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Pulsed electromagnetic fields modulate bone metabolism via RANKL/OPG and Wnt/β-catenin pathways in women with postmenopausal osteoporosis: A pilot study

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ABSTRACT

Pulsed electromagnetic fields (PEMFs) have been proven to enhance in vitro and in vivo osteogenesis with unknown mechanism. Aim of our study was to explore whether RANKL/OPG and Wnt/β-Catenin pathways could be involved in bone response to PEMFs in a setting of postmenopausal osteoporotic women.

Forty-three women (mean age 62.8 ± 4.5 yr.) were randomized into two groups. The PEMFs group received PEMFs treatment (50 min treatment session/day, 6 treatment sessions/week, for a total of 25 times), by wearing a specific gilet applied to the trunk and connected to the electromagnetic device (Biosalus, by HSD Srl, Serravalle RSM), while women assigned to control group received sham PEMFs with the same device. BSAP as bone formation and CTX as bone resorption markers, RANKL, OPG, β-Catenin, DKK-1 and sclerostin were obtained at baseline, after 30 and 60 days.

In PEMFs group, BSAP levels significantly increased after 30 and 60 days while CTX concentrations decreased at day 60. RANKL levels significantly decreased after 60 days. OPG was not significantly changed, but the RANKL/OPG ratio significantly decreased at day 30. DKK-1 levels decreased, while β-catenin concentrations increased after 30 and 60 days ($P < 0.05$). No significant changes of calcium, phosphorus, creatinine and sclerostin were detected. In the PEMFs group, at day 30, Δsclerostin was associated with ΔRANKL/OPG ratio ($r = -0.5$, $P = 0.03$) and ΔDKK-1 was associated with Δβ-Catenin ($r = -0.47$, $P = 0.02$).

In women with postmenopausal osteoporosis, our data provide evidence of a PEMFs modulation of RANKL/OPG and Wnt/β-Catenin signaling pathways able to explain the metabolic effects of PEMFs on bone.

1. Introduction

An electrical effect in bone, when mechanical loading is exerted on bone surface was originally reported by Fukada and Yasuda, and its consequence on bone biological processes became objects of growing investigations [1, 2]. Therefore, the effects of pulsed electromagnetic fields (PEMFs) on bone tissue were widely investigated [2]. Most but not all the preclinical studies using PEMFs showed recovery of strength and load bearing capability, increasing synthesis of extra-cellular matrix, and formation of bridging bone, and more advanced healing suggesting PEMFs as a booster of bone anabolism [3–7].

PEMFs have been proven to promote osteogenesis and bone mineralization and to preserve bone mass in animal models of disuse or tail-suspension osteoporosis but also in ovariectomy-induced bone loss, a model of postmenopausal osteoporosis [8, 9].

Since the Food and Drug Administration approved PEMFs as safe and effective, a great amount of evidence were cumulated in the past decades showing effects in humans in a wide range of skeletal diseases, such as fresh and non-union fractures, osteoarthritis and osteoporosis [10–12].

The precise mechanism promoting osteogenesis however is still not clear, and although PEMFs therapy is claimed to enhance fracture healing, inconsistent data exist about the treatment of metabolic bone diseases and osteoporosis [13].

In vitro, PEMFs exposure induces the modification of bone morphogenetic protein 2 (BMP-2), transforming growth factor beta (TGF-β)

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and insulin-like growth factor II (IGF-II) levels; but PEMFs induced stimulatory effects on bone may be also dependent from extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase (MAPK) and prostaglandin synthesis [14–18].

Canonical Wnt-signaling is a major regulator of bone homeostasis by promoting bone formation. Activation of Wnt/β-catenin signaling pathway triggers proliferation and differentiation of osteoblast precursor cells, reduces apoptosis of mature osteoblasts and promotes the ability of differentiated osteoblasts to inhibit osteoclast differentiation [19–21]. Stimulation of this pathway leads to the down-regulation of glycogen synthase-3 activity and inhibition of β-catenin phosphorylation and proteasome degradation, resulting in its accumulation in the cytoplasm and its translocation into the nucleus, where it promotes the transcription of Wnt target genes. Inhibitors such as sclerostin and Dickkopf-related protein 1 (DKK-1) may block the canonical Wnt pathway in bone by binding to Wnt co-receptors low-density lipoprotein receptor related protein 5 (LRP5) and LRP6 [19].

