# Unfocused Extracorporeal Shock Wave Therapy as Potential Treatment for Osteoporosis

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**ABSTRACT:** Extracorporeal shock wave therapy (ESWT) influences the differentiation of bone marrow stroma cells towards osteoprogenitors and increases the expression of several growth factors. To assess whether unfocused ESWT might serve as a treatment for osteoporosis, we examined the bone architecture dynamics of ESWT-treated and untreated rat tibiae using in vivo micro-computed tomography (CT) scanning. In addition, the effects of ESWT on fracture healing, using a bilateral fibula osteotomy, were examined. Unilateral unfocused ESWT with 2,000 pulses and an energy flux density of 0.16 $mJ/mm^2$ was applied to the hind leg of ovariectomized and sham-ovariectomized rats. A single treatment with unfocused ESWT resulted in a higher trabecular bone volume fraction (BV/TV) in the proximal tibia of the sham-ovariectomized animals. Three weeks after ESWT, BV/TV was 110% of baseline BV/TV in treated legs versus 101% in untreated contralateral control legs ($p = 0.001$) and 105% of baseline BV/TV versus 95% at 7 weeks after ESWT ($p = 0.0004$). In ovariectomized rats, shock wave treatment resulted in a diminished bone loss. At 7 weeks, the BV/TV of the treated legs was 50% of baseline BV/TV, whereas in untreated control legs this was 35% ($p = 0.0004$). ESWT did not influence acute fracture healing. This study shows that bone microarchitecture can be affected by unfocused shock waves, and indicates that unfocused ESWT might be useful for the treatment of osteopenia and osteoporosis.  J Orthop Res 27:1528–1533, 2009

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Osteoporosis is a disease characterized by low bone mass and a deterioration of the microarchitecture of the bone. Consequently, patients suffering from osteoporosis have an increased risk for bone fractures. These fractures are associated with increased morbidity and mortality. Currently treatment is mainly pharmacological. The necessity for lifelong treatment, the potential negative side effects, and the high costs justify the search for alternative treatments. One such treatment might be extracorporeal shock wave therapy (ESWT).

Extracorporeal shock wave therapy is effective in the treatment of nonunions and fresh fractures.[1–4] Shock waves are acoustical pulses that are characterized by a high amplitude (up to 120 MPa), a short rise time (<10 ns), and a (negative) tensile wave (up to 10 MPa).[5] They can be generated electrohydraulically, electromagnetically, or pneumatically, which has important consequences for the wave characteristics.

In a prospective randomized study, high-energy fractures of the long bones that were treated with ESWT in addition to internal stabilization resulted in a decreased rate of nonunions, less pain, and earlier weight-bearing compared to fractures that only received internal stabilization.[1] Although no prospective double-blind placebo controlled studies examining the effect of ESWT on delayed and nonunions are available, several observational studies had success rates between 72% and 85%.[2–4]

ESWT results in biological responses at an energy flux density (EFD) of 0.16 $mJ/mm^2$ or higher, and 500 or more pulses,[6–8] and might be induced by several mechanisms such as mechanical stimulation, bone marrow hypoxia, subperiosteal hemorrhage, or increased regional blood flow.[9–11] These responses increase the expression of a variety of growth factors, including VEGF-A, IGF-I, TGF-beta, and BMP-2,-3,-4, and -7.[6–8] In studies examining the effects on bone healing, one or more of these growth factors were associated with an increased recruitment of mesenchymal stem cells and an increased differentiation of bone marrow stroma cells towards the osteogenic lineage.[6,7] Furthermore, ESWT also induces neovascularization[12] and enhances the recruitment of endothelial progenitor cells in ischemic hind limbs.[13]

ESWT is noninvasive and is used in a wide variety of musculoskeletal disorders. The development of nonfocusing shock wave generators that act at a relatively large region further expanded its use to dermatologic conditions, such as diabetic ulcers, and acute and chronic wounds.[14] An accompanying advantage of these nonfocused shock waves is that they are less painful for the patient, so analgesia is not required, making this application easily accessible to the clinical practice. Also, this further implies that, with unfocused ESWT, larger areas of bone can be treated, enabling opportunities for the treatment of osteoporosis.

In this study, we have examined the effects of nonfocused electrohydraulically generated shock waves on the bone architecture dynamics of ovariectomized and control rats to determine if nonfocused ESWT can serve

Additional Supporting Information may be found in the online version of this article.

