

ORIGINAL ARTICLE

The effects of electromagnetic fields on plasma levels of corticosterone, free-T3, free-T4 malonyldialdehyde in white male rabbit with normal diet and hyperchlosterol diet

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Received: 11 December 2010**Accepted:** 14 March 2011**Abstract**

The main goal of this study was to evaluate the possible effect of whole-body magnetic field (MF) exposure on the variations of corticosterone, Free-T3, Free-T4 and malonyl dialdehyde in plasma in 48 adult white New Zealand male rabbits. Animals were divided into six groups namely, C1 (normal diet, not exposed), C2 (normal diet, sham exposed), T1 (normal diet, exposed to electromagnetic field), C3 (high-cholesterol diet, not exposed), C4 (high-cholesterol diet, sham exposed) and T2 (high-cholesterol diet, exposed to electromagnetic field). In eight separate experiments, sham exposed groups (C2 and C4), were exposed to sham stimulated (without electromagnetic stimulation) for 5 days, 2 hour/day and the rabbits of the treatment groups (T1 and T2) were treated with triangular form 10 Hz of electromagnetic field for 5 days, 2 hour/day, while the control groups (C1 and C3) had no any exposure. At the end of the exposure, after a 12-hour fasting period, blood samples were taken and level of corticosterone, Free-T3 and Free-T4 were measured by Elisa kits and level of malonyldialdehyde was measured by spectrophotometric method. The results indicated that the blood serum levels of Free-T3, Free-T4 and Corticosterone in the T1 and T2 groups were significantly increased compared to those of their own control groups ($P < 0.05$). Malonyldialdehyde levels in T2 animals showed a significant decrease compared to that of animals of C3 and C4 ($P < 0.05$). We conclude that 10 Hz pulsed electromagnetic field can alter the levels of Free-T3, Free-T4 and corticosterone in animals with both normal diet and hyperchlosterol diet and also alter the amount of malonyldialdehyde in animals with hyperchlosterol diet.

Introduction

Considerable experimental evidence exists indicating that almost all biological systems are highly sensitive to weak pulsed electromagnetic fields (PEMFs), with a wide range of biologic effects. In recent decades, the utilization of electricity has increased and diffused in both households and industries. The frequency of the current used in these systems is 0-300 Hz. This frequency interval is termed extremely low frequency magnetic field (ELF-MF), which is a nonionizing radiation having photon energy too weak to break the atomic bonds. One of the most important fields of research in this topic is the investigation of the possible biological effects of power-line frequency (50/60 Hz) magnetic filed (MF). At these frequencies, male and female reproductive functions have been proposed as possibly sensitive targets for the biological actions of MF. However,

experimental data on male reproduction are quite limited and contradictory.^{1,2}

Data on the effects of electric and magnetic fields on human health and other animals are inconsistent, probably due to differences in the exposure conditions, populations and the parameters studied.³⁻⁵ The effects of these fields on the human body are depended on amperage, frequency and exposure duration.⁶ For a better understanding of the effects of these foregoing technologies, many different researchers have studied the effects of electromagnetic fields on human health.^{6,7} In this study, the effects of 10 Hz extremely low frequency electromagnetic fields on levels of corticosterone, Free-T3, Free-T4 and malonyldialdehyde (MDA) in the blood serum of white New Zealand rabbit were investigated.

Materials and Methods

Experimental Animals. All procedures that involved animals were approved by the Veterinary Ethics Committee of the Faculty of Veterinary Medicine of Urmia University. Forty - eight rabbits were randomly divided into six groups, randomly: C1 (normal diet, not exposed), C2 (normal diet, sham exposed), T1 (normal diet, exposed to electromagnetic field), C3 (high-cholesterol diet, not exposed), C4 (high-cholesterol diet, sham exposed) and T2 (high-cholesterol diet, exposed to electromagnetic field). The animals were housed individually in metabolic cages with sawdust bedding. Rabbits were kept in a 25 °C room with a 12h light:dark cycle, had free access to feed and clean water, and were stabilized for two weeks before the start of the experiment. After the acclimatization period, C1, C2 and T1 were fed with a standard chow diet for five days and C3, C4 and T2 groups were fed with the cholesterol-enriched diet (5% cholesterol) the same way.