Moreover, RANKL, a transmembrane protein belonging to the tumor necrosis factor superfamily, and its decoy receptor osteoprotegerin (OPG) play an important role in regulating osteoclast differentiation and activation and have been associated with bone loss [22].

Since the pathways of Wnt/β-catenin and RANKL are strongly involved in the pathogenesis of postmenopausal osteoporosis, by governing bone formation and bone resorption, the aim of our research was to investigate the effects of PEMFs on these signaling pathways and to evaluate correlations with surrogate biomarkers of bone turn-over in women with established postmenopausal osteoporosis.

2. Materials and methods

This is a double-blinded randomized prospective clinical study involving women with postmenopausal osteoporosis. In the study forty-five postmenopausal Caucasian women were enrolled, who were attending the Center for the Prevention and Treatment of Osteoporosis of the Department of Clinical and Experimental Medicine at the University of Messina (Messina, Italy). Women were eligible for inclusion if they had a bone mineral density T-score of −2.5 SD or less at lumbar spine and/or femoral neck, without evidence of prevalent (clinical or morphometric) vertebral or non-vertebral fractures. Patients with secondary causes of osteoporosis or affected by chronic renal or liver failure, heart failure, hypo/hypercalcemia, diabetes mellitus, prior cancer diagnosis or if they had received treatment with drugs potentially involving bone and mineral metabolism within the last six months (including corticosteroids, heparin, anticonvulsant) were excluded from the study. Women with previous (> 3 months) or current use of active bone agents (bisphosphonates, denosumab, teriparatide, selective estrogen receptor modulators, strontium ranelate) for the treatment of osteoporosis were not considered in this research. Calcium and vitamin D supplements, if administered as part of an ongoing preventive treatment, were not an exclusion criteria when their administration was in accordance with the dietary reference intakes from the Institute of Medicine [23].

At baseline, height and weight were measured according to standard procedures and BMI was calculated as weight divided by the square of height in meters. After baseline assessment, participants were randomized into two groups: PEMFs group and placebo group. Participants allocated to the PEMFs group received PEMFs treatment (50 min treatment session/day, 6 treatment sessions/week, for a total of 25 times as one course of treatment) for the first 30 days after enrolment, while those assigned to the sham PEMFs group received sham PEMFs treatment with the same device but no stimulus generated, indistinguishable from the real one, and served as control group.

PEMFs were generated by an electromagnetic device (Biosalus, by HSD Srl, Serravalle RSM) and treatment was carried out in accordance with the manufacturer's recommendations (osteoporosis program) using low-frequency time-varying fields: 4 frequencies ranging from 16 to 22 (i.e. 16, 18, 20, 22) Hz were provided, with change of frequency occurring every 4 min; at the same times, energy changed from 30 to 36 (i.e. 30, 32, 34, 36) Gauss.

Patients were exposed to PEMFs by wearing a specific gilet connected to the electromagnetic device.

The study was conducted in accordance with the ethical standards and with the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants before entering the study.

Venous blood samples were collected in the fasting status in the morning at baseline and then after 30 and 60 days. Serum was separated from the blood corpuscles by centrifugation and stored frozen at −80 °C until analysed.

Serum levels of bone specific alkaline phosphatase (BSAP), C-telopeptide of type 1 collagen (CTX), as surrogate markers of bone formation and resorption respectively, 25-hydroxyvitamin D (25(OH)D), sclerostin and DKK-1 as antagonists of the Wnt signaling, calcium and creatinine were considered. CTX (Roche, Basel, Switzerland) and BSAP (Beckman Coulter, Fullerton, California) were measured by enzyme-linked immunosorbent assay, with intraassay CV of 1.6% to 3% and interassay CV of 1.3% to 4.3% for CTX, and intraassay CV of 2.3% to 3.7% and interassay CV of 4.9% to 9.8% for BSAP. 25(OH)D were detected by high-performance liquid chromatography; sclerostin and DKK-1were determined by enzyme immunoassay (Biomedica Medizinprodukte GmbH & Co KG, Vienna Austria) with intra-assay and inter-assay CV < 7% for both analytes. Calcium and creatinine were measured using standard laboratory techniques.

