

Effects of 1.8 GHz Radiofrequency Field on Microstructure and Bone Metabolism of Femur in Mice

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To investigate the effects of 1.8 GHz radiofrequency (RF) field on bone microstructure and metabolism of femur in mice, C57BL/6 mice (male, age 4 weeks) were whole-body exposed or sham exposed to 1.8 GHz RF field. Specific absorption rates of whole body and bone were approximately 2.70 and 1.14 W/kg (6 h/day for 28 days). After exposure, microstructure and morphology of femur were observed by microcomputed tomography (micro-CT), Hematoxylin and Eosin (HE) and Masson staining. Subsequently, bone parameters were calculated directly from the reconstructed images, including structure model index, bone mineral density, trabecular bone volume/total volume, connectivity density, trabecular number, trabecular thickness, and trabecular separation. Biomarkers that reflect bone metabolism, such as serum total alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BALP), and tartrate-resistant acid phosphatase 5b (TRACP-5b), were determined by biochemical assay methods. Micro-CT and histology results showed that there was no significant change in bone microstructure and the above parameters in RF group, compared with sham group. The activity of serum ALP and BALP increased 29.47% and 16.82%, respectively, in RF group, compared with sham group ($P < 0.05$). In addition, there were no significant differences in the activity of serum TRACP-5b between RF group and sham group. In brief, under present experimental conditions, we did not find support for an effect of 1.8 GHz RF field on bone microstructure; however, it might promote metabolic function of osteoblasts in mice. *Bioelectromagnetics*. 39:386–393, 2018. © 2018 Wiley Periodicals, Inc.

Keywords: RF field; electromagnetic field; mice; bone microstructure; bone metabolism

INTRODUCTION

According to a recent report [Ministry of Industry and Information Technology of PRC, 2017], the number of mobile phone users in China in 2016 reached 1.32 billion with a popularity rate of 96.2%. Familiarization and dependency on mobile phones has been growing at an alarming pace. Accordingly, the chance of exposure to radiofrequency (RF) field emitted from mobile phones has also progressively increased. Therefore, the biological effects of RF field, especially potential health hazards, have become a hot topic in recent years.

RF field is a kind of electromagnetic field (EMF) in frequencies ranging from 100 kHz to 300 GHz, and has gone through phenomenal development and deployment worldwide during the last several decades, especially in wireless communications such as mobile phones, base stations, and

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laptops. RF field emitted from mobile phones has been mainly at frequencies 800–2000 MHz [Chen et al., 2005], which can induce adverse health effects, such as brain tumors [Morgan et al., 2015], infertility [La Vignera et al., 2012; Merhi, 2012; Sepehrmanesh et al., 2017], genotoxic effects [Atli et al., 2013; Duan et al., 2015], fatigue, rapid exhaustibility [Schoeni et al., 2015], increased anxiety, poor learning [Saikhedkar et al., 2014], and so on. Meanwhile, other studies showed beneficial effects or no effects of RF field on biological tissue [Kismali et al., 2012; Ahlers and Ammermüller, 2014; Gurbuz et al., 2014]. A systematic review in 2012 [La Vignera et al., 2012] found that although RF field was discussed in a lot of published literature, not much literature described the impact of RF field produced by mobile phones on bone microstructure and metabolism, especially in-vivo experiments.

In recent years, RF fields have also been used in osteoporosis therapy. For example, it was found that exposure to RF field significantly slowed bone resorption and improved bone mineral density (BMD) in the treatment of osteoporosis [Jayanand et al., 2003]. In addition, it was reported that 2100 MHz RF field significantly increased energy absorption capacity and positively affected fracture healing in rabbits [Durgun et al., 2016]. However, this report conflicted with Aslan et al.'s [2013, 2014] findings; they found that RF at 900 MHz of frequency emitted from cellular phones had an adverse effect on bone fracture healing in a rat tibia model, and RF at 1800 MHz frequency emitted from cellular phones had no effect on bone fracture healing in rats. Another study [Sieroń-Stołyński et al., 2015] showed that exposure to 900 MHz RF field did not have a direct impact on macro-metric parameters of bone tissues in rats; however, it altered the processes of bone mineralization and intensity of bone turnover processes, thus influencing the mechanical strength of bones. Whether 1.8 GHz (a widely-used frequency for mobile phone communication) RF field affects bone microstructure and metabolism remains unclear. Therefore, in this study, we observed femoral microstructure and bone metabolism after exposing mice to 1.8 GHz RF field for 28 days.

