EXAMINATION OF LONG TERM MAGNETIC FIELDS ON RAT CALVARIAL AND MANDIBULAR BONE MASS

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ABSTRACT
By means of long term extremely low frequency magnetic field (ELF), this study examined the effect of long term magnetic field on rat calvarial and mandibular bone mass. Forty-five female Sprague-Dawley rats were selected for the study. The rats were divided into 3 groups (n=15); ELF MF with 50Hz, 1.5mT was applied for 6 months, 4 hours/day. Total cranial and mandibular bone mineral density (BMD) and bone mineral content (BMC) of rats were determined by dual-energy X-ray absorptiometry (DXA; Discovery QDR series, Discovery A, Hologic, Inc.). Measurements were recorded for all groups before and after the applications, all data were collected and analyzed statistically.

The variations in Group 1, before and after the ELF MF applications with respect to BMC and BMD were considered significant (p< .01, p< .001). The variations in Group 2, that has bone loss with respect to BMC and BMD were considered not significant (p>0.05). The variations in control group, Group 3, with respect to BMC and BMD were considered significant (p<0.05).


Keywords: magnetic field, bone mass, densitometry

Introduction
The biological response to exposure to static magnetic field (SMF) has recently been widely discussed from the perspective of possible health benefits as well as potential adverse effects. With respect to the possible health benefits, it has been reported that local SMF stimulation is beneficial for pain management, nerve regeneration (19), inflammation (16), blood flow (2) and united fractures (12). SMF has been used to provide pain relief from neck and shoulder pain and knee pain due to ischemic conditions of the blood microcirculation (11, 15, 18).

Bone is a complex tissue and remodels continuously throughout life via resorption of old bone by osteoclasts and the subsequent formation of new bone by osteoblasts (4). There is a great interest in understanding the effects that are induced by interactions of many physical agents with bone. After menopause or ovariectomy, women tend to develop osteopenia and menopausal symptoms, including hot flashes, abnormal feelings, palpitations and insomnia. In particular, ovariectomy before menopause has been reported to cause osteoporosis and severe menopausal symptoms because of sudden estrogen deficiency (9, 10, 20).

One important physical agent is the electromagnetic field (EMF). It is now well established that exogenously EMF effects bone metabolism both in vivo and in vitro. Several researches can be summarized as follows: osteogenesis can be promoted by electrical stimulation (13, 21); apoptotic rate of osteoclast can be accelerate by pulsed electromagnetic fields (PEMF) (4); bone remodeling can be controlled by optimization of electric field parameters (14); osteoblast proliferation and differentiation can be regulated by the PEMF stimulation (17); and PEMF has been shown to significantly reduce the loss of bone mass and accelerates the bone formation in animal models (17).

Materials and Methods
Specimens
The study was performed on 45 female Sprague-Dawley rats with initial weights of 157-226 g that were obtained from Medical Science Application and Research Center, aged 4 months at the beginning of the study. All rats were allowed free access to water and standard pelleted food diet (TA VAS Inc., Adana, Turkey) during the experimental period. The rats were divided into three groups of 15 rats each (Table 1).

Ovariectomy was performed to the animals in Group 1 and Group 2, under general anesthesia which was induced by venous administration of 10 mg/kg pentobarbital (Nembutal, Abbott, North Chicago, IL, USA). The rats in the Group 1 were exposed to ELFMF for 12 weeks after ovariectomy. Group 1 animals were subjected to 1.5 mT ELFMF exposure 4 h/day, during 6 months. Group 3, which was the control group, received neither ovariectomy nor exposure to ELMFT. The specimens were kept in 14/10h light/dark environment at constant temperature of 22±3°C, 45±10% humidity. This protocol was approved by the local ethics committee.
### TABLE 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Classification</th>
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<tr>
<td>Group 1</td>
<td>ovariectomized (OVX) and ELF MF exposure</td>
<td>15</td>
</tr>
<tr>
<td>Group 2</td>
<td>ovariectomized</td>
<td>15</td>
</tr>
<tr>
<td>Group 3</td>
<td>cage-control (Cg-Cnt)</td>
<td>15</td>
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**Cages and ELFMF Stimulators**

The MF was generated in a device that had two pairs of Helmholtz coils, 70 cm in diameter, in a Faraday cage (130x65x80 cm) that earthed shielding against the electric component (Fig. 1, Fig. 2). This magnet was constructed by winding 125 turns of insulated soft copper wire with a diameter of 1.5 mm. Coils were placed vertically as facing one another. The distance between coils was 47 cm. An AC current produced by an AC power supply (DAYM, Turkey) was passed through the device. The current in the wires of the energized exposure solenoid was 40 A for 1.5 mT, which resulted 50 Hz MF. The MF intensities were measured once per week as 1.5 mT in different 15 points of methacrylate cage with a Bell 7030 Gauss/Teslamer (F.W. Bell, Inc., Orlando, FL) to ensure homogeneity of the field during the course of the experiment by a person who is not involved in the animal experiment.

