Effects of 0.4 T Rotating Magnetic Field Exposure on Density, Strength, Calcium and Metabolism of Rat Thigh Bones

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The current study investigated the effects of 0.4 T rotary non-uniform magnetic field (RMF) exposure on bone density in ovariectomized (OVX) rats. Results showed that many bone indexes are significantly elevated after RMF exposure compared to the control OVX group and confirmed mechanistic evidence that strong magnetic field (MF) exposure could effectively increase bone density and might be used to treat osteoporosis. Synergy of daily RMF exposure (30 min a day for 30 days using an 8 Hz rotary 0.4 T MF) with calcium supplement tended to increase the indexes of thigh bone density, energy absorption, maximum load, maximum flexibility, and elastic deformation as compared to those of untreated OVX control group. Results also revealed that the indexes of alkaline phosphatase (ALP), serum phosphate, and serum calcium were higher in rats exposed to RMF with calcium than in the untreated OVX control group. Changes in bone mineral density (BMD) and bone mineral content (BMC) were observed in rats for three months including the first month RMF exposure. Bone density in rats exposed each day for 60 min increased during 1-month exposure and continued to increase during the post-exposure period. Furthermore, bone density and calcium content in rats exposed for 90 min daily decreased initially in the exposure month; however, ratio of increase was well above the control values by the end of the post-exposure period suggesting possible window and delayed effects. The study indicated that RMF exposure to both male and OVX female rats for 120 min a day over 15 day period should effectively promote increase of bone calcium contents (BCC) and bone-specific alkaline phosphatase (BAP) in rats thigh bone as well as a corresponding decrease in deoxypyridinoline crosslinks (DPD). Bioelectromagnetics 27:1–9, 2006.

Key words: rotary non-uniform magnetic field; bone mineral density; bone mineral content; bone-specific alkaline phosphatase; deoxypyridinoline crosslinks; ovariectomy

INTRODUCTION

Exogenous electromagnetic fields have shown healing effects in both bone and cartilage [Pilla, 1993; Trock et al., 1994] and have proved to be effective in alleviating pain and improving motion and function in patients with osteoporosis (OP). Recently, exogenous magnetic fields (MFs) from permanent magnets were suggested as an alternative modality in treating various problems of the musculoskeletal system. A growing body of research supports the impression that permanent magnets stimulate healing of bone fractures and wounds, enhance cartilage metabolism, decrease inflammation, and provide pain relief [Markov, 1995; Weinberger et al., 1996; Jerabek et al., 1998; Lawrence et al., 1998]. Hinman et al. [2002] reported beneficial effects of wearing a set of neodymium—iron–boron permanent magnets for both pain reduction and improved physical function in patients with dysfunctions.

Static MFs not only have healing effects on bone and cartilage, but also come with added benefits such as ease of application, low cost, low maintenance, and most importantly no electromagnetic radiation as in pulsing magnetic and electromagnetic fields [Washnis and Hricak, 1993; Szor and Topp, 1998; Colbert et al., 1999; Weintraub et al., 2003; Panagos et al., 2004]. With rotary non-uniform magnetic field (RMF), rotating frequency of the permanent magnet in

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vertical direction axis of 8 to 10 Hz, response to the MF may increase more than to static MF. Based on the previous studies using RMF applications which demonstrated its ability to enhance bone metabolism and density, we believe that a static MF should play a role in fighting OP beyond pharmaceuticals [Xiaoyun and Weide, 1994; Xiaoyun and Zhenguo, 2001; Xiaoyun and Yan, 2002].

OP is a disease linked with the aged and has been listed as the seventh most common disease in the world [Shibao, 1998]. The prevention and treatment of OP has drawn the attention of governments globally, and researchers in China and other countries began to investigate the potential of MF exposure for the purpose of finding a solution for the treatment of OP and its related symptoms such as bone fracture [Renjie et al., 1998; Xueli et al., 1998; Xiaoyun and Zhenguo, 2001; Xiaoyun and Yan, 2002]. Although, MF treatment has been used for over a thousand years in China, it has not been accepted as common practice in contemporary medicine, mainly because the mechanism of MF treatment has not been explicitly established.