MATERIALS AND METHODS

Animals

A total of 30 C57BL/6 mice (male, age 4 weeks) at the adolescent stage, which were purchased from the Experimental Animal Center of the Fourth Military Medical University (Xi'an, China), were randomly

divided into RF group and sham group ($n = 15$ for each group); no cage controls were included in this study. The mice were housed in two plastic cages with ad lib access to standard food pellets and water and moved freely within the confines of the cage, which was maintained on a 12 h light/dark schedule at $22 \pm 2^\circ\text{C}$. No specific dietary supplements were provided. All mice in this study were treated in accordance with The Code of Ethics of Animal Use and Care Committee that was approved by the Research and Education of Fourth Military Medical University (NO: 20161004, Xi'an, China), and all efforts were made to minimize animal suffering. The experiment was performed in blind condition.

Exposure Apparatus

The 1.8 GHz RF-exposure system for live animals was made by East China Normal University (Shanghai, China), which was described elsewhere [Wang et al., 2017]. Briefly, the system had an RF signal source, a control system, and a movable loading platform. The microwave signal source from a horn antenna with an adjustable power of 1–200 W and 40 W was applied in the experiment. A schematic diagram of the experimental setup for RF exposure is presented in Figure 1A [Zhang et al., 2017]. The specific absorption rates (SARs) of 17 tissue types were calculated by EFDTD7.2.3 (Remcom, State College, PA) at 1 meter below the horn antenna, including brain, bone, tongue, eyes, esophagus, heart, liver, lung, spleen, stomach, digestive system, kidney, urinary bladder, penis, testis, skin, and muscle. The SARs of whole body and bone were approximately 2.70 and 1.14 W/kg (occupational exposure limit is 0.4 W/kg). The mice in RF group were placed freely in a plastic box (with bedding, and then exposed to 1.8 GHz RF field for 28 consecutive days, 6 h/day [9:00 AM to 3:00 PM]). During exposure, the mice could access food and water ad lib. The power density in this study was $530 \mu\text{W}/\text{cm}^2$ measured by an EMF meter (PMM8053A, PMM, Milan, Italy). The mice in sham group were treated in the same way as RF group but with the control system off.

During the RF or sham exposure period, the surface body temperature of mice was recorded simultaneously with an infrared thermal camera every 0.5 h (Ti400 Thermal Imager, Fluke, Everett, WA). This result showed that no gross thermal effects were involved (Fig. 1B), which was published elsewhere [Zhang et al., 2017]. In addition, the body weight of mice was measured about every 4 days during the 28 days of exposure. The body weight in both RF group and sham group increased as time was extended. However, the body weight (Fig. 1C) and

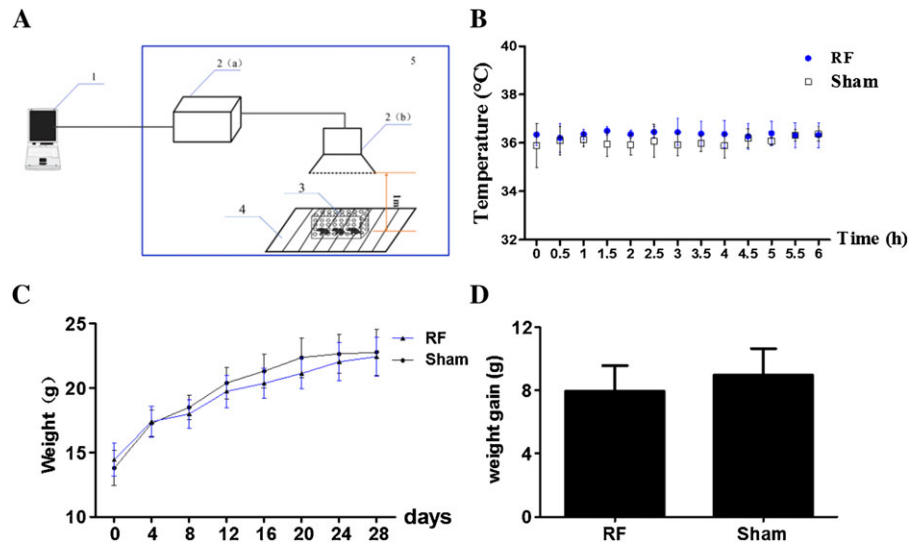


Fig. 1. General case of 1.8 GHz RF field exposure. (A) Schematic diagram of experimental setup of RF-exposure system; 1. Control system: a computer; 2. Microwave signal source. (a). Microwave source; (b). Horn antenna (aperture plane: $21.4 \times 16.3 \text{ cm}^2$); 3. Experiment animals were placed in a plastic box ($35 \text{ cm} \times 24 \text{ cm} \times 24 \text{ cm}$) with many vents on the surface; 4. Experiment platform; 5. Shield room. (B) Time course of changes in surface body temperature of mice during 1.8 GHz RF field exposure, $n = 6$; (C) Growth curve, $n = 15$; (D) Weight gain, $n = 15$. Data are represented as the means $\pm$ SD.

weight gain (weight on 28th day minus weight on 0th exposure day (Fig. 1D)) showed that there were no differences between sham group and RF group.

