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Full Length Research Paper

Influences of extremely low frequency magnetic fields on mineral and trace elements content of rat teeth

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Exposure to extremely low frequency magnetic fields (ELF-MF) emanating from the generation, distribution, and utilization of electricity is widespread. The major debate in recent years has focused on the possibility that exposure to ELF-MF may result in adverse health consequences. The present study was carried out to investigate the effects of ELF-MF on the mineral content in rat teeth. 27 male Sprague-Dawley rats were divided into three groups: two experimental (each, n=10), one control sham (n=7). After ELF-MF and sham exposure, some mineral levels (Ca, Mg, Zn, and P) were determined with Atomic Absorbtion Spectrophotometry (AAS). It was determined that Ca levels increased in the two experimental groups as compared to the sham group (p<.05). The levels of Ca, Mg, Zn, and P in second experimental group rats were also higher than sham group (p<.05). The results demonstrate that ELF-MF can have significant effects on teeth mineral content. Future observations and epidemiological studies of ELF-MF effects should be accompanied by laboratory experiments to evaluate oral and dental effects.

Key words: ELF magnetic field, teeth, trace elements, mineral.

INTRODUCTION

Numerous sources of electromagnetic fields exist in nature and in the occupational and residential environments. In nearly all instances, these fields pose no obvious threat to human health or safety and are generally discussed as an inevitable by-product of modern technology. In fact, the ability of the ELF-MF to produce effects on living systems is still a matter of debate, and contradictory results are available in the literature. However, public awareness of the ubiquitous nature of these fields and the growing controversy over their potential effects on living systems have stimulated the research community to define more precisely the physical properties of these fields and to delineate the thresholds for their possible effects on human health and environment (Tenforde and Kaune, 1987; Knave, 2001).

Some studies revealed that oscillating MFs could influence water–electrolyte balance in experimental animals (Lopucki et al., 2004). Importantly, ionic and membranous effects of the oscillating MF and their severity were related to the intensity and duration of exposure (Lopucki et al., 2004). Lopucki et al. (2004) suggest that the variable MFs used in the experiments may disturb the electrolyte balance in the human placenta (Lopucki et al., 2004). Lopucki et al. (2004) speculate that the MF induced changes in the placental intermolecular protein–protein or protein–lipid interactions may lead to the selective outflow of sodium, calcium, and magnesium ions (Lopucki et al., 2004). There are some studies that investigate the effect of ELF magnetic field cytosolic and intracelluler calcium levels and bone tissues. There has been considerable interest in the role cytosolic calcium and calcium signalling plays in the possible effects of weak ELF MF for two reasons (McCreary et al., 2002). First, calcium is a ubiquitous second messenger in several key biochemical pathways and is thought to be an important mechanism for controlling and

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synchronising physiological responses (McCreary et al., 2002). For example, calcium signalling is essential for lymphocyte proliferation and the immune response to antigens (Guse, 1998). Perturbations in the regulation of cytosolic calcium homeostasis or calcium signalling could have a wide range of downstream effects, including alterations in proliferation, gene transcription, secretion, and motility. Second, the physical mechanism(s) of interaction between ELF EMF and a biological system at the cellular level have yet to be elucidated. The study of cytosolic calcium concentration ($[Ca^{2+}]c$) may lead to further understanding the physical coupling between biological signals and ELF MF (Adey, 1993). Some studies reported that ELF-MF exposure can alter intra-celluler calcium and cytosolic free calcium levels (Pessina et al., 2001; Ikehara et al., 2002; McCreary et al., 2006). Potential associations between bone mineral density (BMD) and dental status have been investigated (Bodic et al., 2005). In a study of 329 postmenopausal women, Krall et al. (1994) found that lumbar spine BMD was significantly lower among the patients who acquired dentures at age 40 or earlier (Krall et al., 1994). In another study by the same group, 189 women were monitored over a 7-year-period (Krall et al., 1996); the relative risk of tooth loss was 4.83 in patients for each 1% per year decrease in wholebody BMD.