Statistical analyses were performed using MedCalc software (version 10.2.0.0; Mariakerke, 173 Belgium). The normal distribution of values was verified through the Kolmogorov-Smirnov test. Comparisons between the groups were performed using Student's *t*-test or Mann-Whitney test as appropriate. A repeated measures ANOVA model was used to assess changes of parameters within each group over time. Spearman's coefficient was used to measure the degree of association between two variables. Values of $P < 0.05$ were considered to indicate statistical significance. All reported *P* values were two-sided.

3. Results

Forty-three postmenopausal women (mean age 62.8 ± 4.5 yr.) completed the study and attended all the PEMFs or placebo sessions; two patients were excluded from the final analysis because missing at follow-up (one in the PEMFs group and one in the placebo group). The main clinical characteristics of recruited women are shown in Table 1. At baseline, no significant differences were detected between PEMFs and control groups in any of the studied variables. 25(OH)D was measured at baseline (29.85 ± 7.36 vs. 28.72 ± 6.82 ng/mL in PEMFs and control group, respectively; P=NS). In PEMFs group, BSAP levels were significantly increased at day 30 and day 60 in comparison with baseline, and at day 60 in comparison with controls; CTX levels were significantly reduced only at day 60 in comparison with baseline and with controls (Table 2). As shown in Table 3, a significant reduction of RANKL levels was observed at day 30 versus baseline and at day 60

Table 1
Clinical characteristics of recruited postmenopausal women.

	PEMFs (n = 22)	Controls (n = 21)	P values
Age (yr.)	62.2 ± 4.8	63.4 ± 4.2	NS
Time since menopause (yr.)	12.6 ± 4.1	13.12 ± 4.6	NS
BMI (kg/m^2)	25.1 ± 3.1	25.42 ± 3.9	NS
Smoking (n)	2	2	NS
L1-L4 T-score (SD)	−2.8 ± 0.6	−2.7 ± 0.5	NS
Femoral neck T-score (SD)	−2.2 ± 0.7	−2.3 ± 0.6	NS

Data are expressed as mean ± SD.

Table 2
Serum levels of RANKL, OPG, β-catenin, sclerostin and DKK-1 in postmenopausal osteoporotic women.

	Groups	Baseline	Day 30	Day 60
RANKL (pmol/L)	PEMFs	4.1 (2.2 to 7.9)	2.54 (1.8 to 6.1)*	2.14 (1.8 to 7.1)*,#
	Controls	3.9 (2.1 to 6.8)	3.71 (2.1 to 6.24)	3.98 (2.31 to 6.1)
OPG (pmol/L)	PEMFs	8.5 (6.9 to 9.9)	8.3 (6.2 to 9.4)	8.7 (7.8 to 10.1)
	Controls	8.16 (6.2 to 8.19)	8.31 (5.9 to 9.32)	7.9 (5.46 to 9.24)
RANK-L/OPG ratio	PEMFs	0.59 (0.22 to 0.97)	0.51 (0.15 to 0.66)	0.24 (0.22 to 0.78)*,#
	Controls	0.51 (0.24 to 0.94)	0.47 (0.21 to 0.87)	0.52 (0.19 to 0.91)
Sclerostin (pmol/L)	PEMFs	26 (16.5 to 31.5)	21.2 (16.6 to 27.1)	26.8 (20.6 to 27.9)
	Controls	24.4 (14.7 to 32.8)	23.9 (15.1 to 28.8)	26.3 (15.6 to 34.9)
DKK-1 (pmol/L)	PEMFs	34.41 (28.8 to 44.2)	28 (20.2 to 30.8)*	27.53 (18.9 to 31.9)*
	Controls	32.84 (20.9 to 45.3)	31.2 (21.2 to 38.9)	34.1 (27.3 to 42.9)
β-Catenin (pmol/L)	PEMFs	17.8 (6.9 to 70.8)	34.46 (8.2 to 84)*	39.7 (5.8 to 78.1)*
	Controls	19.46 (8.2 to 64.2)	23.8 (9.5 to 78.2)	21.9 (8.9 to 62.6)

Data are reported as median (IQR). PEMFs exposure occurred within day 30.
* $P < 0.05$ vs. baseline.
# $P < 0.05$ vs. controls.

