■ RESEARCH

Electromagnetic fields do not affect bone micro-architecture in osteoporotic rats

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Objectives

Electromagnetic fields (EMF) are widely used in musculoskeletal disorders. There are indications that EMF might also be effective in the treatment of osteoporosis. To justify clinical follow-up experiments, we examined the effects of EMF on bone micro-architectural changes in osteoporotic and healthy rats. Moreover, we tested the effects of EMF on fracture healing.

Methods

EMF (20 Gauss) was examined in rats (aged 20 weeks), which underwent an ovariectomy (OVX; n = 8) or sham-ovariectomy (sham-OVX; n = 8). As a putative positive control, all rats received bilateral fibular osteotomies to examine the effects on fracture healing. Treatment was applied to one proximal lower leg (three hours a day, five days a week); the lower leg was not treated and served as a control. Bone architectural changes of the proximal tibia and bone formation around the osteotomy were evaluated using *in vivo* microCT scans at start of treatment and after three and six weeks.

Results

In both OVX and sham-OVX groups, EMF did not result in cancellous or cortical bone changes during follow-up. Moreover, EMF did not affect the amount of mineralised callus volume around the fibular osteotomy.

Conclusions

In this study we were unable to reproduce the strong beneficial findings reported by others. This might indicate that EMF treatment is very sensitive to the specific set-up, which would be a serious hindrance for clinical use. No evidence was found that EMF treatment can influence bone mass for the benefit of osteoporotic patients.

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Article focus

■ Does EMF treatment induce beneficial trabecular and/or cortical bone changes in osteoporotic or healthy rats?
■ Does EMF stimulate bone healing in a fracture model in healthy and/or osteoporotic rats?
■ Might EMF treatment be beneficial for osteoporotic patients?

Key messages

■ No effect of EMF on trabecular and cortical bone was detected during six-week follow-up.
■ In the literature there are contradictory findings on the effect of EMF on bone mass.

■ EMF might be very sensitive to specific set-up, which might impede clinical implementation.

Strengths and limitations

■ *In vivo* microCT imaging is a very sensitive method to analyse bone changes in small animals over time.
■ Both animals which underwent an ovariectomy and healthy animals were examined.
■ *In vivo* microCT imaging was the only analysing technique; no histomorphometric or biomechanical analyses were performed.

Introduction

Osteoporosis is a disease characterised by progressive bone loss and deterioration of the

micro-architecture leading to an increased risk of fracture. Several pharmaceutical treatments that aim to reduce further bone loss or increase bone mass are available. However, these therapies require a regular and probably lifelong intake, with potential side effects and related high costs. Electromagnetic fields (EMF) are also reported to have a beneficial effect on bone micro-architecture and might reduce the risk of fracture.

EMF were developed based on the finding that electrical currents exist in mechanically-loaded bone and are important for physiological regulation of bone metabolism.[1] There is some evidence for the effectiveness of EMF. However, due to the large variation in the characteristics of EMF signals of different generators, comparison of different *in vitro* and *in vivo* studies is difficult.

Experimental studies examining the effects of EMF on bone healing in osteotomy gap models have shown the stimulatory effects of EMF on callus formation and mechanical capacities, even when treated for only one hour per day.[2-5] The underlying stimulatory mechanisms of electromagnetic fields have been studied *in vitro* using mesenchymal stem cells, osteoblasts or osteoblast-like cells and are related to BMP-2, TGF-β, IGF-II, prostaglandins, nitric oxide synthase phosphorylation and mitogen-activated protein kinase activation.[6-13]

Although experimental evidence of the effectiveness of EMF on bone formation seems quite extensive, clinical evidence is lacking. EMF are, for example, widely used for the treatment of nonunion, but their effectiveness is limited to observational studies[14-18] and some smaller control-based studies with suboptimal study design.[19-21] Therefore, the use of EMF in nonunions is still under debate.[22]