Major elements and trace elements play an important role in human health. Deficiency and excess (toxicity) of these, resulting from exposure to both the natural and man made environment, can lead to a wide variety of clinical effects (Brown et al., 2004). The monitoring of trace element status via tissue sampling has important implications for the identification and correction of such effects. Tissues used for studying exposure to trace elements include, blood, urine, finger nails, and hair. Each is associated with particular advantages and dis-advantages. These include contamination by external agents, e.g. dust or shampoo, masking by effective homeostatic mechanisms and cultural taboos such as the collection of finger nails. Teeth are reported to be suitable indicators of trace element exposure for a wide range of elements (Brown et al., 2004). Some ions, such as Cu and Zn, have multiple functions as cofactors of enzyme activity, and their variations have been suggested as part of the biological effects caused by EMF (Burchard et al., 1999). Some authors suggested that these effects are caused by a direct interaction of the EMF and the cell membrane (Burchard et al., 1999). Burchard et al. (1999) reported that exposure to electric and magnetic fields resulted in decreased concentrations of Mg in blood Plasma and in increased concentrations of Ca and P and decreased concentrations of Fe and Mn in cerebrospinal fluid. Very little is known concerning the MF influence on electrolyte balance and concentration of some trace elements, and no studies have quantified the changes of Ca, Mg, Zn and P in rat teeth subjected to sinusoidal oscillating MF.

As humans have no way to escape ELF-MF in modern life, the aim of the present work was to experimentally investigate the effects of prolonged exposure of animals to 50 Hz, 100 µT and 500 µT magnetic fields on the mineral contents of rat teeth.

MATERIAL AND METHODS

Animal care

The experiments were performed on 27 male Sprague-Dawley rats obtained from Medical Science Application and Research Center of Dicle University, aged 4 months at the beginning of the study, weighing 342.4±38.89 g, and fed with standard pelleted food (TAVAS Inc. Adana, Turkey). The rats were divided into three groups: two experimental and one control (sham). The animals were kept in 14/10 h light/dark environment at constant temperature of 22±3°C, 45±10% humidity. All animal procedures were in agreement with the Principles of Laboratory Animal Care and the rules of Scientific and Ethics Committee of Dicle University Health Research Center.

Magnetic field generation and exposure of rat to magnetic field

The MF was generated in a device designed by us that had one pairs of Helmholtz coils of 25 cm in diameter in a Faraday cage (130 × 65 × 80 cm) that earthed shielding against the electric component. This magnet was constructed by winding 225 turns of insulated soft copper wire with a diameter of 1.0 mm. Coils were placed horizontally facing one another. The distance between coils was 25 cm (Figure 1). 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 0.12 A for 100 µT and 0.50 A for 500 µT, which resulted in 50 Hz MF.

The MF intensities were measured once per week as 100 µT and 500 µT in different 15 points of methacrylate cage by using digital teslameter (Phywe, 209101074, Göttingen, Germany) to ensure homogeneity of the field during the course of the experiment by a person who was not involved in the animal experiment. Magnetic field measurements showed that, under the conditions of the experiment, the magnetic field exposure system produced a stable flux density of 100 µT, 500 µT and stable frequency of 50 Hz with neglible harmonics and no transients. The 50 Hz stray fields in the sham-exposure system were 0.1 µT. The static earth magnetic field was measured with a Bell 7030 Gauss/Teslameter (F.W. Bell, Inc., Orlando, FL). The component parallel to the exposure field was 14 µT, and the component perpendicular to the exposed field was 34 µT.

All field measurements were performed by persons not involved in the animal experiments. Observers were not aware of which group of rats was ELF Magnetic Field-or sham-exposed, i.e., the whole study was done blind. No temperature differences were observed between exposure and sham coils during the exposure. The first and second experimental group rats (n=10) were exposed to 100 µT and 500 µT ELF MF throughout 10 months, 2 h a day respectively and thirth (n=7) group was sham that were treated like experimental group except ELF-EMF exposure (corresponding to first and second groups, respectively) in methacrylate cagees (17 × 17 × 25 cm). The rats were free to move in a methacrylate cage inside the coils. Immediately after the last exposure, teeth of the animals were collected under ketamine anesthesia (100 mg/kg, intramuscularly) in sterile saline to measure the levels of mineral amount.