Microcomputed Tomography (Micro-CT) Scanning and Analysis

After exposure, the mice were deeply anaesthetized with 1% pentobarbital sodium anesthesia (60 mg/kg). During deep sedation, bilateral femur samples of all mice were harvested in randomized sequence. Then right femur distal end samples ($n = 15$ for each group) were vertically fixed along the long axis in a 10-mm diameter sample tube-perpendicular immobilization device and scanned with a high-resolution Micro-CT (GE Healthcare, Milwaukee, WI). The scanning protocol was as follows: 15 ml tube, $8 \mu\text{m}$ scanning resolution, 120 min total scan time, and total rotation angle 360 degrees. The basic scanning parameters for the measurements were as follows: reconstructed image size 2048×2048 pixels, voltage 80 kV, current $80 \mu\text{A}$, integration time 300 ms, and rotation angle of increment 0.5 degrees.

For the analysis of femoral cancellous bone microarchitecture, the region of interest (ROI) of cancellous bone, which started at the lowest end of the femoral growth plate and extended 2.0 mm distally from the growth plate, was scanned every 0.5 mm and used for three-dimensional (3D) recombination. All

3D microstructural image data were reconstructed by using Recon Interface software (GE Healthcare, Milwaukee, WI). Structural indices were calculated with Micro View software (GE Healthcare). Within the ROI, bone structural parameters were calculated directly from the reconstructed images, including structure model index (SMI, describing degree of plate-like and rod-like trabecular by calculating the curvature of the bone surface), BMD (presenting bone mineral content calculated according to phantom and standard CT value), trabecular bone volume/total volume (BV/TV), connectivity density (Conn.Dens), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp). Quantitative analyzes of the ROI were carried out three times after scanning once, and the results were then averaged.

Histology Assay

The left femoral samples were harvested as previously described and then fixed in 4% paraformaldehyde for 48 h and decalcified in 20% ethylenediamine tetraacetic acid (EDTA) for 30 days at 37°C , followed by gradient dehydration with ethanol and then embedded in paraffin. Sections with a thickness of $5 \mu\text{m}$ in the longitudinal direction were stained with Hematoxylin and Eosin (H&E) and Masson triplicate, respectively. Morphology of trabecular bone and cells at the growth plate was observed by Nano Zoomer with NDP.scan SQ1.0 (Hamamatsu,

Shizuoka, Japan) and Leica DMI4000B (Leica, Heidelberg, Germany), respectively. Each group contained 15 mice.

Serum Biochemical Assay

Ten mice from each group were anesthetized as previously described, and blood samples were taken from the left ventricle of the heart. After that, the samples were left at room temperature for at least 30 min, and then centrifuged at 3000 rpm for 10 min at 4 °C to separate serum. Finally, the serum samples were stored at −70 °C. The activity of serum total alkaline phosphatase (ALP) (NO. A059-2, Jiancheng Bioengineering Institute, Nanjing, China), bone-specific alkaline phosphatase (BALP), and tartrate-resistant acid phosphatase 5b (TRACP-5b) (m1001892 and m1001944, mlbio, Shanghai, China) were determined according to the manufacturers' instructions. Briefly, after adding stop solution, gently shaking the plate, and taking blank well as zero, optical density (OD) values were measured at 520, 450, and 450 nm, respectively, on a microplate reader and the activity of ALP, BALP, and TRACP-5b in each well were calculated according to their standard curves. Two duplicate wells were measured on each serum sample and the results were then averaged.

Statistical Analysis

All data are presented as mean values with standard deviation (SD), and were analyzed using SPSS version 19.0 (SPSS, Chicago, IL). Shapiro-Wilk test and Levene's test were used to evaluate for normality and homogeneity of variance, respectively. Analyses suggested that all parameters of the two groups obeyed normal distribution and variance homogeneity. Student *t*-test was used to evaluate the significance of differences between RF group and sham group. For all comparisons, value of *P* less than 0.05 was indicated statistically significant.

RESULTS

Effects of RF Field on Femoral Microstructure Examined by Micro-CT

The location of ROI selected for analyzing femoral microstructure is shown in Figure 2A-1. Micro-CT visualization imaging shows that no obvious structural alteration in trabecular bone mass, trabecular bone connection, or trabecular bone micro-architecture was found in RF group, compared with sham group (Fig. 2A-2). Quantitative analysis results also show that there were no significant differences in bone parameters between RF group and sham group,

including SMI, BMD, Conn.Dens, BV/TV, Tb.N, Tb.Th, and Tb.Sp (Fig. 2B-1 to Fig. 2B-7).

Effects of RF Field on Histology of Distal Femurs

As shown in Figure 3A, no obvious differences were observed in the structure and area of trabecular bone between RF group and sham group. In addition, compared with sham group, the number of trabecular bone, distance between the trabecular, the continuity, integrity, and thickness of the trabecular bone in distal femurs did not change in RF group (Fig. 3B). Additionally, cells at the growth plate in two groups were all arranged regularly and compacted to plate layer, and no obvious change was found between RF group and sham group after 1.8 GHz RF field exposure.