Few studies have investigated the potential role of EMF treatment for osteoporosis. In 1990, Tabrah et al[23] reported a positive effect of EMF on the bone mineral density (BMD) of radii in older women. Furthermore, few animal studies address the topic. Chang and Chang[24] showed that whole-body EMF completely prevented OVX-induced trabecular bone loss in rats that were treated for eight hours per day. Jing et al[25] also demonstrated preventive effects of EMF on OVX-induced trabecular bone loss using BMD analysis. In another study by Sert et al,[26] it was shown that electromagnetic treatment induced an almost two fold increase in cortical bone mass in the tibias of OVX rats. In our previous work, we examined several systemic EMF signals and treatment protocols with the use of *in vivo* microCT imaging, but we were unable to demonstrate positive effects on bone micro-architecture in OVX rats.[27] One difference between this previous study and those that found positive effects is that we used lower magnetic fields, which might explain the lack of success.

Since many *in vitro* and *in vivo* studies show strong positive effects of EMF on bone formation, this non-invasive treatment has a potentially important role in the treatment of low bone mass and osteoporosis. To justify clinical follow-up experiments, we examined the effects of 20 Gauss EMF on the bone micro-architecture using *in vivo* microCT scanning. This is a stronger EMF than we used in our previous paper and is similar to signals that did find anabolic effects.[26,27] *In vivo* microCT scanning is a sensitive technique in which both cancellous and cortical bone changes can be analysed in longitudinal and 3D fashion. The effects were examined in both ovariectomised[28] and sham-ovariectomised rats. As a putative positive control, a bilateral fibular osteotomy was added to the model to examine the effect of EMF on fresh fracture healing.

Materials and Methods

A total of 16 female Wistar rats were obtained, age 20 weeks (Harlan, the Netherlands). All were housed in the institute's animal facility with a 12-hour light/dark regime, and all received standard food pellets and water *ad libitum*. The study protocol was approved by the local Animal Experiments Committee (EUR 687) and was in accordance with Dutch law on animal experimentation.

Research model. Two groups were made three weeks prior to EMF treatment. The rats underwent either a bilateral ovariectomy (OVX) to simulate osteoporosis (n = 8) or a sham-ovariectomy (n = 8). This procedure was performed under sterile conditions using gas anaesthesia (oxygen with 3% isoflurane; Rhodia Organique Fine Ltd, Bristol, UK). Analgesics were given for three days: 0.05 mg/kg/12 hour buprenorphine (Schering-Plough, Kenilworth, New Jersey). In sham-ovariectomy, the procedures were exactly the same, except that the ovaries were only manipulated and not removed.

A bilateral fibula osteotomy was performed in all rats 19 days after (sham-)OVX. It has been shown that EMF stimulated fracture healing using the fibula osteotomy model.[5] To include a positive control, this fresh-fracture model was added to our osteoporosis model. In a previous paper, we demonstrated that there is no interaction of OVX on mineralised callus volume and *vice versa*.[29] The hind legs were shaved under general anaesthesia (oxygen/3% isoflurane). Then, under sterile conditions, a 1 cm incision at the lateral side of the calf muscle was made through the skin and fascia. Under microscopic control, the fibula was presented in a blunt manner. At 0.4 cm distal to the fibulo-tibial joint, an osteotomy (including the periosteum) was made using a high-speed mini-saw. The thickness of the osteotomy was the same as that of the saw blade, i.e. 0.1 mm. Fascia and skin were stitched. These rats received analgesics (buprenorphine 0.05 mg/kg/12 hour) for two days.

EMF. EMF treatment was started 21 days after (sham-)OVX and two days after fibular osteotomy. During EMF treatment, the rats were placed in a custom-made harness.