Our laboratory has been actively involved with RMF research work on the various effects on cellular function, promotion of blood circulation by removing blood stasis, relieving pain, and stopping emesis. Recently, we conducted a series of explorative studies on the influence of MF exposure on bone density, strength, and metabolism in OVX rats [Xiaoyun and Weide, 1994; Xiaoyun and Zhenguo, 1998, 2001; Xiaoyun and Yan, 2002]. The current study intends to further explore those findings and assesses the magnitude and duration of RMF exposure effect on improving bone density and increasing calcium content. It also offers insight and explanation towards the mechanism by characterizing various biochemical indexes relevant to bone metabolism including bone-specific alkaline phosphatase (BAP) and deoxypyridinoline crosslinks (DPD).

MATERIALS AND METHODS

Animals and Treatment Groups

Sprague Dawley (SD) rats 5 months old from Beijing Experiment Animal Centre (Grade II) or from the Animal Centre of Guangdong Chinese Medicine College, Guanzhou, Guandong, China (Grade III) were divided randomly into experimental and control groups, housed in polyethylene cages, under a 12/12 h light/dark cycle (light 08:00–20:00 h) at 21 ± 2 °C with $60 \pm 10\%$ relative humidity. Experiments were run in a well-lit room with an ambient geomagnetic field. Food and water were freely available. The ovaries of female rats in all groups were removed except for untreated control group that were surgically opened without ovariectomy. RMF Treatment with or without calcium supplementation was administered 30 days (for experiments I and II) or 15 days (for experiment III), respectively, in various treatment groups after all rats were fully recovered and their estrogens remaining in the body to be thoroughly metabolized (about 1 month after the surgical procedure).

Treatment groups with calcium supplement in experiment I were given calcium gluconate by gavage for dosage of 50 mg/kg of body weight every 3 days and a total of six times; in experiment III diet was adjusted accordingly for rats on either normal (0.26%) or low (0.10%) calcium diets 15 g per day to every rat from surgery to euthanization. Urine samples for analysis were collected before euthanization. All anesthetized rats were euthanized by exsanguination from the main artery in abdomen, and then serum and femeral samples were also collected. The procedures conducted in experiments here with proper approval from the local ethics committee were completely in accord with the USA guidelines for the use of animals in research.

In experiment I, 60 purebred female Sprague Dawley rats (5 months old with an average weight of 261 ± 14 g), were divided into six groups as shown in Table 1. In the second and separate experiment, another 100 5-month old OVX Sprague Dawley rats were separated randomly into six groups including three identical control groups with identical experimental conditions (see Table 3). Rats in all experimental groups were uniformly exposed to RMF non-stop for 30 days. The first experimental group T1 were treated in RMF 60 min/d and, with the first control group C1, were euthanaized after completion of the 30 day exposure period, while the other two control and two experimental groups were allowed to recover without RMF exposure. Following a 30 day recovery period, the second control C2 and experimental T2, treated in RMF

TABLE 1. Treatment Groups for Experiment I

Group	Sex	Number	Process method
A	F	10	Sham (operated but not ovariectomized)
B	F	10	OVX
C	F	10	OVX + Ca
D	F	10	OVX + Ca, 30-day exposure in the MF for 30 min/d
E	F	10	OVX, 30-day exposure in the MF for 30 min/d
F	F	10	OVX, 30-day exposure in the MF for 60 min/d

TABLE 2. Treatment Groups for Experiment II

Group	Sex	n	Process method
C1	F	10	OVX, control of T1
T1	F	10	OVX, 30-day exposed in magnetic field for 60 min/d then measured
C2	F	10	OVX, control of T2
T2	F	10	OVX, 30-day exposed in magnetic field for 90 min/d, measured after 30 days
C3	F	10	OVX, control of T3
T3	F	10	OVX, 30-day exposed in magnetic field for 90 min/d, measured after 60 days
C4	F	10	OVX, control of T4
T4	F	10	OVX, 30-day exposed in magnetic field for 60 min/d, measured after 3 months
C5	F	10	OVX, control of T5
T5	F	10	OVX, 30-day exposed in magnetic field for 60 min/d, measured after 6 months

90 min/d, were euthanized. Finally, the third group of control C3 and experimental T3, treated in MF 90 min/d, were euthanized after a 60 day recovery period. The groups T4 and T5 were treated like group T1 and euthanized after 3 or 6 months with their control groups. When all animals were euthanized, BMD, BMC, blood serum, and Bone Growth Protein (BGP) were collected and analyzed in extracted femurs from the rats in each group (Table 2).