Effects of RF Field on Bone Metabolism

As shown in Figure 4, the activity of serum total ALP (Fig. 4A) and BALP (Fig. 4B) increased $29.47 \pm 4.08\%$ and $16.82 \pm 2.74\%$, respectively, after exposure to 1.8 GHz RF field, compared with sham group ($P < 0.05$). In addition, there were no significant differences in the activity of serum TRACP-5b (Fig. 4C) between RF group and sham group.

DISCUSSION

The potential human health hazards of increased and chronic exposure to RF field have gotten much attention in recent years. Based on the evidence from epidemiological and laboratorial studies, the International Agency for Research on Cancer (IARC) issued an official release in May, 2011 labeling radiofrequency electromagnetic fields as “possibly carcinogenic to humans” and added it to the list of a possible human carcinogen (Group 2B) [International Agency for Research on Cancer, 2013].

In recent years, some experiments have been carried out to investigate the effects of EMF on bone, and the results showed some positive effects on bone cells. For example, Bagnato et al. [2016] reported that pulsed EMF therapy was safe and effective in improving knee osteoarthritis symptoms. It was believed that pulsed EMF could mimic physiologic processes to aid in bone healing [Cook et al., 2015]. In addition, Cicek et al. [2009] reported that 900 MHz or 1800 MHz RF (30 min/day, 5 days a week for 4 weeks) promoted maturation of the bone trabecula, bone volume, and formation. However, other authors dispute these claims. For instance, Van der Jagt et al. [2012, 2014] found that EMF at 15 and 50 Hz did not induce any changes in cancellous or cortical bone compared

