reduction and immobilization should not be the target of the LIPUS therapy. Its function may be
more useful for some comminuted and/or open fractures involving risk patients with associated comorbidities, including elderly, smokers, DM, malnourished. LIPUS also may reduce healing time, and could produce substantial cost savings and decreases in disability associated with delayed union, non-union fractures, and long periods of limb lengthening. Any reduction in treatment translates into a reduction in complications, lower costs and a quicker return of the patient to his/her regular activities. The role of LIPUS in the management of either simple or complex fractures is still unknown because of the heterogeneity between in vitro, animal studies and their application to human studies. Although it is not clear how ultrasound accelerates bone healing, we found substantial evidence to support that LIPUS stimulates the bone healing process under different conditions. However, further randomized controlled trials of high methodological quality are needed to determine the optimal role of ultrasound therapy in fracture healing.

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