Table 3
Bone turn-over markers, creatinine and calcium in postmenopausal women receiving PEMFs.

	Groups	Baseline	Day 30	Day 60
BSAP (ng/mL)	PEMFs	15.1 (14.5 to 16.2)	15.85 (14.9 to 16.87)*	15.65 (14.35 to 16.75)*,#
	Controls	15.2 (15.1 to 15.8)	15.2 (14.2 to 15.84)	15.1 (14.8 to 16.93)
CTX (ng/mL)	PEMFs	0.46 (0.36 to 0.58)	0.39 (0.26 to 0.46)	0.3 (0.24 to 0.53)*
	Controls	0.39 (0.36 to 0.58)	0.35 (0.23 to 0.56)	0.35 (0.25 to 0.58)
Creatinine (mg/dL)	PEMFs	0.81 ± 0.19	0.82 ± 0.18	0.8 ± 0.19
	Controls	0.79 ± 0.18	0.82 ± 0.21	0.81 ± 0.2
Calcium (mg/dL)	PEMFs	9.12 ± 0.42	9.01 ± 0.36	9.18 ± 0.44
	Controls	9.08 ± 0.33	9.13 ± 0.31	9.11 ± 0.39

Data are expressed as mean ± SD or median (IQR) as appropriate. BASP = bone specific alkaline phosphatase; CTX = C-telopeptide of type 1 collagen.
* $P < 0.05$ vs. baseline.
# $P < 0.05$ vs. controls.

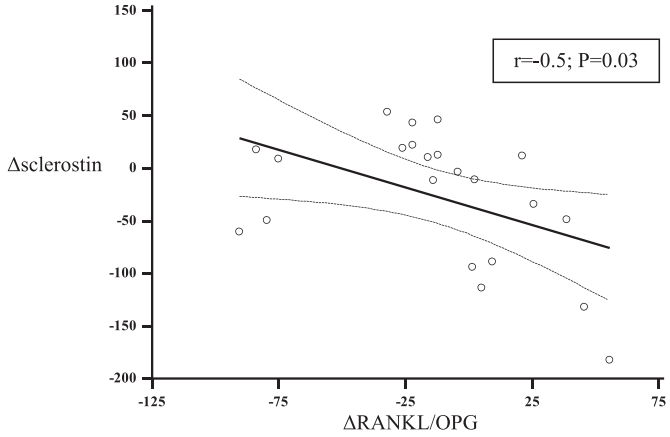

Fig. 1. Correlation between Δsclerostin with ΔRANKL/OPG ratio.

both versus baseline and versus controls. However, the difference in OPG was not significant over time. After 30 and 60 days, DKK-1 levels significantly decreased, while β-catenin concentrations increased significantly only in PEMFs group. Sclerostin slightly, but not significantly, decreased at day 30 in PEMFs group in comparison with baseline. No significant changes of calcium, phosphorus and creatinine were detected in both groups over the observation period (Table 3). In PEMFs group, at day 30, Δsclerostin was significantly associated with

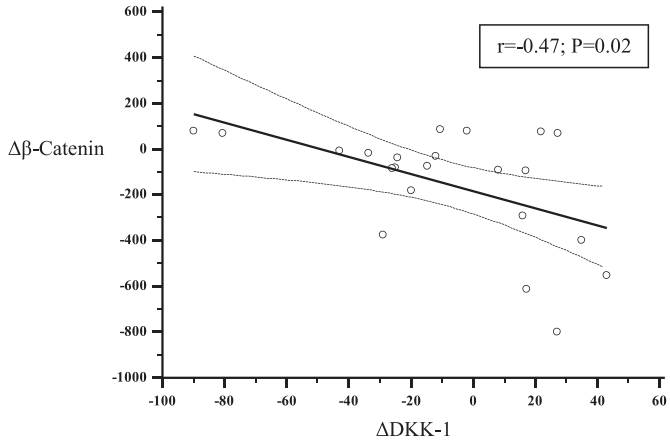

Fig. 2. Correlation between ΔDKK-1 with Δβ-Catenin.

ΔRANKL/OPG ratio ($r = -0.5$, $P = 0.03$) (Fig. 1) and ΔDKK-1 was associated with Δβ-Catenin ($r = -0.47$, $P = 0.02$) (Fig. 2). None of the patients reported any adverse events during this study.