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as a treatment for osteoporosis. Additionally, the effects of ESWT on fracture healing, using a bilateral fibula osteotomy, were examined.

## MATERIALS AND METHODS

### Animal Models and Surgical Procedures

Thirty-six 13-week-old female Wistar rats (Harlan Netherlands BV, Horst, the Netherlands) were housed in the animal facility of the ErasmusMC, with a 12-h light–dark regimen, at 21°C during the experimental period. Animals received standard food pellets and water ad libitum. All procedures were examined and approved by the animal experiments committee of the institution (EUR 939) and conformed with Dutch law on animal experiments.

Six groups were examined; all groups consisted of six rats. At 20 weeks of age, the animals received an ovariectomy (OVX) to simulate osteoporosis or a sham-ovariectomy (sham-OVX), in which the operative procedure was the same except that the ovaries were left intact. Since positive effects of ESWT on fresh fracture healing have been described,[1,15–18] we added a fracture model as a positive control in the study and analyzed the effects of unfocused ESWT on fracture healing as well.

Three groups received ESWT at the right tibia (Fig. 1). In addition to the sham-OVX rats, all rats that received ESWT also received a bilateral fibular osteotomy to examine the effects of unfocused ESWT on fracture healing. Group A consisted of sham-OVX rats (non-osteoporotic) that received 2,000 shock waves 3 weeks after sham operation. Group B consisted of OVX rats that received 2,000 shock waves 3 weeks after OVX. Group C consisted of OVX rats that received 1,000 pulses 3 weeks after OVX and received another 1,000 pulses 3 weeks later. This group was added to examine whether two repetitive ESWT treatments would affect bone mass more than a single treatment.

To evaluate a potential effect of the fibular osteotomy on bone loss and vice versa, another three control groups were added. The first got both a bilateral OVX and fibular osteotomy, the second got only an OVX, and the third group got only a bilateral fibular osteotomy.

The osteotomy was performed 2 days prior to the first shock wave treatment. Because the fibula is proximally attached to the tibia with a syndesmosis, and at the distal side with a bony union, a stabilized fracture can be created.[19,20] A 1-cm incision at the lateral side of the calf muscle was made through the skin and fascia. Under microscopic view, an osteotomy, including the periosteum, was made using a high-speed mini saw 0.4 cm distal to the fibulotibial joint. The osteotomy thickness was the same as that of the saw blade, 0.1 mm. Fascia and skin were stitched. All operative procedures were performed under sterile conditions with general anesthesia ($O_2$ with 2% isoflurane; Rhodia Organique Fine Ltd., Bristol, UK). Analgesics were given for 3 days as in 0.05 mg/kg/12 h buprenorfine (Schering-Plough, Kenilworth, NJ).

### Shock Wave Therapy

Unfocused, electrohydraulically generated shock waves with a treatment area of 3.8 cm in diameter, an energy flux density of 0.16 mJ/mm$^2$, and a frequency of 5 Hz were given using a commercially available generator (Dermagold/Orthowave 180, Tissue Regeneration Technologies, Woodstock, GA). After general anesthesia was established, both hind legs were shaved from ankle to knee. The rat was placed on its left dorsal-lateral side when the right tibia was treated. The applicator was placed at the anterolateral side of the hind leg, covering the surface from the proximal to the distal tibia. An ultrasonic gel was used as coupling media between the applicator and the skin. The contralateral left tibia served as a control and was not treated. Animals did not receive additional analgesics during or after treatment.

| weeks / group | -3 | 0 | 3 | 7 |
|---|---|---|---|---|
| | | µCT | µCT | µCT |
| A | Sham-OVX | # + ESWT 2000 pulses* | | † |
| B | OVX | # + ESWT 2000 pulses* | | † |
| C | OVX | # + ESWT 1000 pulses* | ESWT 1000 pulses | † |
| X | OVX | # | | † |
| Y | OVX | | | † |
| Z | | # | | † |

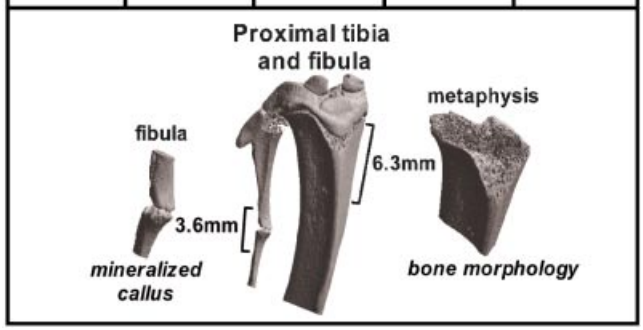


**Figure 1.** Schema of the study design representing the treatment groups and the analyzed regions (*, unilateral to the right leg; #, a bilateral fibula osteotomy; †, sacrifice).