In experiment III, 120 purebred 5-month-old Sprague Dawley rats provided by the Animal Centre of Guangdong Chinese Medicine College (Guanzhou, Guandong, China, Grade III), 75 female and 45 male (average weights 259 ± 24 g and 351 ± 28 g, respectively) were separated into 8 groups randomly as listed in Table 3. The ovaries of female rats in treatment groups were removed except for female rats with sham surgery in the untreated control group. In the treatment groups, rats were exposed to RMF 120 min a day for 15 days after the OVX surgery as to test the hypothesis that increased time of RMF exposure may reduce the necessary number of treatment days.

Magnetic Field Exposure

The HMF-6000 rotary non-uniform MF treatment device (Chinese Patent number 2L93118017.1 and US Patent number 5.667.469, prototyped by Shenzhen University) was used for all RMF exposures. The rotary non-uniform magnetic field (RMF) was derived from two anti-parallel stacks of 30 neodymium–iron–boron permanent magnets magnets as shown in Figure 1. The magnet array was rotated on a vertical axis at 8–10 Hz and the MF strength was 0.6 T at the treatment table top and 0.4 T 5 cm above surface, with 0.6–0.32 T spatial variation horizontally within the animal cage region (measured when the MF was stationary). The rotation introduces an approximately sinusoidal 0.21 T average fluctuation in the MF intensity with a frequency of 8–10 Hz. Measurements were made with a Hall Magnetometer (ETM-13-Achsen, Bedienungsanleitung, Geneva, Switzerland) and a three-axis fluxgate magnetometer (Mag-03IEHV100, GMW Associates, San Carlos, CA). Based on our previous research, we have found that the use of this RMF intensity is more feasible than other modalities available for its safety and efficacy in these preclinical trials. Rotation makes a time variation in the MF due to the change of MF magnitude horizontally within the animal cage region and this will induce stronger biomagnetic effects on the treatment area. It is also noted that the distribution of RMF intensity differs little theoretically in the center and this was considered as the test area with stronger intensity, so the caged rats were put on the central treatment area with plenty of space to move around. Control groups stayed in the same room but far from RMF with only 1.5 G in their surrounding area. Exposures were given 30, 60, 90, or 120 minutes a day for either 30 or 15 days to different groups according to the experimental protocol.

TABLE 3. Treatment Groups in Experiment III

Group	Sex	Feedstuff	Number	Process method
G	F	Normal calcium	15	Sham surgery
H	F	Normal calcium	15	OVX for control
I	F	Lower calcium	15	OVX for control
J	F	Lower calcium	15	OVX, exposed 15 days to magnetic field for 2 h/d
K	F	Normal calcium	15	OVX, and exposed 15 days to magnetic field for 2 h/d
L	M	Normal calcium	15	Male rats, normal controls
M	M	Lower calcium	15	Male rats exposed 15 days to magnetic field for 2 h/d
N	M	Normal calcium	15	Male rats exposed 15 days to magnetic field for 2 h/d