This fixating procedure enabled application of the EMF coil to the hind leg without the use of an anaesthetic. Three weeks prior to the start of EMF treatment, rats were placed in the harness with an increasing time period in order to get acclimatised to the harness.
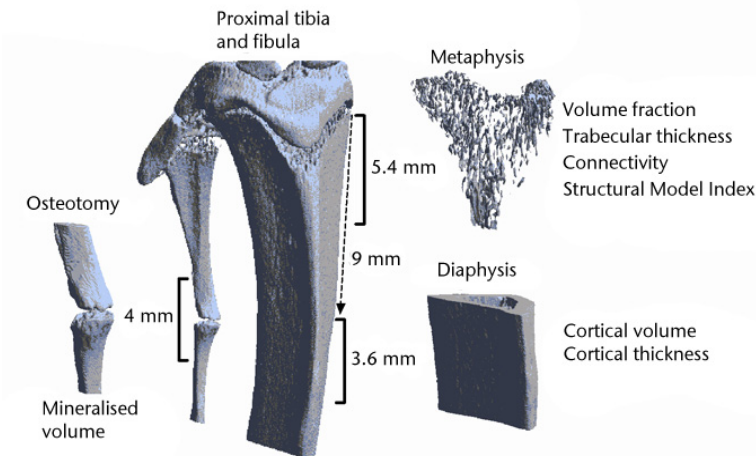
Fig. 1

Three-dimensional reconstruction of a microCT scan of the proximal tibia and the proximal fibula. The analysed regions of interest are indicated.

EMF was given to one hind leg, five days a week for three hours a day. The EMF signal was produced by a custom-made generator producing a 20 Gauss sinusoid waveform at 50 Hz. This is a higher EMF than we used in our previous paper. For technical reasons, this has consequences for the characteristics of the signal (sinusoidal instead of pulse bursts). This higher EMF field is similar to signals that did find anabolic effects.[26,27] The coil extended from the knee to the ankle joint.

The other hind leg was left untreated and served as a control. μ-copper foil was placed around the control leg to exclude any effect of the coil on the contra lateral side. **MicroCT scanning.** To evaluate bone changes in the proximal tibia, *in vivo* microCT scans were made at the start of EMF treatment and three and six weeks after EMF treatment was started, so at three, six and nine weeks after (sham-)OVX. Under gas anaesthesia, the hind leg of the rat (from distal femur to the tibial diaphysis) was scanned with an *in vivo* microCT scanner (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 rotation step of 1°, taking eight minutes per scan.

Using NRecon (NRecon software version 1.5, Skyscan, Kontich, Belgium) three-dimensional reconstructions (isotropic voxelsize of 18 microns) of two regions of interest were made, one at the proximal metaphysis which mainly contains cancellous bone and another at the mid-diaphysis, which contains mainly cortical bone (Fig. 1).

The reconstruction of the proximal metaphysis was selected manually, starting just distally of the epiphysis and continuing 5.4 mm beyond that. The reconstruction of the diaphysis was defined by a region of 3.6 mm, starting 9 mm distally from the epiphysis. Bony and non-bony structures were separated using a local threshold algorithm (software freely available)[30] resulting in binary datasets.[31] Cortical and trabecular bone were automatically separated using in-house software. Trabecular architecture in the proximal metaphysis was characterised by determining trabecular volume fraction (BV/TV), connectivity density (Conn/TV), structure model index (SMI) and 3D trabecular thickness (TbTh). Cortical architecture was assessed in the diaphysis and was characterised by cortical volume (CtV) and cortical thickness (CtTh).

Analysis of the mineralised callus formation at the fibular osteotomy was performed on the same microCT scans. In reconstructed datasets, the osteotomy site was selected and an area of 2 mm proximal and 2 mm dorsal to the fracture was selected for further analysis (Fig. 1).

Because the degree of mineralistion within the callus site varied, a global threshold was used. The threshold was set in such a way that the cortical bone visually included the selection; in this way the mineralised callus volume was measured.

Statistical analysis. Differences between the treated legs and the untreated control legs were statistically analysed for each time point using paired *t*-tests for all parameters (GraphPad Software, San Diego, California). A p-value < 0.05 was considered statistically significant.