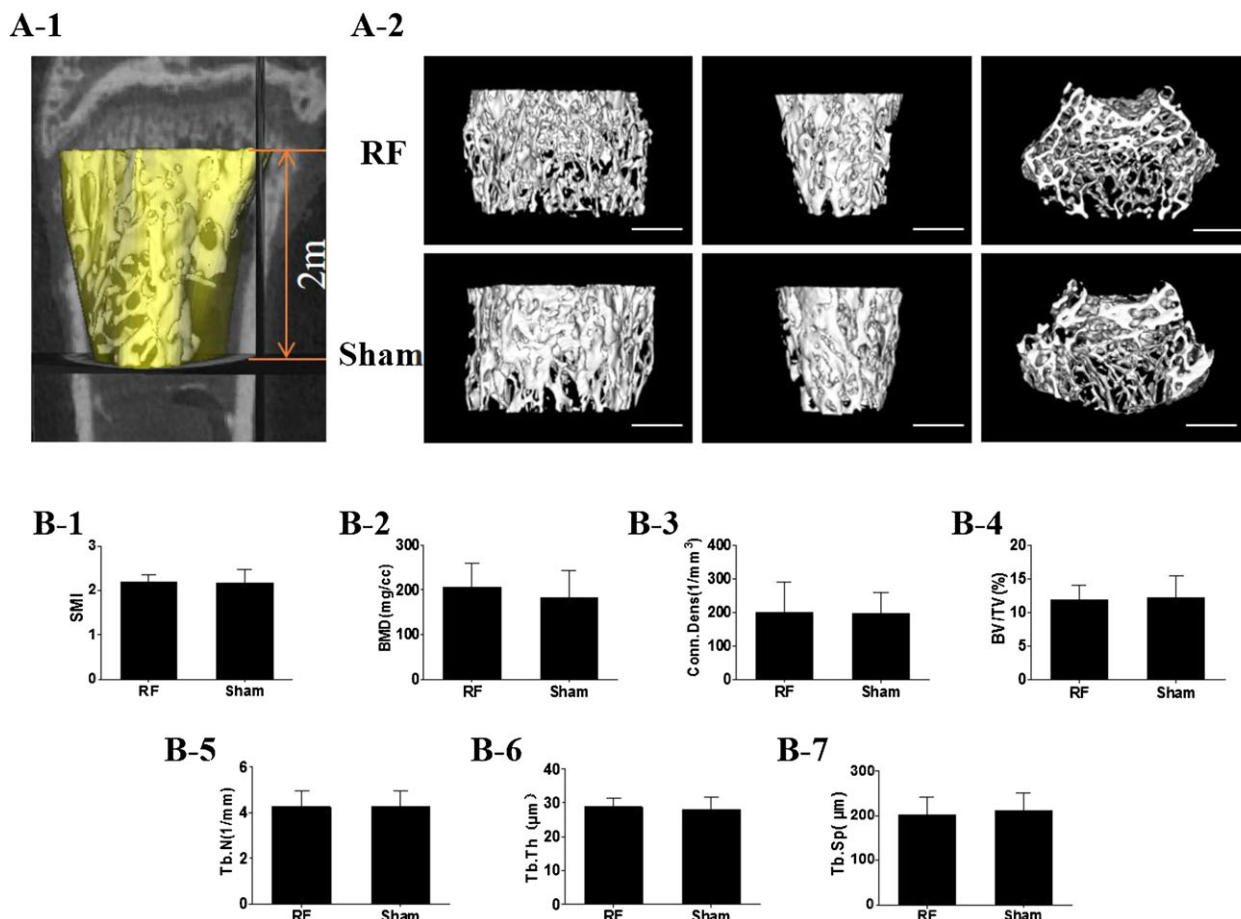


Fig. 2. Effects of RF field on femoral microstructure examined by micro-CT. **A:** 3D reconstruction of distal femur in mice by micro-CT after exposure to 1.8 GHz RF field. (A-1) ROI: 2 mm cancellous bone tissue under femoral plate was selected as analysis area; (A-2) 3D reconstruction of ROI cancellous bone from front, side, and top views (from left to right) of same mouse. Bars = 0.8 mm, $n = 15$ for each group. **B:** Quantitative analysis of ROI microstructure of trabecular bone in distal femur of mice after exposure to 1.8 GHz RF field. (B-1) SMI, (B-2) BMD, (B-3) Conn.Dens, (B-4) BV/TV, (B-5) Tb.N, (B-6) Tb.Th, and (B-7) Tb.Sp. Data are represented as means $\pm$ SD, $n = 15$ for each group.

to untreated controls in osteoporotic rats. In brief, the results available were controversial.

In this study, we first investigated the effects of 1.8 GHz RF field on cancellous bone microstructure by micro-CT in mice. Micro-CT is a widely used technology to detect bone microstructure through X-ray scanning and 3D reconstruction, which enables highly quantitative and qualitative measurement of bone augmentation [Kochi et al., 2010]. According to the results from 3D reconstruction of mouse femoral ROI and quantitative analysis, no obvious difference in bone microstructure was found between RF group and sham group. BMD is a critical parameter that reflects cancellous bone mass. It was found that exposure to 1.8 GHz RF field for 28 days had no dramatic effects on BMD of mice femur, which was

consistent with Aslan et al.'s [2011] results. They found that exposure to 900 MHz EMF had no obvious effects on BMD of rat's bone tissue. In addition, the histology results from H&E staining and Masson staining confirmed that the bone microstructure of mice did not change after exposure to 1.8 GHz RF field.

Bone metabolism not only reflects the activities of osteogenesis and bone resorption, but also reflects the level of osteoblasts and osteoclasts. Osteoblasts are deposited in matrix that is mineralized by calcium (Ca) and phosphate shifting from interstitial fluid into bone and are responsible for bone formation. Osteoclasts are derived from hematopoietic progenitors in bone marrow and are responsible for bone resorption [Boyce, 2013]. These two kinds of cells keep a

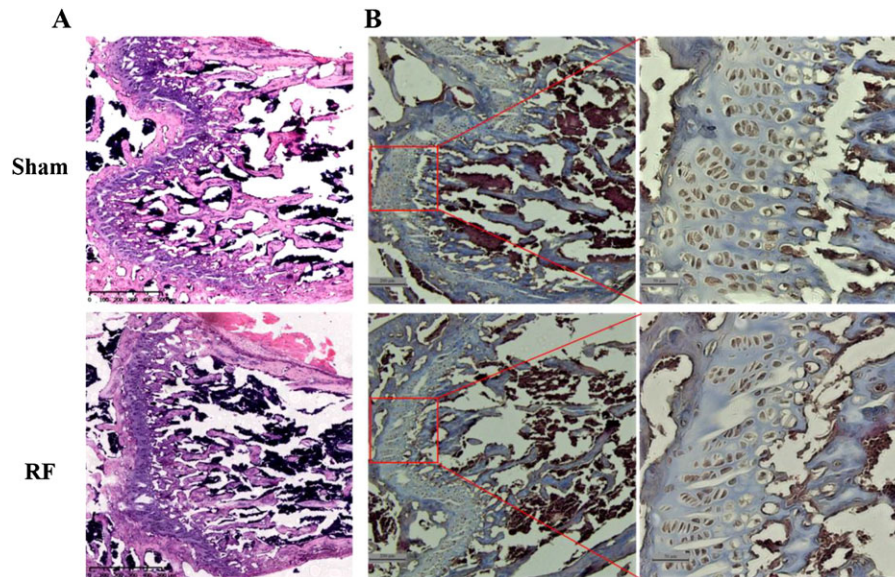


Fig. 3. Histology of distal femurs in mice after exposure to 1.8 GHz RF field. **(A)** H&E staining. Bar = 500 μm . **(B)** Masson staining. The two images on the right are magnified with a red outline in the left two images. Bar = 200 μm (left two images) and 50 μm (right two images), $n = 15$ for each group.

balance in normal skeletal development and keep the integrity of bone throughout a person's lifetime. It was reported that bone formation and resorption biomarkers of bone were the most useful indicators of bone metabolism [Kuo and Chen, 2017].