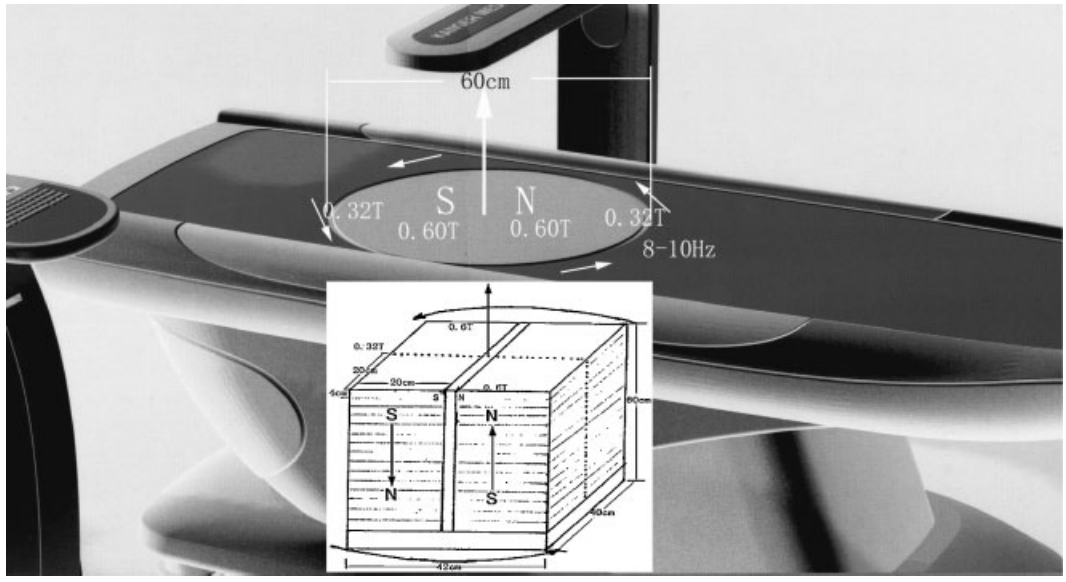

Fig. 1. HMF-6000 rotary non-uniform magnetic field (RMF). The rotary magnetic field (RMF) was derived from two anti-parallel stacks of 30 neodymium–iron–boron permanent magnets magnets as shown in Figure 1. The magnet array was rotated on a vertical axis at 8 to 10 Hz and the magnetic field (MF) strength was 0.6 T at the treatment table top and 0.4 T 5 cm above surface, with 0.6–0.32 T spatial variation horizontally within the animal cage region (measured when the MF was stationary). The rotation introduces an approximately sinusoidal 0.21 T average fluctuation in the MF intensity with a frequency of 8–10 Hz.

Bone Evaluation

Femur BMD and BMC were measured using dual energy X-ray densitometer (DXA) with DPX-L (Lunar Instruments Co, Madison, WI). The femur was initially dried at a temperature of 105 °C for 10 h until there was no more decrease in weight, and then the ash was weighed after being incinerated in a Muffle incinerator (600 °C, 2 h) [Hanna et al., 2003]. Bone calcium content (BCC) was determined from ash after dissolving in 10 ml, 4 mol/l concentrated nitric acid, then diluted with 790 ml double distilled water, and placing little of the solution in an inductively coupled plasma-atomic emission spectrometry (ICP-AES method, in co-operation with Shenzhen Epidemic Prevention Station, China) [Krejcova and Cernohorsky, 2003]. The BCC was taken as the ratio of ash weight to the dry weight (g/g). Bone mechanical parameters of femur was also measured using QTS-25 Texture (Stevens/Mechtric Company, UK) [Jones et al., 2003]. Bone sections for pathological analysis were made in paraffin, stained with metachromasia, observed under a OLYMPUS BH2-RFCA microscope (Japan), then analyzed using VIDAS software by ZEISS (Germany).

Serum and Urine Evaluation

BAP in the serum and deoxypyridinoline cross-links (DPD) in the urine were determined using an EIA reagent kit produced by American Quidel Company (San Diego, CA). Blood calcium and phosphorous content were also verified using EIA liquid reagent kits produced by Beijing Zhongsheng Biological Engineering Hi-tech Company (Beijing, China). All measurements were then confirmed on the XD-811 multifunctional biochemical analyser produced by Shanghai Xunda Medical Instrument Company (Shanghai, China).

Statistical Analysis

Mean values, standard deviation, and standard error of mean were calculated for every group and were compared in order to identify significant differences among groups for the parameters measured using ANOVA. *P* values of less than .05 were considered significant if verified by rank sum analysis.

RESULTS

Experiment I

From Tables 4 and 5, OVX treatment (group B) showed decreased BMD of the Female as compared to the control rats in Group A indicating loss of calcium from ovariectomy. The calcium supplementation in Group C reflected recovery with BMC and ash weight increasing 5.4 and 13.8%, respectively, compared to