Results

Due to complications during sham-OVX, one rat died. In addition, because the fibular osteotomy resulted in additional fractures in the fibular, two additional animals were excluded from analyses of the mineralised callus volume.

At the start of EMF, the mean treatment body weight in sham-OVX rats was 251.3 g (SD 12.0) and the mean increase in body weight was 10.7 g (SD 2.9). In OVX rats, the mean body weight was 299.9 g (SD 15.0) at start of
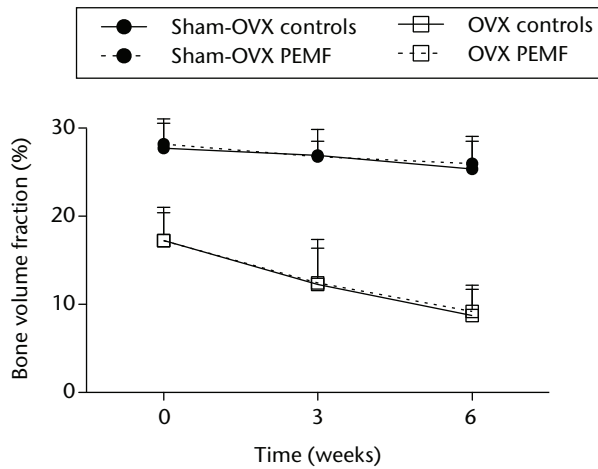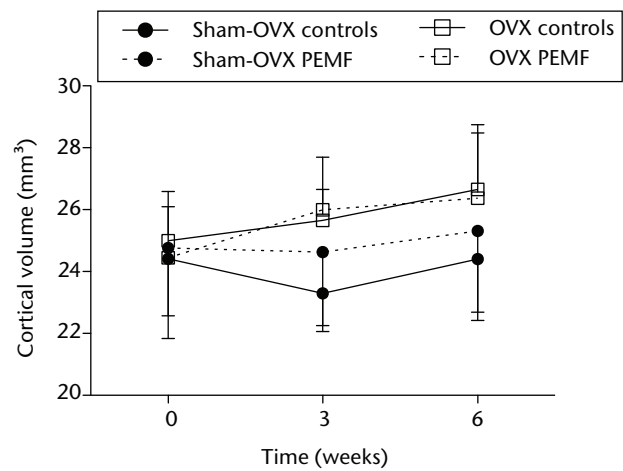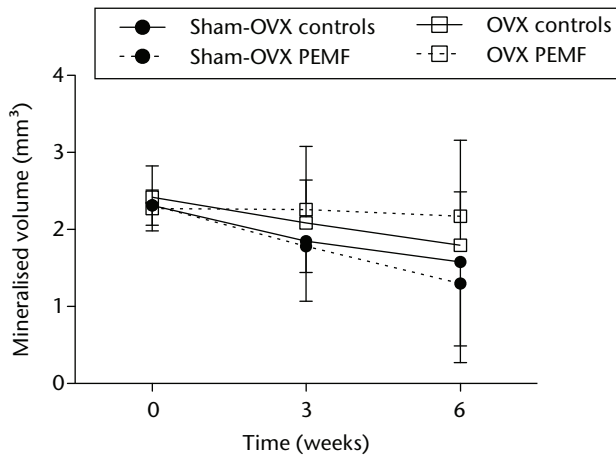
Fig. 2a

Fig. 2b

Fig. 2c

Graphs showing a) trabecular volume fractions, b) cortical volume and c) mineralised callus volume of electromagnetic field-treated and non-treated control tibias in sham-ovariectomised (n = 7) and in ovariectomised (n = 8) rats. Data are presented as mean values and standard deviations.

Table I. Bone changes in the tibia of non-treated tibias and electromagnetic field (EMF)-treated tibias of sham-ovariectomised rats. Mean values with standard deviations and p-value (paired *t*-tests) are given (n = 7).