Usually, serum ALP is used as an indicator of bone formation in mammalian bone research [Halling et al., 2013; Fink et al., 2016]. ALP could be produced in liver, bone, intestine, and kidney, and about 50% of total ALP was produced from bone in healthy adults [Kuo and Chen, 2017]. In the present study, the activity of ALP increased statistically in the RF group compared with sham group, which was consistent with Yuan et al.'s [2004] report. They found that ALP activity significantly increased after exposure to 170 MHz RF field in the experimental group. To

confirm the results of serum ALP, the level of serum BALP (an indicator of osteoblast activity) was determined, and it was found that the results of BALP were consistent with that of ALP. BALP is essential for the mineralization of bone matrix during the process of bone formation [D'Oronzo et al., 2017]. Thus, our results suggest that 1.8 GHz RF field might promote the metabolic function of femoral osteoblasts in mice. Similar findings were also reported by other researchers. For example, Lei et al. [2018] found that after exposing ovariectomized BALB/c mice to pulsed EMF, the level of BALP markedly increased compared to the control group.

Serum TRACP-5b is normally secreted by osteoclasts during the process of bone resorption and used as a bone resorption marker [Sarvari et al., 2015;

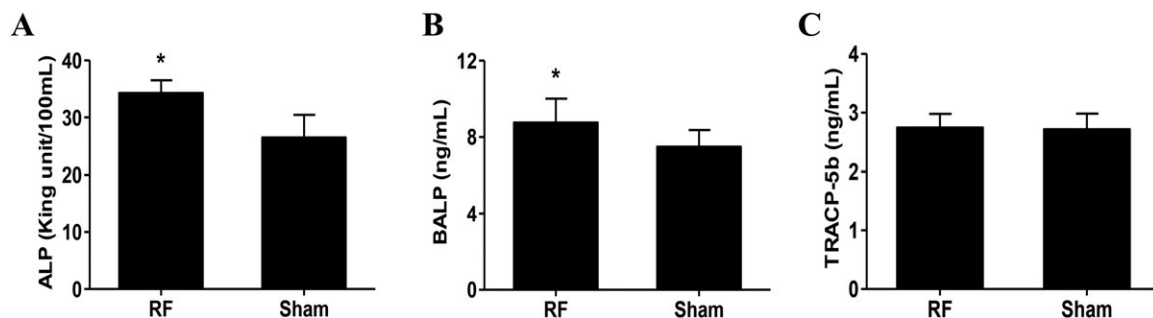


Fig. 4. Effects of RF field on bone metabolism. Activity of serum **(A)** ALP, $n = 7$, **(B)** BALP, $n = 10$, and **(C)** TRACP-5b, $n = 10$ in mice after exposure to 1.8 GHz RF field. Data are represented as means $\pm$ SD. * $P < 0.05$ versus sham group.

Wu et al., 2015; D'Oronzo et al., 2017; Kuo and Chen, 2017]. TRACP-5b is extensively used and has revealed good specificity and high sensitivity in comparison with other bone biomarkers [Kuo and Chen, 2017]. Recently, Gao et al.'s reports [2014, 2017] showed that 50 Hz EMF could decrease TRACP 5b expression in young rats. In the present study, we found that there was no significant difference in the activity of TRACP-5b between RF group and sham group. The inconsistent results may be due to the different experimental conditions and EMF parameters.

Recently, a meta-analysis study showed that pulsed RF energy was effective in the treatment of postoperative and non-postoperative pain and edema, and in wound-healing applications [Guo et al., 2012]. Also, RF fields have been successfully used in physiotherapy for the treatment of bone disorders and osteoarthritis, as well as for cartilage regeneration or pain reduction [Maziarz et al., 2016]. The results of this study have provided some theoretical basis.

In brief, under present experimental conditions, we did not find support for an effect of 1.8 GHz RF field on bone microstructure; however, it might promote metabolic function of osteoblasts in mice.