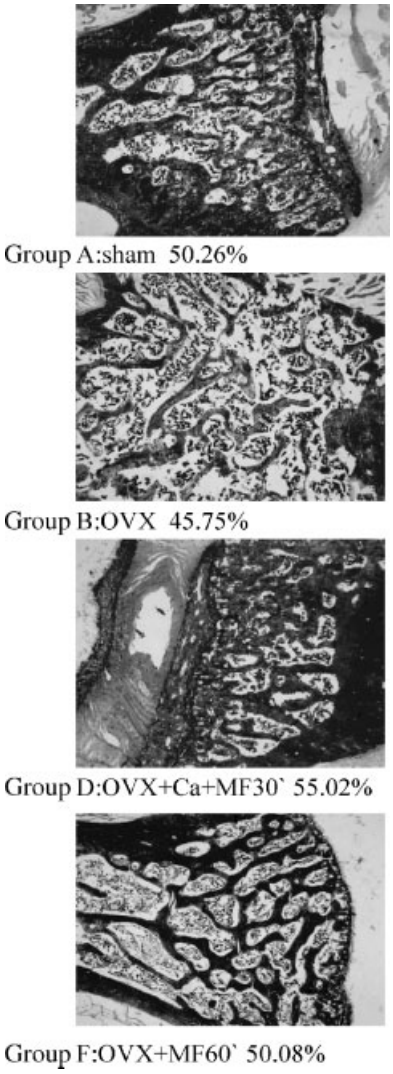

Fig. 2. Trabecular bone area, expressed as percentage of total area, in sections of femur from representative rats in groups A, B, D, and F in Experiment I. OVX and OVX + Ca + MF30' differ from sham ($P < .05$), but OVX area is close to that of sham.

Group B. When calcium supplementation and RMF exposure were co-administered, BMC, energy absorption, elastic energy absorption, maximum load, maximum flexibility, elastic deformation, and bone density were increased as compared to control group A and OVX group B. Elastic deformation, ash weight, and BMC in Group E were increased with RMF alone and the above increases were also the case in OVX treated Group B. Short RMF treatment in group D did not show much increase in all the indexes, but calcium supplementation could enhance these indexes such as in Group E. BMC, BMD, dry weight, and ash weight in Group F with longer RMF treatment were also increased as compared to OVX treated Group B. In Table 5, ALP, serum calcium, and serum phosphorus levels were elevated more than those of both untreated and OVX controls ($P < .05$) by calcium supplementation and RMF exposure. Treatment with either calcium or RMF did not affect the weight of the uterus significantly as compared to OVX treated rats. Determination of bending points in the rats thigh bones was performed and it showed that, except for Group D (treatment with both RMF and calcium supplement), other groups had no remarkable differences in the various parameters of bone mechanics as compared with the OVX groups.

Sections of femur from representative rats in groups A, B, D, and F in experiment 1 were used to compare changes in the trabecular bone. In metachromasia stained sections, the trabecular bone in the OVX rats became thinner and contained irregular changes in the three-dimensional bone microstructure. Based on the analysis, the trabecular area in sham group was found to be 50.26%, in OVX 45.75%, in the OVX + Ca + MF30'group 55.02%, and in the OVX + MF60' group 50.08%. These data suggested that mean trabecular width and trabecular area were remarkably reduced in OVX group, but increased in OVX + Ca + MF30' group ($P < .05$ vs. sham).

The energy absorption of sham-OVX groups was higher than that of the OVX groups, since after the ovary was removed there was a marked increase in bone absorption with a corresponding damage of capitellum structure and a decrease in bone mechanical properties. In Group D rats receiving RMF exposure for 30 min per day plus calcium supplement, had a higher maximum load, elastic deformation, energy absorption and elastic energy absorption than that in OVX models, indicating that the joint treatment of RMF and calcium had a favorable influence on repairing or maintaining the structure of the capitellum.

Experiment II

Table 6 indicates that the 90 min RMF exposure (T2) initially lowered BMD and BMC in the T1 experiment group as compared to the sham group. However, after 1 month of post-exposure recovery, BMD and BMC levels in T3 had increased to levels above that in the sham group. In contrast, RMF exposure for 60 min a day as was performed in the Experiment I (Group F) resulted in an increase in BMD and BMC during the initial exposure month and lasted for more than two months, but no change in serum E_2 (estradiol) and BGP during RMF exposure were found (Table 7).

Experiment III

From Table 8, the uterine weight of female rats in Group G was higher than that in other groups, indicating that ovariectomy were successful. Femur dry weight in

TABLE 4. Comparison of Femoral BMC and Bone Mechanics Biomechanics in Experiment I*