Figure 1. The experimental setup.

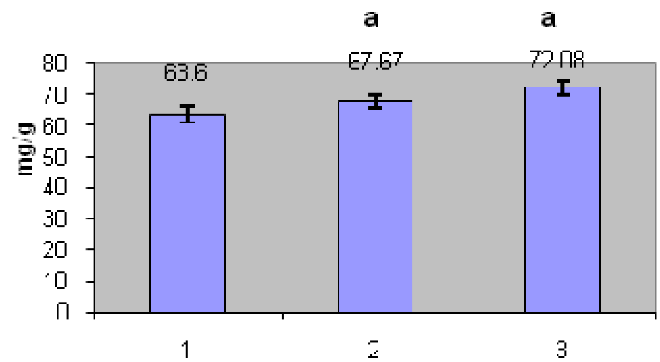

Figure 2. The changes of Concentrations of Ca (mg/g) in rats' teeth of sham (1), first (2) and second (3) experimental group. Values are expressed as median (n=10). [a]*P*<.05 as compare to sham group by non-parametric Kruskal–Wallis one-way analysis of variance and Mann Whitney U test.

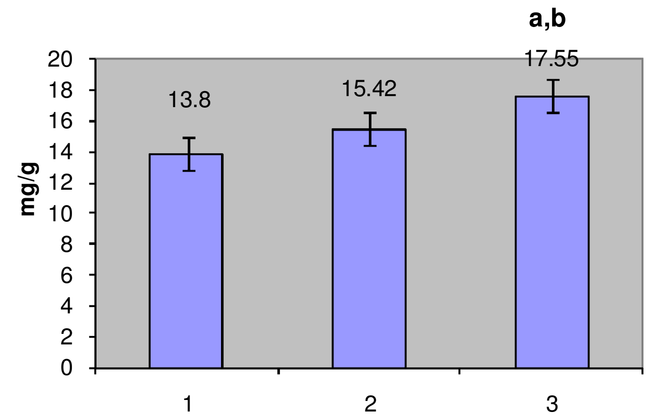

Figure 3. The changes of Concentrations of Mg (mg/g) in rats' teeth of sham (1), first (2) and second (3) experimental group. Values are expressed as median (n=10). [a]*P*<.001 as compare to sham group by non-parametric Kruskal–Wallis one-way analysis of variance and Mann Whitney U test. [b]*P*<.001 as compare to first experimental group by non-parametric Kruskal–Wallis one-way analysis of variance and Mann Whitney U test.

The teeth were placed in a porcelain crucible. The furnace temperature was slowly increased from room temperature to 500°C in 1 h. The samples were ashed for about 4 h until a white or grey ash residue was obtained. The residue was dissolved in 3 ml of the mixture of the 65% nitric acid (HNO_3), 30% hydrogen peroxide (H_2O_2) (3:1), when necessary, was heated slowly to dissolve the residue. The solution was transferred to a 10 ml volumetric flask and made up to the volume.

The concentrations of Ca^{2+}, Mg^{2+} and Zn^{2+} in the solution were determined by an atomic absorption spectrophotometer (AAS) using a unicam 929 (Cambridge, UK) equipped with an air-acetylene flame burner and hollow-cathode lamps. The atomic absorptions were measured as 422.7 nm for calcium, 285.2 nm for magnesium, 213.9 nm for zinc, respectively. The spectral band-width was 0.5 nm. The standards stock solution for calcium, magnesium and zinc were obtained from sigma. The Standard solutions and samples were dilluted in double-distilled water.

The concentrations of phosphorus in solution were determined by phospho molibdic acid method by ultraviolet spectrophotometry (UV) at 830 nm. All units of measurement is mg/g.