### Analyses of Morphologic Parameters and Mineralized Callus Volume

In vivo micro-computed tomography (CT) scanning was performed under gas anesthesia (isoflurane/oxygen).[21] In supine position, the hind leg of the rat was fixed, allowing scanning of both the proximal tibia and the osteotomy site in a single session. Scanning was performed with a resolution of 18 µm using a Skyscan 1076 microtomograph (Kontich, Belgium) at a voltage of 60 kV, a current of 167 µA, and a 0.5-mm aluminium filter, over 196° with a 1° rotation step, taking 8 min. per scan.

For assessment of changes in cancellous and cortical bone, 3D reconstructions of the proximal tibia were made (Nrecon software version 1.5, Skyscan). To discriminate bony structures from non-bony structures, binary datasets were made using a local threshold algorithm (3D Calculator software available at: http://www.erasmusmc.nl/orthopaedie/research/klinres).[22] Analyses were made on the proximal 6.3 mm of the tibial metaphysis, which was manually selected. Cortical and trabecular bone were automatically separated using in-house software. Trabecular architecture was characterized by determining trabecular volume fraction (BV/TV), connectivity density, Structural Model Index (SMI), and 3D Trabecular Thickness. Cortical architecture was characterized by Cortical Volume and Cortical Thickness.

**Table 1.** Average Trabecular Bone Volume Fraction (BV/TV) in Control Groups (± SD)

| | BV/TV in Ovariectomy Group with Osteotomy ($n = 6$) | BV/TV in Ovariectomy Group with Sham-Osteotomy ($n = 6$) | $p$-Value |
|---|---|---|---|
| Week 0 | 0.19 (0.06) | 0.17 (0.03) | 0.54 |
| Week 3 | 0.13 (0.03) | 0.12 (0.02) | 0.70 |
| Week 7 | 0.10 (0.03) | 0.07 (0.02) | 0.18 |

For assessing mineralized callus volume, 3D reconstructions of the fibula were made using the same software. The osteotomy site and areas of 1.8 mm proximal and dorsal were selected for further analysis. A global threshold at the cut-off point of cortical bone was used to select mineralized callus. The mineralized volume was measured using 3D Calculator software.

### Histology

Directly after euthanasia, the hind legs were harvested and fixed in paraformaldehyde. The proximal half of the tibia was dehydrated and block embedded in methylmethacrylate; 6-μm-thick sagittal sections were made throughout the proximal tibia. Overall appearance and new bone formation was evaluated using a thionine staining (0.05% thionine in 0.01 M aqueous sodium phosphate, pH5.8 for 5 min). Sections that were stained in 2% Toluidine blue were analyzed under polarized light to observe the presence of woven bone.

### Statistics

Results are presented relative to baseline at start of treatment (time point, $t = 0$, is 100%). In the treatment groups, differences between means of the ESWT-treated right side and the untreated left side were evaluated for all parameters using paired $t$-tests (GraphPad Software, San Diego, CA). In the control groups that did not receive ESWT, differences between group means of all parameters were evaluated using the Mann–Whitney $U$ test.

## RESULTS

The surgical procedures did not result in complications. Directly after ESWT, redness of the skin and minor bleedings were observed at the treated site. When rats awoke from anesthesia, they did not use the treated leg directly. This rapidly improved, and after 10 min no difference was observed between treated and untreated legs. No weight loss occurred the days following ESWT. The average weight gain over the 10 weeks of the experimental was 85.2 g (67–121 g) in treated OVX rats and 77.8 (63–89 g) in control OVX rats, which was not significantly different ($p = 0.36$). In treated sham-OVX rats, the weight gain was 21.8 g (4–31 g), and in control sham-OVX rats, 23.8 (15–34 g) ($p = 0.34$).

No difference in cancellous and cortical bone morphometric parameters was found between OVX rats with a bilateral fibular osteotomy and rats that received ovariectomy only (Table 1). Bone healing at the osteotomy site was not significantly different in OVX or sham-OVX rats (Table 2).