Electromagnetic field exposure. One low-intensity magnetic field exposure apparatus (made in German PHYWE factory) was applied to generate a pulsed electromagnetic field. In pulsed electromagnetic field, in contrast to static electromagnetic field, the poles of the field are constantly being changed. By the way the pace of this changing is depended on frequency of the field. In this apparatus, one pair of identical Helmholtz coils, each of which contained 600 turns of enameled copper wire with diameters of 0.8 mm, were mounted coaxially at a distance of one coil radius (14.5 cm) from each other to produce a highly uniform horizontal field between them. The coils were connected to an amplifier driven by a pulse generator. This was set to produce a pulsed triangular form with a frequency of 10 Hz. During 5 days with special diets (chow diet and high-cholesterol diet) subjected to each groups, the animals of groups C2 and C4 were exposed to sham field. It means animals were only put under exposure of the apparatus with turned off-electromagnetic field 1h/day and animals of groups T1 and T2 were exposed to electromagnetic field 1h/day and animals of groups C1 and the C3 were not exposed to any electromagnetic field.

Serum analyses. On the last day of the study the overnight fasted animals were anesthetized with a ketamine and xylazine (35 mg kg⁻¹ and 5 mg kg⁻¹ IM, Alfasan, The Netherlands) combination and blood samples for sera preparations were collected from the marginal ear vein of each rabbit into sterile plain tubes. Serum samples were separated from the clot by centrifugation at 3000 rpm for 15 min using a bench top centrifuge (MSE Minor, England). Serum samples were separated into sterile plain tubes and stored in the refrigerator for analyses. Malonyldialdehyde was determined using the spectrophotometric method.⁸ The level of free-T3, free-T4 and corticosterone were measured by ELISA kits (Pars Azmoon, Tehran, Iran).

Statistical analysis. The results were expressed as mean ± SEM. Differences between means were analyzed using one-way ANOVA, and then the means were compared with Duncan. P values of 0.05 or less were taken as being statistically significant. Data were analyzed using version 13 of SPSS software (SPSS Inc., Chicago, IL, USA).

Results

All plasma levels of Free-T3, Free-T4 and corticosterone were considerably affected by the administration of ELF-EMF (extremely low frequency electromagnetic field) ($P = 0.001$), in such a way that both Free-T3 and Free-T4 were significantly increased and, conversely, corticosterone was considerably decreased in groups subjected to the electromagnetic field (T1 and T2) in comparison with the sham exposed (C2, C4) and control groups (C1, C3).

Malonyldialdehyde showed a significant reduction ($P = 0.001$) in animals with the high-cholesterol diet exposed to the electromagnetic field compared to the C3 (hyper cholesterol, not exposed) and C4 (hyper cholesterol, sham exposed).

Discussion

MDA is one of the end products of lipid peroxidation and belongs to the compounds reacting with thiobarbituric acid. MDA level was used as a marker of lipid peroxidation. Aldehydes, including MDA, can diffuse to distant cellular structures where they can cause further damage, including DNA damage. Owing to these properties they are said to have cytotoxic, mutagenic and carcinogenic potentials.⁹ Lipid peroxidation the course of which is rapid and leads to oxidation of unsaturated fatty acids by reactive oxygen substances (ROS), which in turn results in the generation of lipid peroxides, is an important process that needs to be taken into consideration. Cell membranes are a frequent site of attack because they contain phospholipids, which are made up of polyunsaturated fatty acids. Thus, lipid peroxidation ends up numerous consequences including changes in the structure and fluidity of the cell membrane, disturbances in membrane transport and changes in the activity of cell membrane enzymes or damage of protein receptors located in membrane structures.¹⁰ Increased lipid peroxidation was observed for steady MF of the flux density of ca. 8 mT.¹¹ A statistically significant decrease in the level of MDA was found in group T2 (hyper cholesterol, exposed) compared to that in group C3 (hyper cholesterol, not exposed). In contrast, an experimental study indicated *in vitro* exposure of blood to an extremely low frequency electromagnetic field (ELF-EMF) considerably increased the MDA level,¹² also Zwirska-Korczala *et al.* (2005) showed that using ELF-EMF on pre-adipocytes caused a significant elevation in MDA level.¹³ It appears a hyper cholesterol diet increased lipid peroxidation in groups C3 and C4 with high level of MDA, though using the

Table 1. Concentration of corticosterone, Free-T3, Free-T4 and MDA in treatment groups T1 (normal diet, exposed), T2 (high cholesterol, exposed), sham control groups C2 (normal diet, sham-exposed), C4 (high cholesterol, sham-exposed) and control groups C1 (normal diet, not exposed), C3 (normal diet, sham-exposed).