A computer program (SPSS 11.5, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data were analyzed by Kruskal–Wallis One-way analysis of variance ANOVA and Mann-whitney U tests. The number of specimens were low that is why non-paramentric tests instead of parametric tests were used for comparisons. All hypothesis tests used a criterion level of α=0.05.

RESULTS AND DISCUSSION

The results in relation to the teeth mineral (Ca, Mg, Zn and P) amounts in exposed and sham groups are shown in Figures 2 to 5. Ca concentration in first and second experimental groups was found to be significantly higher than in the sham group. ($p<0.05$) (Figure 2). However, no statistical difference was found in Ca concentrations between first and second experimental groups. The increase of Mg concentrations in second experimental group were found to be statistically significant relative to the sham exposed group ($P<0.001$) (Figure 3). Statistically significant difference was also noted between first and second experimental groups with respect to Mg concentrations ($p<0.001$). No significant difference was determined between sham and first experimental groups in terms of Mg concentration ($P>0.05$). Similar to findings regarding Mg concentrations, Zn and P concentrations in first and second experimental group were higher than sham group. However, the difference between second experimental and sham groups were found to be statistically significant with respect to P and Zn concentrations, respectively ($p<0.05$, $p<0.001$) (Figures 4, 5). P and Zn concentrations in rat teeth in the second experimental groups were found to be higher than those in first experimental group ($P < 0.05$). No significant differences in P and Zn concentrations were found in first experimental group compared to the sham group.

Earlier studies on the health effects of ELF-MF have not provided clear answers regarding a possible biological

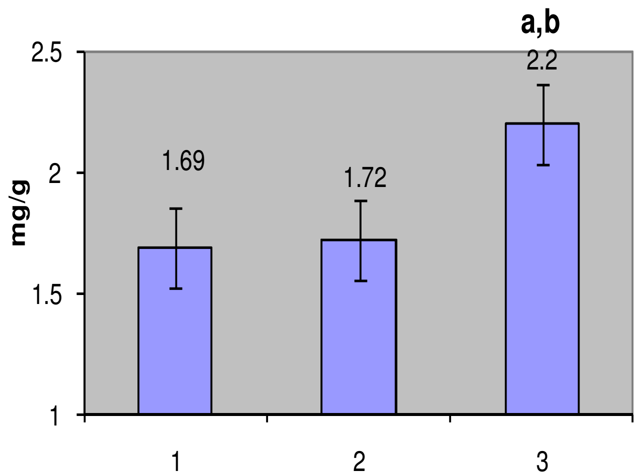

Figure 4. The changes of Concentrations of P (mg/g) in rats' teeth of sham (1), first (2) and second (3) experimental group. Values are expressed as median (n=10). [a]*P*<.05 as compare to sham group by non-parametric Kruskal–Wallis one-way analysis of variance and Mann Whitney U test. [b]*P*<.05 as compare to first experimental group by non-parametric Kruskal–Wallis one-way analysis of variance and Mann Whitney U test.

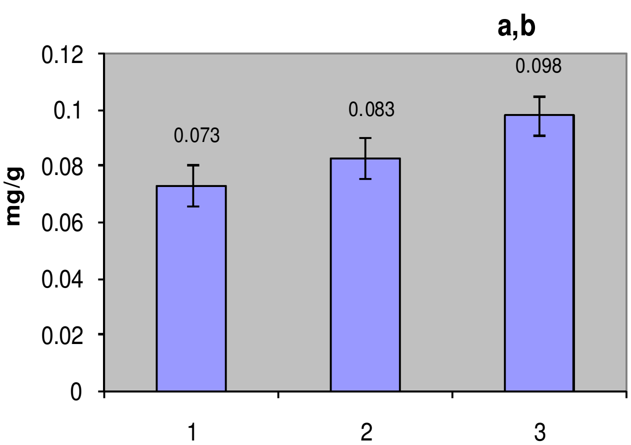

Figure 5. The changes of Concentrations of Zn (mg/g) in rats' teeth of sham (1), first (2) and second (3) experimental group. Values are expressed as median (n=10). [a]*P*<.001 as compare to sham group by non-parametric Kruskal–Wallis one-way analysis of variance and Mann Whitney U test. [b]*P*<.05 as compare to first experimental group by non-parametric Kruskal–Wallis one-way analysis of variance and Mann Whitney U test.