### Microarchitectural Bone Changes after ESWT

In sham-OVX rats (group A), BV/TV was 0.26 (range 0.21–0.30) at start of ESWT treatment. After 3 weeks of treatment, BV/TV of the ESWT-treated legs was 110% of baseline, and BV/TV of untreated control legs was 101% of baseline (Fig. 2a), a significant increase in difference ($p = 0.001$). Seven weeks after treatment, BV/TV was 105% of baseline in treated legs, whereas the non-ESWT–treated control side lost bone to 95% of baseline, a significant decrease in difference ($p = 0.0004$).

At baseline, connectivity density in the legs of sham-OVX rats was 66.5 (range 49.4–78.6). At 3 and 7 weeks after treatment, it increased in the treated legs compared to the untreated control legs ($p = 0.03$ and $p = 0.018$, respectively) (Fig. 3). The SMI (1.8 on average at baseline), in which an index of 3 indicates the presence of rods and an index of 0 indicates the presence of plates, was significantly lower in the treated legs compared to untreated control legs at 3 weeks ($p = 0.008$), indicating that the structures were more plate-like in the treated legs (Fig. 3). Cortical volume and trabecular thickness were not different in the treated legs compared to control legs.

In OVX rats that received 2,000 shock waves at one time point (group B), BV/TV was 0.19 (0.16–0.24) at start of treatment. Three weeks after treatment, 83% of the BV/TV at baseline remained in the treated legs (Fig. 2b). BV/TV in the non-ESWT control legs was 74% of baseline. The difference between treated and control legs was not significant ($p = 0.12$). Seven weeks after treat-

**Table 2.** Average Mineralized Callus Volume ($\mu m^3$) in Control Groups (± SD)

| | Callus Volume in Ovariectomy Group with Osteotomy ($n = 6$) | Callus Volume in Sham-Ovariectomy Group with Osteotomy ($n = 6$) | $p$-Value |
|---|---|---|---|
| Week 0 | 2.2 (0.36) | 2.2 (0.26) | 0.94 |
| Week 3 | 2.0 (0.8) | 1.9 (0.59) | 0.82 |
| Week 7 | 1.7 (1.6) | 1.6 (1.1) | 1.0 |

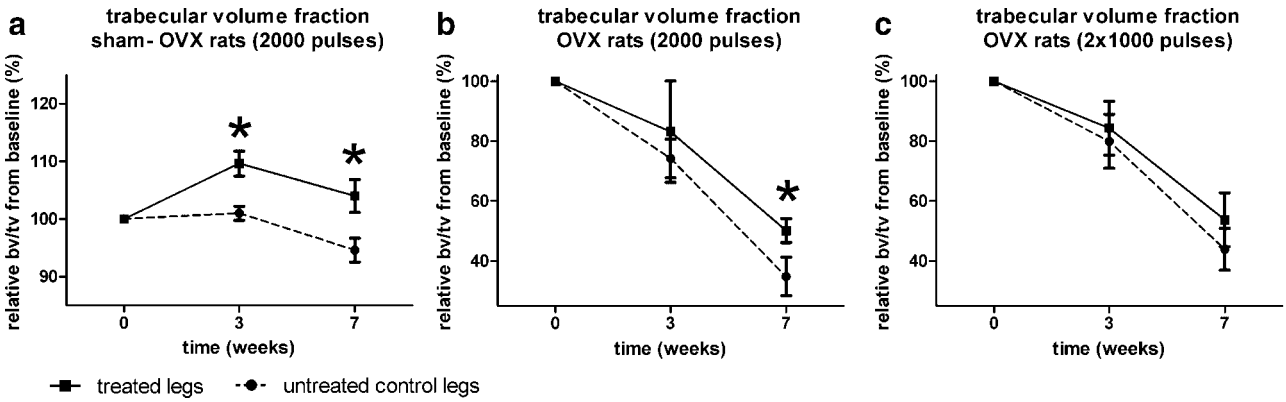


**Figure 2.** (a–c) BV/TV as percentage from baseline in sham-OVX rats receiving 2,000 pulses (a), and OVX rats (b, c) receiving 2,000 (b) or $2 \times 1{,}000$ (c) pulses. (*, $p < 0.05$.)

ment, the BV/TV was 50% in the treated legs and 35% in the control legs, a significant difference ($p = 0.0004$). At 7 weeks, bone loss of the treated leg was diminished compared to the nontreated control leg in every animal. The morphometric parameters and the cortical bone were not affected by ESWT.