	C1 normal diet, not exposed	C2 normal diet, sham-exposed	T1 normal diet, exposed	C3 high cholesterol, not exposed	C4 high cholesterol, sham-exposed	T2 high cholesterol, exposed
Free-T3 (pg mL ⁻¹)	1.54 ± 0.01 ^b	1.55 ± 0.01 ^c	1.61 ± 0.00 ^{bc}	1.54 ± 0.43 [*]	1.54 ± 0.04 ^a	1.85 ± 0.01 ^{a*}
Free-T4 (ng dL ⁻¹)	1.74 ± 0.01 ^b	1.74 ± 0.03 ^c	1.82 ± 0.01 ^{bc}	1.80 ± 0.01 [*]	1.77 ± 0.04 ^a	1.91 ± 0.02 ^{a*}
Corticosterone(µg 100 mL ⁻¹)	2.38 ± 0.02 ^b	2.26 ± 0.10	2.13 ± 0.03 ^b	2.40 ± 0.01 [*]	2.61 ± 0.16 ^a	2.01 ± 0.02 ^{a*}
Malonyldialdehyde (ng mL ⁻¹)	1.17 ± 0.01	1.23 ± 0.03	1.21 ± 0.03	1.41 ± 0.03 [*]	1.46 ± 0.15 ^a	1.20 ± 0.02 ^{a*}

a, b and c values are significantly different in comparison with together of ANOVA ($P < 0.05$).

electromagnetic field in rabbits of group T2, a statistically significant decrease was observed.

In rodents such as rabbits, the major glucocorticoid acting agent is corticosterone. Our results revealed a marked decreased in level of corticosterone in rabbits exposed to the electromagnetic field (groups T1 and T2). Similar findings were reported in the studies on the effect of electromagnetic field on corticosterone level in chickens and mice.¹⁴ Bastide *et al.* (2001) observed that electromagnetic fields induced a considerable drop in the ACTH level in mice¹⁵. In the present study the decreased amount of corticosterone in exposed groups (groups T1, T2) could be due to the falling level of ACTH.

Actually thyroid hormones in their free forms (Free-T3 and Free-T4), act more efficiently rather than bound forms (bound to plasma proteins) generally called T3 and T4 in current studies. Therefore concentrations of Free-T3 and Free-T4 are better criteria to assess the activity of thyroid hormones. In this study it can be seen that the groups subjected to EMF (T1 and T2) present a lower serum concentration for Free-T3 and Free-T4 when compared to the control groups (C1, C2, C3 and C4). This data is in agreement with data reported by Rajkovic *et al.* (2003) that found increased levels of circulating T4 and TSH in rats exposed to 50 Hz EMF of 20mT for 18 hours. Elevated Free-T3 and Free-T4 levels can be explained by the increasing level of TSH, the result obtained by Rajkovic *et al.* (2003).¹⁶

Rajkovic *et al.* (2006) exposed rats to electromagnetic field. The results of this study showed the stimulative effect of power-frequency EMF on thyroid gland at both the light microscope and the ultrastructural level.¹⁷ As a matter of fact, this effect of electromagnetic field will naturally result in augmentation of synthesis and secretion activity of thyroid gland and probably increased amounts of thyroid hormones. Parafollicular (PF) cells reacted to electromagnetic field exposure by their decreased activity.¹⁸ PF cells in the thyroid gland are known to produce mainly calcitonin, involved in the homeostasis of calcium, but also a number of other regulatory peptides affecting the TSH (thyroid stimulating hormone)-regulated thyrocyte activity.¹⁹ Some of regulatory peptides like somatostatin, katecalcin I (CCP-I), CCP-II, gastrin-releasing

peptide, thyroliberin and helodermin have been found in the PF cells, exclusively.¹⁹ Probably decreased activity of PF cells,¹⁸ could change thyrocyte activity and consequently concentration of thyroid hormones.

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