Group	BMC (g)	BMD (g/cm^2)	Energy absorption (J)	Elastic energy absorption (J)	Maximum load (N)	Maximum flexibility (mm)	Elastic deformation (mm)
A	0.359 ± 0.013	0.234 ± 0.011	0.148 ± 0.014	1.500 ± 0.260	14.070 ± 2.020	0.616 ± 0.017	0.299 ± 0.048
B	0.367 ± 0.014	0.226 ± 0.011	0.136 ± 0.013	1.700 ± 0.220	14.590 ± 0.765	0.617 ± 0.033	0.303 ± 0.029
C	0.387 ± 0.012^{a}	0.228 ± 0.012	0.143 ± 0.020	1.790 ± 0.215	14.910 ± 0.922	0.638 ± 0.049	0.328 ± 0.050
D	0.386 ± 0.014^{a}	0.234 ± 0.012	0.157 ± 0.027^{a}	1.910 ± 0.200	15.700 ± 0.807^{a}	0.653 ± 0.057	0.339 ± 0.094^{a}
E	0.358 ± 0.012	0.234 ± 0.011	0.110 ± 0.020	1.760 ± 0.115	14.360 ± 1.112	0.600 ± 0.050	0.333 ± 0.030^{a}
F	0.391 ± 0.013^{a}	0.245 ± 0.012^{a}	0.143 ± 0.022	1.172 ± 0.172	15.010 ± 1.068	0.604 ± 0.051	0.313 ± 0.042

$^{a}P < .05$, $^{aa}P < .01$ vs group B. Data ($\bar{k} \pm$ SD).
*Energy and elastic energy absorption reflect the internal rigidity of bones. These features depend on the arrangement of capitellum and changes in bone density.

TABLE 5. Comparison of Uterine Weight, Femur Dry Weight, Ash Weight, Serum Alkaline Phosphatase, Calcium and Phosphorus in Experiment I

Group	Weight of uterus (g)	Dry weight (g)	Ash weight (g)	ALP(U/l)	Pi (mmol/l)	Ca (mmol/l)
A	0.103 ± 0.029^{aa}	0.515 ± 0.064	0.339 ± 0.031	104.00 ± 20.60^{a}	1.67 ± 0.43^{a}	2.40 ± 0.45^{aa}
B	0.041 ± 0.043	0.540 ± 0.048	0.348 ± 0.027	74.50 ± 12.30	0.96 ± 0.40	1.13 ± 0.61
C	0.030 ± 0.011	0.569 ± 0.041	0.369 ± 0.059^{a}	130.30 ± 35.10^{aa}	1.51 ± 0.26^{a}	2.99 ± 0.24^{aa}
D	0.029 ± 0.007	0.586 ± 0.059	0.411 ± 0.099	127.60 ± 9.50^{aa}	2.00 ± 0.50^{aa}	2.90 ± 0.38^{aa}
E	0.030 ± 0.080	0.563 ± 0.037	0.378 ± 0.021^{a}	103.90 ± 19.780^{a}	1.65 ± 0.45^{a}	2.42 ± 0.41^{aa}
F	0.060 ± 0.031	0.584 ± 0.053	0.376 ± 0.029^{a}	106.00 ± 27.80^{a}	1.70 ± 0.30^{a}	2.74 ± 0.71^{aa}

$^{a}P < .05$, $^{aa}P < .01$ vs Group B. All data ($\bar{x} \pm$ SD).

the OVX group was lower in sham treated female rats as well as in female rats exposed to the RMF. BCC in female sham and female groups with RMF exposure were higher in Group H and Group I. Table 8 also listed comparisons among untreated male rats (Group M) and male rats with RMF exposure on either low (Group M) or normal (Group N) calcium diets. BCC of male rats with RMF exposure was lower but femur dry weight did not change significantly.

In Table 9, serum calcium in female rats (Groups H and I) was lower than that in the sham group as well as groups with RMF exposure. In addition, serum phosphorus showed some differences as well in some groups. In Table 9, the serum calcium in male rats (Group N) was lower in groups with RMF exposure. The content of BAP in female rats in groups with RMF exposure was higher than that in Group G and H. The contents of DPD in groups with RMF exposure were also found lower than that in Group G and H. Furthermore, Table 9 showed higher contents of BAP in male rats groups with RMF exposure than that in Group N and the contents of DPD in groups RMF exposure was lower than Group N. These data confirmed that a lower calcium diet did not alter the actions of RMF on bones.

TABLE 6. Changes in BMD and BMC Over Time With Exposure to RMF in Experiment II

Group	n	BMD (g/cm^2)	BMC (g)
C1	10	0.264 ± 0.014	0.420 ± 0.028
T1	10	0.287 ± 0.019^{a}	0.453 ± 0.029^{a}
C2	10	0.266 ± 0.012	0.433 ± 0.026
T2	10	0.265 ± 0.010	0.437 ± 0.020
C3	10	0.269 ± 0.018	0.434 ± 0.027
T3	10	0.295 ± 0.015^{a}	0.464 ± 0.024^{a}
C4	10	0.261 ± 0.013	0.436 ± 0.021
T4	10	0.284 ± 0.015^{a}	0.466 ± 0.011^{a}
C5	10	0.263 ± 0.012	0.435 ± 0.026
T5	10	0.278 ± 0.016^{a}	0.454 ± 0.019^{a}

$^{a}P < .05$ vs their corresponding control. Data ($\bar{x} \pm$ SD).