response. The study of the possible general health and especially oral and dental effects of ELF-MF has been particularly complex and given to us no clear knowledge. Therefore, we investigated the effect of prolonged exposure of animals to 50 Hz, 100 μT and 500 μT magnetic field on the concentrations of Ca, Mg, P and Zn minerals in the teeth of rats.

Several organizations have established guidelines for occupational exposures to power frequency ELF-MF. Power frequency is the frequency at which AC electricity is generated. For electric utilities, the power frequency is 60 Hz in North America, Brazil, and parts of Japan. Electric power is 50 Hz in most of the rest of the world (NIEHS, 1998). The sources of power frequency electromagnetic fields (EMFs) are divided broadly into those produced by natural processes and those generated by human activity (Health Protection Agency, 2006). Naturally occuring EMFs arise from electrical processes associated with the earth and the atmosphere (Health Protection Agency, 2006). In most environments the dominant source of exposure is that associated with the generation, transmission and use of electricity. People are exposed directly through the use of electrical appliances or equipment, or incidentally through working close to heating systems and power supplies (Health Protection Agency, 2006). At 50 Hz magnetic fields exposure limits are 500 μT for occupational and 100 μT for public by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) (ICNIRP, 1998). Therefore, the 100 μT and 500 μT magnetic field strengths were choosen to investigate the effects of prolonged ELF-MF exposure on mineral content of teeth. In the present study, it was determined that the teeth mineral content of rat such as Ca, Mg, P and Zn increased after prolonged ELF-MF exposure and that this increase was dose-dependent. According to the results, it can be suggested that prolonged 500 μT magnetic field exposure can affect mineral content of rats' teeth. These results are not directly comparable to any exisiting studies due to the lack of similar reports. However, there are some studies in relation to ELF-MF and trace elements that have been carried out on blood, bone and cerebrospinal fluid in rats. Sert et al. (2002) reported that Ca, Mg and Li concentrations of rats' tibia did not change after exposure to ELF-MF (1mT) Sert et al., 2002). Akdag et al. (2006) did not observe significant differences serum concentrations of Cu^{2+}, Zn^{2+} and Fe^{3+} in rats exposed to ELF-MF (1.35 mT) (Akdag et al., 2006). Burchard et al. (1999) reported that exposure to electric and magnetic fields (60 Hz, 10 kV/m, and 30 μT) resulted in decreased concentrations of Mg in blood plasma and in increased concentrations of Ca and P and decreased concentrations of Fe and Mn in cerebrospinal fluid (Burchard et al., 1999). The discrepancies between some studies and present study can be attributed to experimental factors like intensity and duration of exposure or the cell types used in the different studies. Beside the dependence of ELF magnetic field effects on physical parameters, several biological variables characterizing genetic background and physiological state are of importance for ELF MF effects (Belyaev et al., 1999). In the present study, the increasing of mineral content in rats' teeth after ELF-MF exposure are in good agreement with the results of the previous studies where increase in bone formation and mineral content was found with exposure to ELF MF (Burchard et al., 1999; Frank et al., 1998; Stephen, 1998;

Buch et al., 1985).

The body of an adult normally contains about 1200 g of calcium. At least 99% of this is present in the skeleton, where calcium minerals provide the hard structure of the bones and teeth (Brown et al., 2004). Calcium absorption may be impaired either by lack of Vitamin D, by any conditions causing intestinal hurry or by the combination of calcium with certain substances in the diet to form insoluble minerals and soaps (Brown et al., 2004). The increasing of calcium concentrations both 100 and 500 μT ELF-MF exposure supported some studies that reported increase in calcium concentrations after ELF-MF exposure (McCreary et al., 2006; Walleczek and Liburdy, 1990; Lindstrom et al., 1993; Lindstrom et al., 1995; Barbier et al., 1996; Lyle et al., 1991). According to the results of Ca concentrations in the present study, it was suggested that prolonged ELF-MF exposure can affect the hard structure of the teeth.