	Week three			Week six		
	Controls	EMF	p-value	Controls	EMF	p-value
Trabecular volume fraction (BV/TV)(%)	26.9 (3.0)	26.8 (1.7)	0.42	25.3 (3.1)	25.9 (3.1)	0.68
Mean trabecular thickness (μm)	106.7 (5.8)	108 (5.0)	0.19	108.7 (4.1)	109.6 (6.0)	0.74
Connectivity density (mm³)	75.4 (5.1)	69.5 (10.5)	0.14	63.7 (7.7)	52.0 (10.5)	0.28
Structure model index	1.85 (0.11)	1.83 (0.18)	0.15	1.87 (0.15)	1.81 (0.24)	0.33
Cortical volume (mm³)	23.3 (1.2)	24.6 (2.4)	0.3	24.4 (2.0)	25.3 (2.6)	0.27
Cortical thickness (μm)	458.5 (11.1)	469.7 (20.4)	0.74	476.6 (18.4)	487.4 (30.0)	0.44

EMF treatment and the mean increase in the six-week follow-up period was 14.1 g (SD 10.3). None of the rats experienced weight loss during follow-up.

In sham-OVX rats, the BV/TV in non-treated control tibias was 27.7% (SD 2.8), 26.9% (SD 3.0) and 25.3% (SD 3.1) at weeks 0, three and six, respectively; this was not significantly different from EMF-treated tibias (Fig. 2a).

In sham-OVX rats, no differences were observed in morphometric or cortical parameters between non-treated control tibias and EMF-treated tibias at three and six weeks after the start of treatment (Fig. 2b, Table I).

Mineralised callus formation at the osteotomy site showed a wide variation between and within rats. The average mineralised callus volume decreased during

Table II. Bone changes in the tibia of non-treated tibias and electromagnetic field (EMF)-treated tibias of ovariectomised rats. Mean values with standard deviations and p-value (paired *t*-tests) are given (n = 8)

	Week Three			Week Six		
	Controls	EMF	p-value	Controls	EMF	p-value
Trabecular volume fraction BV/TV (%)	12.3 (5.1)	12.4 (3.9)	0.78	8.7 (3.0)	9.2 (3.0)	0.85
Mean trabecular thickness (μm)	101.0 (6.3)	102.6 (5.74)	0.27	100.3 (3.8)	101.7 (4.8)	0.66
Connectivity density (mm^3)	16.9 (13.4)	17.1 (10.4)	0.71	8.5 (7.5)	8.7 (7.0)	0.68
Structure model index	2.50 (0.20)	2.48 (0.18)	0.91	2.62 (0.16)	2.58 (0.17)	0.38
Cortical volume (mm^3)	25.7 (1.0)	26.0 (1.7)	0.54	26.7 (1.8)	26.4 (2.4)	0.41
Cortical thickness (μm)	469.2 (22.0)	477.9 (23.8)	0.45	504.0 (28.4)	495.8 (27.5)	0.24

follow-up in both the controls and the EMF-treated tibias but the differences were not significant (Fig. 2c).

In OVX rats, the BV/TV in non-treated control tibias was 17.2% (SD 3.2), 12.3% (SD 5.1) and 8.7% (SD 3.0) at weeks 0, three and six, respectively (Fig. 2a).

BV/TV and morphometric parameters showed no significant differences from EMF-treated tibias at any time point (Table II).

CtV in non-treated control tibias was 25.0 (SD 1.6), 25.7 (SD 1.0) and 26.7 (SD 1.8) at 0, three and six weeks, respectively. Neither CtV nor CtTh were significantly different from that in EMF-treated tibias (Fig. 2b, Table II).

Mineralised callus formation showed a wide variation; there were no significant differences between controls and EMF-treated tibias (Fig. 2c).