DISCUSSION

Okazaki's research (2001) suggested that while 4.7 T static magnetic field (SMF) exposure promoted the endochondral ossification of chondrocytes, it did not

TABLE 7. Change in serum E_2 (Estradiol) and BGP (Bone Growth Protein) During Exposure to RMF in Experiment II

Group	n	E_2 (ng/l)	BGP (ng/ml)
C1	10	10.0 ± 8.3	7.2 ± 1.7
T1	10	10.1 ± 7.3	6.6 ± 2.1

Data ($\bar{x} \pm$ SD).

TABLE 8. Comparison of Weight of Uterus, Dry Weight and Bone Calcium Content of Femur in Rats in Experiment III

	Group	Weight of uterus (g)	Dry weight (g)	BCC (g/g)
G	Sham	1.245 ± 0.218^{a}	0.310 ± 0.022^{a}	0.212 ± 0.014^{a}
H	OVX	0.294 ± 0.167^{bb}	0.274 ± 0.025	0.204 ± 0.013
I	OVX + LCa	0.286 ± 0.135^{bb}	0.329 ± 0.018^{aa}	0.206 ± 0.015
J	OVX+MF2h+LCa	0.271 ± 0.129^{bb}	0.308 ± 0.024^{ab}	0.220 ± 0.021^{ab}
K	OVX + MF2h	0.289 ± 0.119^{bb}	0.292 ± 0.031^{ab}	0.224 ± 0.022^{ab}
L	Control		0.402 ± 0.015	0.199 ± 0.014
M	MF2h + LCa		0.380 ± 0.016	0.206 ± 0.025^{c}
N	MF2h		0.397 ± 0.013	0.206 ± 0.031^{c}

$^{a}P < .05$, $^{aa}P < .01$ vs OVX; $^{b}P < .05$, $^{bb}P < .01$ vs. sham, $^{c}P < .05$ vs control L. Data ($\bar{x} \pm$ SD).

induce any harmful effects on fetal development in mice [Okazaki et al., 2001; Stuchly and Dawson, 2002]. All data from these rat models in various groups tended to confirm that RMF is in fact capable of increasing density, strength, calcium, and metabolism in bones with further clinical protocol to be developed.

The present rat model involving OVX female rats with decreased bone density, BCC, and ash weight were in agreement with previous studies in the published literature [Hosokwa et al., 2000], and the use of such models for OP were validated in the current study. RMF exposure applied daily over a period of $15 \sim 30$ days was able to increase thigh bone density in untreated and OVX rats with the lasting effect for months post-exposure. The increase of bone density did not appear to be the result of an influence on circulating hormone levels [Lei and Qian, 2000; Yan et al., 2000]. To investigate whether the mechanism of RMF exposure in sham-OVX and OVX rats caused the direct increase in the uptake of calcium by bone tissue, or whether other factors involved in bone formation led to the indirect result in an increase in bone calcium, BAP, and DPD levels were assessed for the above considerations. RMF exposure resulted in an increase in BAP levels in the serum as well as in bone forming cells indicating increased osteoblast growth and activity. These observations were supported by previous research by Qichang [2001], Qian [1997], and Qingquan [2001] that explained why MF exposure effectively could enhance the growth and multiplication of bone cells [Qian and Guoxian, 1997; Qing and Nanming, 1999; Qichang and Tianzhixiu, 2001; Qingquan and Yi, 2001; Yunshan and Xizhen, 2002; Yuge et al., 2003]. DPD, a product of bone metabolism, was reduced after RMF treatment indicating a decrease in osteoclast activity.