There is limited study in dental sciences which one of them is reporting that ELF-MF treatment not only appears to increase bone formation, as previously reported in the literature, but, acting on osteoclast activity, also seems to improve bone quality during orthodontic treatment (Stephen, 1998).

The previous studies indicate that Mg is a cofactor in many enzyme system and play an important role in regular development of enamel and dentin, mineralization and binding of Ca and P (Ozbek et al., 2001). In the present study, a parallel increase in Ca and Mg was seen. It was indicated that Zn has an an essential role on bone metabolism (Ozbek et al., 2001). In the present study, it was determined an increase in Zn concentrations on rats was exposed to ELF-MF. Almost all enzymes, including endonucleases, topoisomerases, and polymerases, contain ions such as Mg, Ca, and Zn, which are important for the conformational stability of these proteins and their enzymatic activity (Sarimov et al., 2005). Ions are often bound in special protein pockets by four amino acids, such as histidine or cysteine (Sarimov et al., 2005). Lack of ions in the protein pockets results in significant changes of protein conformations and the activity of the enzymes (Sarimov et al., 2005). According to several models, ELF magnetic fields affect cells by influencing unhydrated ions inside proteins if the parameters of exposure (frequency, magnetic intensities of alternating, AC, and static, DC, fields) are tuned to these ions (Sarimov et al., 2005). Among other ions, Zn^{2+} is important and has been implicated in the biological effects of magnetic fields. Presumably this is associated with effects on Zn^{2+}-containing proteins, including transcription factors and topoisomerases (Sarimov et al., 2005). The mineral increasing of rats' teeth in the present study can be attributed to the increase in P concentrations of teeth.

Walter et al. (2004) observed that the effect of EMF stimulation on the bone tissue formation is most likely associated with the increase in the number of cells (Walter et al., 2004). The results of the our investigation can be related with increase in the number of odontoblast cells. Stephen (1990) reported that ELF-MF can have dramatic effects on ligament cells, which may be useful for bioelectrical stimulation of growth and repair (Stephen, 1990). Glassman et al. (1986). suggested that since fibroblast behavior in bone healing can be altered electrically, it is plausible to hypothesize that fibroblast proliferation and function in soft tissue healing also would respond to an electromagnetically induced pulse (Glassman et al., 1986). These data giving to us opinion which is ELF-MF could be useful for dental treatment and on the pulp tissue repair, that could be a subject of further research.

Further studies on these topics will enhance our understandings on the cellular mechanism of ELF-MF stimulation on the osteoblast cells activities, also growth and repair.

Conclusion

There is a positive effect ELF-MF stimulation on increasing amount of mineral such as Ca, Mg, Zn and P on rat teeth. Particular attention should be directed toward long-term, ELF-MF exposure on humans. *In vivo* studies should focus on the potential for possible synergistic, genotoxic, immunological, and carcinogenic effects in combination with appropriate chemical agents, such as hormones, bisphosphonates, calcium, and Vitamin D associated with prolonged exposure to ELF-MF. It is now clear that ELF-MF can produce statistically highly significant biological and dental responses. Further research such as on histological, endochrinal epidemiological fields are required to determine whether ELF-MF could lead to stimulation on the odontoblast cells activities, cells number, growth and repair ability of the pulp tissue.

This study adds to the literature the effects of ELF-MF to the mineral acumulation in rat teeth structure, such as Ca, Mg, Zn, and P. This should be important to paediatric dentists due to Ca, Mg, Zn, and P important elements for groving of tooth which are effective from ELF-MF coming from environment to growing child. Data from this article can guide future epidemiological research on related relationship growing of teeth and ELF-MF.

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