Magnetic field measurements showed that, at the conditions of the experiment, the magnetic field exposure system produced a stable flux density of 1.5 mT and stable frequency of 50 Hz with negligible harmonics and no transients. ELFMF group animals were exposed to 1.5 mT ELFMF in methacrylate boxes (43x42x15 cm). The rats were free in methacrylate cage inside the coils.

**Densitometric Analysis**

After the last exposure, total body images of the animals were obtained with a DXA scanner having coefficient variations (CV) of 1% for BMC and 0.8% for BMD. Accuracy was within 1% (based on hydroxyapatite phantom) (Hologic, Discovery QDR 4500A Series bone densitometer, Hologic Corp, USA) using small animal scan software available from Hologic.

**Statistical analysis**

The mean and standard deviation (X ± SD) for continuous variables were calculated. The outcomes of all groups were analyzed with Wilcoxon Single Rank test and p<0.05 was accepted as a statistically significant value. Statistical analyses were carried out by using the statistical packages for SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA).
Results and Discussion

According to the densitometric measurements datas, Wilcoxon Single Rank test detected significant difference between the first and after the six months values of total calvarial and mandibular bone BMC and BMD for Group I (exposed to the ELFMF), Group II (ovariectomized) and Group III (cage control) rats (Table 2). Two-sided p-values were considered statistically significant at p<0.05. As shown in Table 2, the outcomes of first and after six months values for Group I rats were statistically significant (p<0.01, p<0.001). No statistically significant result was found for the outcomes of Group II (p>0.05). Finally, the outcomes for Group III were statistically significant (p<0.05).

Although there are many devices that produce extremely low-frequency magnetic field (ELFMF) and are used in daily life, many experimental and clinical researches have used mostly short term PEMF. In this paper, long term ELFMF was used for evaluation of effects on rat calvarial and mandibular bone mass.

Chang and Chang (5) demonstrated that the extremely low intensity, low frequency single pulse electromagnetic fields significantly suppressed the trabecular bone loss and the trabecular bone structure in bilateral ovariectomized rats. Chang et al. (7) suggested that osteoclastogenesis can be inhibited by PEMF (300µs, 7.5Hz) stimulation. Furthermore many in vitro experiments have shown that potential mechanisms for bone loss prevention and treatment by PEMF, in in vivo animal experiments and clinical trials were regulation of osteoblasts and osteoclasts.

Conclusions

In this study, the effects of long term (6 months) ELFMF exposure on rat total calvarial and mandibular bone mass was investigated. It was found that the final BMC and BMD values for Group I (exposed to the ELFMF) and Group III (cage control) were greater than the values for Group II (ovariectomy).

Many studies proved positive effects of the ELFMF treatment on bone fractures. No significant side effects have been reported. However, no standard method has been suggested. As a result, herein a set of criteria for reporting ELFMF treatment parameters in future clinical trials is proposed.

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th><strong>ELFMF Group</strong>&lt;sup&gt;a&lt;/sup&gt;</th>
<th><strong>Ovariectomy Group</strong>&lt;sup&gt;b&lt;/sup&gt;</th>
<th><strong>Cage Control</strong>&lt;sup&gt;c&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
<td>Group III</td>
</tr>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td><strong>BMC gr (Total calvarial)</strong></td>
<td>18±0.05</td>
<td>2.42±0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.40±0.09</td>
</tr>
<tr>
<td><strong>BMD gr/cm&lt;sup&gt;2&lt;/sup&gt; (Total calvarial)</strong></td>
<td>30±0.02</td>
<td>30±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30±0.02</td>
</tr>
<tr>
<td><strong>BMC gr (Total mandibular)</strong></td>
<td>41±0.11</td>
<td>76±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>53±0.09</td>
</tr>
<tr>
<td><strong>BMD gr/cm&lt;sup&gt;2&lt;/sup&gt; (Total mandibular)</strong></td>
<td>28±0.02</td>
<td>48±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29±0.03</td>
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<sup>a</sup>The outcomes on the first and after six months of each group were analyzed with Wilcoxon single rank test. The significant amounts were shown as: * = p < .05; ** = p < .01; *** = p < .001; **** = p > .05 for each comparison.
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REFERENCES