4. Discussion

Electromagnetic fields have been experimentally investigated in several conditions, including fracture healing, osteoarthritis, cardiovascular and neurological disorders, and PEMFs have been mainly used as a non-invasive alternative method to accelerate bone healing usually in long bones such as femur and tibia [2, 24].

Although largely used in clinical practice, to our knowledge, little is known on the molecular mechanism of PEMFs driving their biological effects on bone in humans. Our study, for the first time, evaluated the modifications of regulatory molecules interfering with bone formation and bone resorption mechanisms under PEMFs exposure (Fig. 3).

Shen et al., in a rat model of disuse osteoporosis, previously reported that PEMFs stimulation efficiently suppressed bone mass loss by enhancing TGF-β secretion and inhibiting IL-6 expression, thus boosting bone formation and reducing bone resorption respectively [25]. Consistently, in postmenopausal osteoporotic women, we found that Wnt/β-catenin and RANKL/OPG signaling could be involved in the bone response to PEMFs, highlighting a potential way of interference with mechanisms of bone formation and resorption.

Our data are in accordance with Chang et al. who reported in a murine marrow culture system that PEMFs could regulate osteoclastogenesis, bone resorption, OPG, RANKL, and M-CSF concentrations [26]. Indeed, the RANKL/OPG ratio was decreased in postmenopausal women from the PEMFs group. Additionally, the reduction of CTX

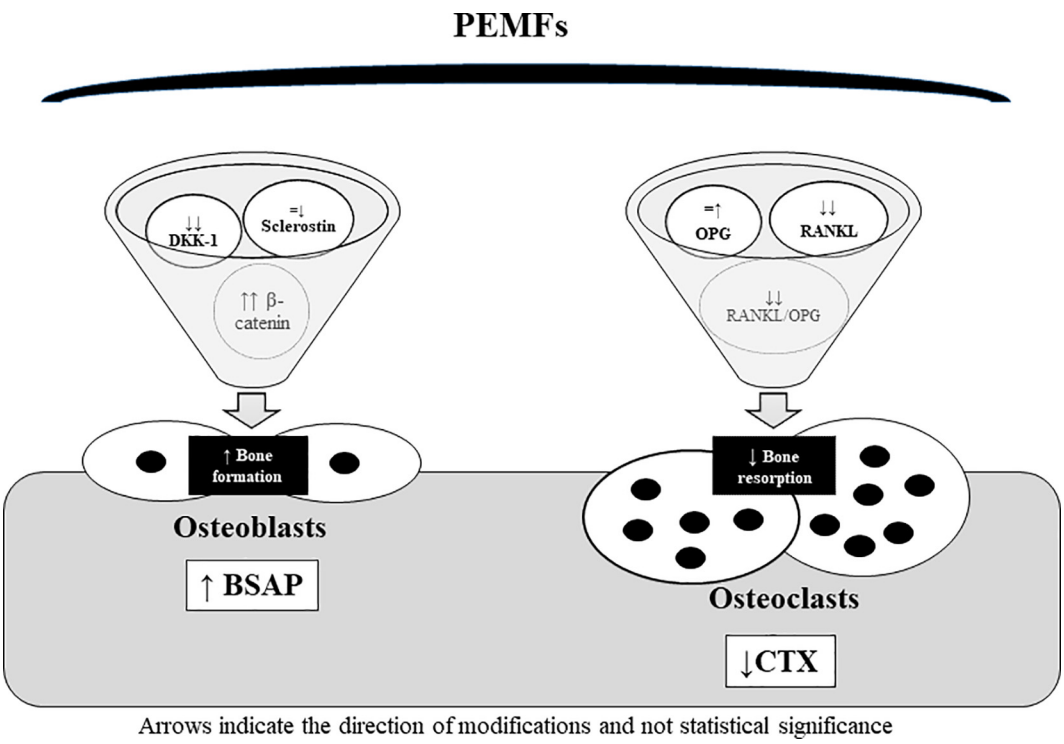

Fig. 3. Schematic proposed mechanism of action of PEMFs on bone tissue.