The increase in BAP and decrease in DPD suggested that RMF exposure should bring about an increase in bone minerals and calcium by stimulating bone forming osteoblasts and inhibiting the activity of osteoclasts and subsequently preventing bone breakdown. The initial difference in BMD and BMC between rats exposed to the same RMF for 60 vs. 90 min/d revealed that a window effect may be involved and a 90 min exposure time may be too long for clinical applications. After several months away from RMF, the increase of BMD in the T3, T4, T5 groups exposed to RMF suggested that a delayed effect be involved as well. In OVX rats, serum ALP, calcium, and phosphorous levels were decreased as compared to sham-OVX control groups, while those levels in rats treated with

TABLE 9. Comparison of Calcium, Phosphorus, BAP and DPD in Rats Serum or Urine in Experiment III

	Group	Ca (mmol/l)	Pi (mmol/l)	BAP (U/l)	DPD (nmol/l)
G	Sham	2.678 ± 0.569^{aa}	1.413 ± 0.139^{a}	9.44 ± 2.57^{aa}	148.69 ± 51.57^{aa}
H	OVX	1.348 ± 0.287	1.984 ± 0.421	15.68 ± 3.68^{bb}	378.97 ± 81.64^{bb}
I	OVX + LCa	1.057 ± 0.195	2.129 ± 0.228	15.69 ± 2.11^{bb}	401.57 ± 79.34^{bb}
J	OVX+MF2h+LCa	2.714 ± 0.317^{aa}	1.398 ± 0.321^{a}	20.11 ± 2.31^{ab}	109.74 ± 20.73^{aab}
K	OVX + MF2h	2.859 ± 0.273^{aa}	1.373 ± 0.257^{a}	20.52 ± 1.78^{ab}	98.45 ± 30.57^{aab}
L	Control	2.981 ± 0.236	1.345 ± 0.115	8.53 ± 2.54	168.71 ± 53.19
M	MF2h + LCa	3.015 ± 0.197^{c}	1.279 ± 0.209	17.69 ± 3.78^{c}	107.49 ± 40.18^{c}
N	MF2h	3.143 ± 0.169^{c}	1.265 ± 0.251	15.54 ± 3.56^{c}	97.87 ± 31.97^{c}

$^{a}P < .05$, $^{aa}P < .01$ vs. OVX; $^{b}P < .05$, $^{bb}P < .01$ vs. sham, $^{c}P < .05$ vs control L. Data ($\bar{x} \pm$ SD).

either RMF or calcium supplementation were higher than those in OVX and sham-OVX groups, indicating RMF exposure may also promote the absorption of calcium and phosphorus in small intestine and increase the levels of blood calcium and phosphorus.

Many research efforts have been reported that pulsed MF exposure might be useful in the treatment of bone fracture, spinal fusion, bone formation, and bone transplant [Takano-Yamamoto et al., 1992; Feng and Erping, 1996; Neil, 2002]. However, studies using lower strength or pulsed MF exposures did not show lasting duration of the effect [Cruess et al., 1983; Bilotta et al., 1994; Lulu and Li, 2001]. In contrast, the present results corresponding to the previous conclusions from Hiroko of Tokyo University Medical College confirmed that high and constant strength MF exposure have an equal or better influence on bone [Kotani et al., 2002].

Although the exact mechanism of RMF exposure that causes calcium to enter bone tissue in OVX female rats is not fully elucidated, it seems clear from the present study that RMF exposure can promote the growth osteoblast and control the activity of osteoclast resulting in increase of overall bone density. RMF exposure also had similar effects on bone calcium and BAP and DPD in male rats suggesting that the mechanism is not related to sex hormone pathways. This finding conformed with the same test result by Hosokwa et al. [2000] from Tokushima Bunri University in Japan, in which MF exposure might accelerate the formation of bone collagen in combination with calcium. He stated that MF exposure may effectively promote fixation of dissociated intracellular calcium and prevent release of calcium from bone cells. The current study also demonstrated that RMF exposure does not have any obvious negative effects on the rat uterus. Furthermore, when bone structural mechanics were measured, RMF treatment with calcium was found to have a positive effect on repairing or retaining the structure of the capitellum leading to higher maximum load, elastic deformation, energy absorption, and elastic energy absorption in OVX rats.

CONCLUSION

RMF showed the efficacy of increasing bone density and mineral composition in rat OP models while time and duration of RMF exposure and calcium supplement also contributed to the effect. The findings of both window and delayed components of RMF suggested that further clinical applications of RMF exposure should be adequately characterized and strictly controlled. MF exposure to promote bone healing has been used for many years around the world, the current study revealed that RMF should be further explored as a potential therapeutic medical device for the treatment of OP patients.

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