Discussion

This study examines the effects of EMF on changes in both cancellous and cortical bone to establish whether EMF might serve as a treatment for osteoporosis. Although *in vivo* microCT scanning allowed us to follow morphometric bone changes in high detail over time, we were unable to detect any differences between EMF-treated and untreated legs.

There are many indications from *in vitro* studies that EMF might have beneficial effects on the bone architecture for the benefit of those suffering with osteoporosis. However, few animal experiments using ovariectomised animals exist. Chang and Chang[24] showed that OVX-induced trabecular bone loss was prevented by EMF treatment using histomorphometric analyses at 30 days of treatment. EMF was given with a single pulse at 7.5 Hz and a maximum magnetic field of 8 Gauss for eight hours per day in the whole animal. In another study using the OVX rat model, whole-body EMF led to an increase of 71% in cortical thickness compared with non-treated controls.[26] EMF was applied for four hours a day with a 50 Hz sinusoidal waveform of 10 Gauss. In our previous work, we could not demonstrate positive effects on cortical or trabecular bone when OVX rats were treated with systemic EMF.[27] In that study, different EMF signals, including those with both pulse bursts and single pulses, were examined, however, the magnetic field of 1 G was lower than the studies of

Chang and Chang[24] and of Sert et al.[26] In the current study we therefore used a EMF signal with 20 G. Although the characteristics of EMF used in previous studies by others and in the current paper are comparable, especially between the paper of Sert et al[26] and the current study, we were unable to confirm the pronounced effects of EMF on cancellous or cortical bone. These contradictory findings suggest that the effects of EMF on osteoporosis are very sensitive to the specific experimental set-up used. This is confirmed in a more recent paper in which ovariectomised rats that received EMF during the day showed a higher BMD than those receiving EMF at night.[25] The cause of this difference is unknown.

Although many pre-clinical experiments show positive effects of EMF on cortical and trabecular bone, it has not resulted in many clinical studies. Tabrah et al[23] found that the BMD of both the treated and the opposite untreated radius of women prone to osteoporosis increased during a 12-week period, suggesting that a systemic effect might be induced by EMF that influenced BMD. However, this study has never been repeated in a randomised controlled trial. According to a review in 2008, more clinical studies are described in Chinese literature.[32] These data suggest that the effects of EMF on BMD in osteoporosis are controversial.

In contrast to other studies, we examined the effects of locally applied EMF on osteoporosis. As a putative control we added a fracture model to that of osteoporosis. We demonstrated earlier that there is no interaction between the OVX and the fracture model.[29] It has been shown that EMF stimulate fracture healing at the fibular osteotomy.[5] As in the study of Midura et al,[5] we found large variations in mineralised callus volume. However, the amount of nonunions, characterised by progressive gap size due to resorption of the bony ends was much higher in the current study. In our hands, 11 of the 30 fibular osteotomies resulted in a nonunion, irrespective of the study group. We speculate that (micro-)movement is the cause of the large number of failures. Although the EMF signal was highly comparable with that of Midura et al[5] (both maximum electrical field of 20 Gauss, treated for three hours a day), we were unable to confirm the stimulating effects of EMF on fresh fracture healing.

A limitation of the current study is that only bone micro-architecture was analysed during follow-up and no mechanical testing or histology was performed. Regarding histological parameters, it is still possible that EMF affected bone turnover, without inducing changes in bone architecture.

In conclusion, we could not confirm any positive effects of EMF for the treatment of osteoporosis or for fracture healing. It is also possible that EMF works under specific circumstances, but that its effects are highly sensitive to the precise set-up, e.g. signal characteristics (including exact waveform of the electromagnetic field), timing of the EMF treatment, and animal strain. This would be a serious hindrance to clinical use. However, the present findings provide no evidence to support EMF as a beneficial treatment for osteoporotic patients.

Supplementary material

A photograph showing a rat hanging in a custom-made harness is available with the online version of this paper at www.bjr.boneandjoint.org.uk.

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