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REFERENCES

- Ahlers MT, Ammermüller J. 2014. No influence of acute RF exposure (GSM-900, GSM-1800, and UMTS) on mouse retinal ganglion cell responses under constant temperature conditions. *Bioelectromagnetics* 35:16–29.
- Aslan A, Aydoğan NH, Atay T, Çömlekçi S. 2011. The effects of electromagnetic field exposure at short and long term of 900MHz frequency emitted from mobile phones on rat bone tissue. *Dicle Med J* 38:452–457.
- Aslan A, Atay T, Gulle K, Kirdemir V, Özden A, Çömlekçi S, Aydoğan NH. 2013. Effect of 900MHz electromagnetic fields emitted from cellular phones on fracture healing: an experimental study on rats. *Acta Orthop Traumatol Turc* 47:273–280.
- Aslan A, Kirdemir V, Kocak A, Atay T, Baydar ML, Özerdemoglu RA, Aydoğan NH. 2014. Influence of 1800MHz GSM-like electromagnetic radiation exposure on fracture healing. *Arch Med Res* 45:125–131.
- Atli SZ, Akar A, Sekeroglu V. 2013. Evaluation of the cytogenotoxic damage in immature and mature rats exposed to 900MHz radiofrequency electromagnetic fields. *Int J Radiat Biol* 89:985–992.
- Bagnato GL, Miceli G, Marino N, Sciortino D, Bagnato GF. 2016. Pulsed electromagnetic fields in knee osteoarthritis: a double blind, placebo-controlled, randomized clinical trial. *Rheumatology* 55:755–762.
- Boyce BF. 2013. Advances in the regulation of osteoclasts and osteoclast functions. *J Dent Res* 92:860–867.
- Chen G, Xu Z, Jiang H. 2005. In vitro study on the relationship between radiofrequency electromagnetic field of mobile phone and health. *Chin J Prev Med* 4:285–287.
- Cicek E, Gokalp O, Varol R, Cesur G. 2009. Influence of electromagnetic fields on bone fracture in rats: Role of CAPE. *Biomed Environ Sci* 22:157–160.
- Cook JJ, Summers NJ, Cook EA. 2015. Healing in the new millennium: bone stimulators. *Clin Podiatr Med Surg* 32: 45–59.
- D'Oronzo S, Brown J, Coleman R. 2017. The role of biomarkers in the management of bone-homing malignancies. *J Bone Oncol* 9:1–9.
- Duan W, Liu C, Zhang L, He M, Xu S, Chen C, Pi H, Gao P, Zhang Y, Zhong M, Yu Z, Zhou Z. 2015. Comparison of the genotoxic effects induced by 50Hz extremely low-frequency electromagnetic fields and 1800MHz radiofrequency electromagnetic fields in GC-2 cells. *Radiat Res* 183:305–314.
- Durgun M, Dasdag S, Erbatur S, Yegin K, Durgun SO, Uzun C, Ogucu G, Alabalik U, Akdag MZ. 2016. Effect of 2100MHz mobile phone radiation on healing of mandibular fractures: an experimental study in rabbits. *Biotechnol Biotech Eq* 30:112–120.
- Fink HA, Litwack-Harrison S, Taylor BC, Bauer DC, Orwoll ES, Lee CG, Barrett-Connor E, Schousboe JT, Kado DM, Garimella PS, Ensrud KE. 2016. Clinical utility of routine laboratory testing to identify possible secondary causes in older men with osteoporosis: the Osteoporotic Fractures in Men (MrOS) Study. *Osteoporos Int* 27:331–338.
- Gao YH, Cheng K, Ge BF, Zhen P, Zhou J, Ma XN, Li SF, Chen KM. 2014. Effect of different-intensity SEMFs on bone mineral density and histomorphometry in SD rats. *China J Orthop Trauma* 27:933–937.
- Gao YH, Zhou YF, Li SF, Li WY, Xi HR, Yang FF, Chen KM. 2017. Effect of 50Hz 1.8 mT sinusoidal electromagnetic fields on bone mineral density in growing rats. *China J Orthop Trauma* 30:1113–1117.
- Guo L, Kubat NJ, Nelson TR, Isenberg RA. 2012. Meta-analysis of clinical efficacy of pulsed radio frequency energy treatment. *Ann Surg* 255:457–467.
- Gurbuz N, Sirav B, Colbay M, Yetkin I, Seyhan N. 2014. No genotoxic effect in exfoliated bladder cells of rat under the exposure of 1800 and 2100MHz radio frequency radiation. *Electromagn Biol Med* 33:296–301.
- Halling Linder C, Englund UH, Narisawa S, Millán JL, Magnusson P. 2013. Isozyme profile and tissue-origin of alkaline phosphatases in mouse serum. *Bone* 53:399–408.
- International Agency for Research on Cancer 2013. Non-ionizing radiation, part 2: radiofrequency electromagnetic fields. In IARC monographs on the evaluation of carcinogenic risks to humans. Geneva, Switzerland: WHO Press.
- Jayanand, Behari JJ, Lochan R. 2003. Effects of low level pulsed radio frequency fields on induced osteoporosis in rat bone. *Indian J Exp Biol* 41:581–586.
- Kismali G, Ozgur E, Guler G, Akcay A, Sel T, Seyhan N. 2012. The influence of 1800MHz GSM-like signals on blood chemistry and oxidative stress in non-pregnant and pregnant rabbits. *Int J Radiat Biol* 88:414–419.
- Kochi G, Sato S, Ebihara H, Hirano J, Arai Y, Ito K. 2010. A comparative study of microfocus CT and histomorphometry