In OVX rats that received $2 \times 1{,}000$ shock waves (group C), BV/TV was 0.17 (0.12–0.22) at start of treatment. Three weeks after treatment, BV/TV was 84% of baseline (Fig. 2a). In the control legs, BV/TV was 80%, which was not significantly different ($p = 0.26$). Seven weeks after treatment, BV/TV in the treated legs was 54% (43.2%–67.9%) of baseline, whereas BV/TV in the control legs was 44%, not a significant difference ($p = 0.13$). Again, the morphometric measurements and cortical bone were not affected by ESWT.

### Mineralized Callus Formation

In all treatment groups, a wide variation was seen in the amount of mineralized callus both in the ESWT-treated and the untreated control legs (Fig. 4). At 3 and 7 weeks after ESWT, no beneficial or unfavorable effect of ESWT on bone healing in sham-OVX ($p = 0.10$ and $p = 0.16$, respectively) or OVX rats (group B, $p = 0.20$ and $p = 0.08$, respectively; group C, $p = 0.82$ and $p = 0.79$, respectively) could be found.

### Histology

No differences in mineralization or osteoid appearance was observed between untreated control and treated legs in thionine-stained sections of the proximal metaphysis. Examination of toluidine-stained sections under polarized light did not show the presence of woven bone in any of the shock wave treated legs (see Supporting Information online).

## DISCUSSION

We examined the effects of unfocused shock waves on the microarchitecture in an osteoporosis rat model and sham control. A single treatment with unfocused electrohydraulically generated shock waves resulted in increased trabecular bone volume and diminished age-dependent bone loss in healthy, nonosteoporotic bone. Moreover, unfocused shock waves with 2,000 pulses in

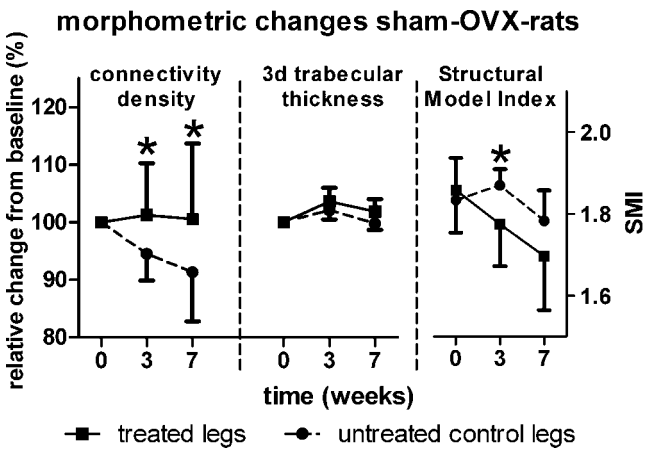


**Figure 3.** Morphometric bone changes in sham-OVX rats. Connectivity density and 3D trabecular thickness as percentage of baseline. For SMI, 3 indicates the presence of rods and 0 indicates the presence of plates.

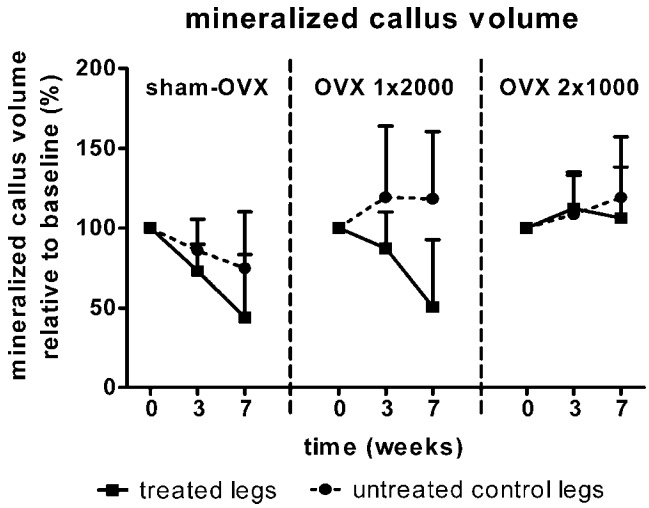


**Figure 4.** Mineralized callus volume presented as percentage from baseline in sham-OVX rats receiving 2,000 shock waves (sham-OVX), and OVX rats receiving 2,000 shock waves (OVX $1 \times 2{,}000$) or two times 1,000 shock waves (OVX $2 \times 1{,}000$).

one session diminished trabecular bone loss in ovariectomized rats with established bone loss treatment at 3 weeks post-OVX. These data suggest that ESWT may be a potential treatment for osteopenia and osteoporosis.