levels, allows us to speculate on a possible reduction of bone resorption. DKK-1 and sclerostin, which compete with the Wnt/β-catenin for binding to LRP5/6, disrupting (Dkk-1) or antagonizing (sclerostin) LRP5/6 mediated Wnt signaling, these were investigated, and we found a significant reduction of DKK-1 serum concentrations in PEMFs group, in accordance with previous data by Zhou J et al. in ovariectomized rats [27]. These findings suggest that Wnt signaling could be improved, and are reinforced by the observation of increased β-catenin levels after PEMFs exposure. β-catenin is the downstream component of Wnt protein-mediated signal transduction pathway and finally promotes transcription of Wnt responsive genes [19]. Sclerostin levels were not significantly changed, but we suppose sclerostin may contribute to the cross-talk between bone cells, as we observed its variation was associated with changes in RANKL/OPG ratio after PEMFs exposure [28].

Working on Wnt/β-catenin pathway, PEMFs could promote osteoblast activity [29]. We recorded a significant elevation of BSAP levels, accordingly with published data showing a significant increase in serum surrogate markers of bone formation such as osteocalcin and serum procollagen type I C-terminal propeptide [30]. Tabrah F et al. reported that PEMFs treatment of the wrist induced an increase of BMD values in the distal radius of osteoporosis-prone women [31]. Interestingly, a similar but weaker response occurred in the opposite non-treated arm, suggesting systemic effects of PEMFs that could be promoted by soluble modulators of bone metabolism as we have shown.

In our study, women were exposed to PEMFs by wearing a specific gilet connected to the electromagnetic device. The exposed region was represented by the trunk, a site where vertebrae and ribs represent bones rich in metabolically active trabecular tissue. This feature could have contributed to the metabolic changes of bone we detected.

Furthermore, PEMFs preserved lumbar vertebral bone mass, microarchitecture and strength in ovariectomized mouse model via a combination of increased bone formation and suppressed bone resorption related to Wnt and OPG/RANKL/RANK signaling pathways [32].

In leptin receptor-deficient db/db mice with type 2 diabetic symptoms, real-time PCR showed that PEMFs upregulated tibial gene expression of osteogenic-related proteins implying the activation of canonical Wnt/β-catenin signaling, and a μCT evaluation showed a significant improvement of both cancellous and cortical bone microarchitecture [33]. Cay J et al. reported also that diabetic rabbits subjected to PEMFs showed increased femoral osteocalcin, BMP-2 and Runx 2 mRNA expression [34].

The osteogenic potential of other electromagnetic application modality different from PEMFs as static magnetic fields (SMFs) and rotating magnetic fields (RMFs) were also studied, but conflicting results have been shown especially for RMFs [35–37]. Recently Jing D et al. showed that RMFs do not affect bone quality and bone remodeling in a rat model of disuse osteoporosis [37].

Nevertheless, clinical data on BMD are inconsistent, someone showing benefits [31, 38], others null effects [30], depending also on the type of stimulation, device manufacturer, output waveform parameters, treatment regimen and observation period.

No data exist on fractures prevention or regarding long-term benefits on BMD by the use of PEMFs. It could be even speculated that if PEMFs inhibit bone resorption for a longer time, the effect on bone formation could be underestimated by the progressive decrease in the number of metabolic units; thus, the use of PEMFs is actually not encouraged in postmenopausal osteoporosis.

Beyond bone metabolism and BMD, however, falls could increase fracture risk especially in the elderly, and gait speed is known to be associated with several clinical outcomes in older adults, including falls. It is quite interesting that Giusti et al. have recently reported the potential beneficial effects of PEMFs on gait characteristics in community-dwelling older adults with low BMD, pointing out a mechanism of fracture risk reduction [39].

We must acknowledge this study has some limitations due to the small sample size and the short observation period not allowing us to observe if the metabolic effects persist over time.

In conclusion, in a setting of postmenopausal osteoporotic women, we have shown that PEMFs induce changes in bone metabolism and that the Wnt/β-Catenin and the RANKL/OPG signaling pathways could play a role in explaining the effects on bone tissue.

Since several line of evidences suggest possible and favorable effects of PEMFs on bone tissue, and because alternative safe and effective treatment options for osteoporosis are needed, further research looking

into BMD and fractures are urgently required.

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Declarations of interest

None.

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