- in the evaluation of bone augmentation in rat calvarium. *J Oral Sci* 52:203–211.
- Kuo T, Chen C. 2017. Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. *Biomark Res* 5:1–9.
- La Vignera S, Condorelli RA, Vicari E, D'Agata R, Calogero AE. 2012. Effects of the exposure to mobile phones on male reproduction: a review of the literature. *J Androl* 33: 350–356.
- Lei T, Liang Z, Li F, Tang C, Xie K, Wang P, Dong X, Shan S, Jiang M, Xu Q, Luo E, Shen G. 2018. Pulsed electromagnetic fields (PEMF) attenuate changes in vertebral bone mass, architecture and strength in ovariectomized mice. *Bone* 108:10–19.
- Maziarz A, Kocan B, Bester M, Budzik S, Cholewa M, Ochiya T, Banas A. 2016. How electromagnetic fields can influence adult stem cells: positive and negative impacts. *Stem Cell Res Ther* 7:54–66.
- Merhi ZO. 2012. Challenging cell phone impact on reproduction: a review. *J Assist Reprod Genet* 29:293–297.
- Ministry of Industry and Information Technology of the People's Republic of China (MIIT). 2017. Communications statistics bullet in 2016. Available from: <http://www.miit.gov.cn/n1146312/n1146904/n1648372/c5498087/content.html> [Last accessed 22 September 2017].
- Morgan L, Miller A, Sasco A, Davis D. 2015. Mobile phone radiation causes brain tumors and should be classified as a probable human carcinogen (2A) (Review). *Int J Oncol* 46: 1865–1871.
- Saikhedkar N, Bhatnagar M, Jain A, Sukhwai P, Sharma C, Jaiswal N. 2014. Effects of mobile phone radiation (900MHz radiofrequency) on structure and functions of rat brain. *Neurol Res* 36:1072–1079.
- Sarvari BKD, Sankara Mahadev D, Rupa S, Mastan SA. 2015. Detection of bone metastases in breast cancer (BC) patients by serum tartrate-resistant acid phosphatase 5b (TRACP 5b), a bone resorption marker and serum alkaline phosphatase (ALP), a bone formation marker, in lieu of whole body skeletal scintigraphy with technetium99m MDP. *Indian J Clin Biochem* 30:66–71.
- Schoeni A, Roser K, Rösli M. 2015. Symptoms and cognitive functions in adolescents in relation to mobile phone use during night. *PLoS ONE* 10:e0133528.
- Sepehrimanesh M, Kazemipour N, Saeb M, Nazifi S, Davis DL. 2017. Proteomic analysis of continuous 900-MHz radio-frequency electromagnetic field exposure in testicular tissue: a rat model of human cell phone exposure. *Environ Sci Pollut Res Int* 24:13666–13673.
- Sieroń-Stońny K, Teister Ł, Cieślak G, Sieroń D, Śliwinski Z, Kucharzewski M, Sieroń A. 2015. The influence of electromagnetic radiation generated by a mobile phone on the skeletal system of rats. *Biomed Res Int* 2015:1–11.
- Van der Jagt OP, van der Linden JC, Waarsing JH, Verhaar JA, Weinans H. 2012. Systemic treatment with pulsed electromagnetic fields do not affect bone microarchitecture in osteoporotic rats. *Int Orthop* 36:1501–1506.
- Van der Jagt OP, van der Linden JC, Waarsing JH, Verhaar JA, Weinans H. 2014. Electromagnetic fields do not affect bone micro-architecture in osteoporotic rats. *Bone Joint Res* 7: 230–235.
- Wang K, Lu J, Xing Z, Zhao Q, Hu L, Xue L, Zhang J, Mei Y. 2017. Effect of 1.8GHz radiofrequency electromagnetic radiation on novel object associative recognition memory in mice. *Sci Rep* 7:44521.
- Wu Q, Zhong ZM, Pan Y, Zeng JH, Zheng S, Zhu SY, Chen JT. 2015. Advanced oxidation protein products as a novel marker of oxidative stress in postmenopausal osteoporosis. *Med Sci Monit* 21:2428–2432.
- Yuan ZQ, Li F, Wang DG, Wang Y, Zhang P. 2004. Effect of low intensity and very high frequency electromagnetic radiation on occupationally exposed personnel. *Chin J Ind Hyg Occup Dis* 4:267–269.
- Zhang J, Zhang K, Guo L, Chen Q, Gao P, Wang T, Li J, Guo G, Ding G. 2017. Effects of 1.8 ghz radiofrequency fields on the emotional behavior and spatial memory of adolescent mice. *Int J Environ Res Public Health* 14:1344.