Several studies showed the effectiveness of shock wave therapy in healing of fresh fractures.[1,15–18] Because this was the first time shock wave therapy was performed in an osteoporosis model, a positive control was added, allowing to conclude the potential ineffectiveness on osteoporosis in the presence of an osteogenic stimulator. Therefore, we added a fresh fracture model as a positive control. Although the usefulness of this model was described in other studies examining biophysical stimuli,[19,20] we found a wide variation with a high number of nonunions within all groups, including the untreated control legs and control groups. This variation might have contributed to the negative finding of ESWT on fresh fracture healing. Retrospectively, this model did not serve as a positive control and might, unfortunately, hamper the interpretation of our results. However, mineralized callus was observed in many animals, concluding that, at least in this model, unfocused shock waves were ineffective. Besides, all previous studies used focused shock waves instead of unfocused shock waves, so the lack of an effect might also be explained by this difference. Furthermore, as shown in our control groups, the bilateral fibular osteotomy did not affect BV/TV, therefore BV/TV findings can be interpreted irrespectively of the fracture model.

We did not analyze the mechanism behind the biological effects. Minor bleeding and a transient disuse of the treated leg were seen directly after ESWT therapy, as described in other studies.[9,23] Disruption of trabeculae or fracture of the cortex were described when focused shock wave therapy with high energy levels (0.54–0.9 mJ/mm$^2$) or when a high number of pulses ($\geq$1,500) were applied.[9,10,24] However, these side effects occur in a dose-related manner, and since we used unfocused shock waves with an energy flux density of 0.16 mJ/mm$^2$, it is not surprising that we did not find these side effects.[10,24] Our results contribute to the assumption that shock waves induce biological responses without gross damaging effects.[6–9,11]

Although bone loss was diminished by EWST, the effects were small, of the magnitude of a low-dose bisphosphonate treatment.[25] In sham-OVX rats, only connectivity density and SMI were affected, and in OVX rats, no morphometric parameters were significantly different in shock wave-treated legs. A limitation of our study is that we only examined shock waves with an energy flux density of 0.16 mJ/mm$^2$. We assume that optimizing the treatment protocols in terms of pulse number and EFD might increase the effectiveness of this therapy. We examined if the effect of a single treatment was different from the effect of two treatments, keeping the amount of shock waves the same in the two conditions. We could find a significant effect in using 2,000 pulses in one treatment, but no significant effect when two treatments of 1,000 pulses were applied. This suggests that the number of shock waves applied in one session is more important for the therapeutic effect. Whether a higher number or higher energy flux densities are more effective should be examined. Finally, it would be interesting to examine whether shock waves are effective in combination with other pharmaceutical osteoporotic treatments.

We demonstrate that unfocused shock wave therapy can induce bone formation in healthy bone, whereas in OVX, estrogen-deficient rats with established bone loss (3 weeks after OVX), bone loss can be diminished. We used an osteoporosis model with established bone loss because it might be a better representation of the clinical situation than when treatment is given directly after estrogen deficiency is created. In additional experiments in which animals were treated with unfocused ESWT 10 weeks after OVX (2,000 shock waves and an energy flux density of 0.16 mJ/mm$^2$), in which the trabecular bone volume fraction was 8% (6%–11%) at time of treatment, no effect was found (data not shown). This might lead to the idea that the more pronounced effects in the sham-OVX animals than in the animals with established bone loss might be related to the amount of bone that remained rather than the estrogen status of the animals. This suggests that unfocused shock waves mainly affect the bone dynamics of existing surfaces and does not induce de novo bone formation. In patients with osteoporosis, a distinctive amount of trabeculae are left in the hip and vertebrae. Therefore, ESWT might be effective both in osteopenia and osteoporosis.[26–28]

A limitation of our study is the difficulty in comparing the effect of the sham-OVX and OVX rats, since the amount of bone tissue volume was much lower at the time of treatment in the OVX animals, and therefore the energy of shock waves was distributed differently, which might lead to other biological responses.

Our current findings indicate that unfocused shock waves can play a role in osteopenia and osteoporosis and justify further experiments on the effects of unfocused ESWT on bone. Clinically, unfocused shock waves can be applied without anesthesia to skeletal sites that are specifically prone for fracturing, which might contribute to a reduction in fracture risk.

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