Effects of Different Electromagnetic Fields on Circadian Rhythms of Some Haematochemical Parameters in Rats

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Objective To investigate the effects of different electromagnetic fields on some haematochemical parameters of circadian rhythms in Sprague-Dawley rats. **Methods** The study was carried out in 18 male and 18 female rats in good health conditions exposed to 50 Hz magnetic sinusoid fields at the intensity of 1000 μT, 100 μT, and 0 μT (control group) respectively, and in 18 male and 18 female rats in good health conditions exposed to 1.8 GHz electromagnetic fields at the intensity of 50 V/m, 25 V/m and 0 V/m (control group), respectively. Following haematochemical parameters for glucose, triglycerides, and total cholesterol were measured. **Results** Different effects of electromagnetic fields on circadian rhythms of both male and female rats were observed. Different changes occurred in some haematochemical parameters for glucose, triglycerides, and total cholesterol ($P<0.05$). **Conclusion** Exposure to different electromagnetic fields is responsible for the variations of some haematochemical parameters in rats.

Key words: Extremely low frequency electromagnetic fields (50 Hz); Radiofrequency electromagnetic fields (1.8 GHz); Haematochemical parameters; Circadian rhythm; Sprague-Dawley rat

INTRODUCTION

Man-made electromagnetic fields are many thousand-time greater than natural fields arising from either the sun or the earth. Electromagnetic fields with a frequency range between 0 and 300 Hz, called extremely low frequency electromagnetic fields (ELFEMFs), are generally diffuse in modern human life. Their most important sources are generation, transmission, and consumption of electricity. Therefore, high-voltage line and electrical devices are the principal cause of their presence in domestic and working environment. ELFEMFs have an "industrial frequency" of 50 Hz in Europe (transmission lines voltage 380-400 kV) and 60 Hz in USA, Canada, and Japan (transmission line voltage 735 kV). Since low frequency electromagnetic fields are supposed to influence the ionic membrane exchange, causing the opening of cationic channels (Na^+, K^+, Ca^+), they may also produce some metabolic changes in the normal activity of organism cells, especially those in the central nervous anatomic districts that are involved in the regulation of metabolic rhythms[1]. Several studies have tried to demonstrate the effects generated by the exposure of animal organisms to ELFEMFs[2-3]. Some authors have observed no effect, but a growing number of reports have revealed that exposure to ELFEMFs can produce an amazing array of effects[5]. Also the effects of high frequency electromagnetic fields, especially radiofrequency electromagnetic fields (RF-EMF), have been previously studied. RF waves have long been used for different types of wireless broadcast, such as wireless Morse code, radio, television, cellular telephones, *etc*. The radio-wave spectrum spans the frequency range from about 0.5 MHz in the AM radio band up to about 30 000 MHz in the radar band. Over the past 50 years, RF-emitting devices have been ever commonly used in homes, offices,

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and schools. High frequency electromagnetic fields (EHF-EMF) are able to modify the temporary organization of physiological systems[6]. Many biological variables, hormonal and biochemical factors, and a diversity of physiological parameters oscillate in animals, and circadian rhythmicity in particular, represent a ubiquitous property of mammalian physiology[7-8]. Circadian rhythms have also been described in various studies in different species[9-10]. The circadian timing system facilitates adaptation of an organism to the environment by the rhythmic regulation of a variety of physiological processes. Synchronization of the endogenous circadian clock to the 24 h environmental cycle occurs due to the combined actions of internal and external stimuli. These time givers (so called Zeitgebers) include light, feeding, activity, and the hormone melatonin[11-12]. Numerous physiological functions have rhythmic cycles with various frequencies (ultradian, circadian, circatrigentan, circannual)[13]. Some parameters linked to rhythmic metabolic processes can have variations which have rhythmic cycles themselves; their biochemical evaluation indicates the stability of organs with clocked functions.

On the basis of these considerations, we have evaluated the effects of different electromagnetic fields on some haematochemical parameters in male and female rats. The aim of the present work was to determine the interactions between these fields and circadian rhythms of glucose, triglycerides and total cholesterol in Sprague-Dawley rats (*Rattus norvegicus*).

MATERIAL AND METHODS

Farms and Animals

Male and female rats from the colony of the Cesare Maltoni Cancer Research Centre (CMCRC) of the Ramazzini Foundation (RF) were used. This colony of rats has been employed for various experiments in the CMCRC/RF Laboratory for nearly 30 years. We conducted two experiments in this study according to the Italian low regulating use of animals for scientific aims (Dlgvo 116/92).

After weaning, at 4-5 weeks of age, experimental animals were identified by ear punch, housed in groups of 5 in makrolon cages (41 cm × 26 cm × 15 cm) with a shallow layer of white wood-shavings as bedding and stainless-steel wire tops for animals exposed to ELFEMFs, and plastic tops for animals exposed to RF-EMFs to avoid interferences with the electromagnetic field generated. Animals were kept in one single room for the first experiment and in three different anechoic rooms for the second experiment, at 21±3 ℃, 40%-60% relative humidity, in a light/night cycle of 12 hours under uniform illumination.

Water and feed were administered *ad libitum*. Mean daily drinking water, feed consumption and body weight were measured every 2 weeks for the first 8 weeks, and then every 4 weeks until 110 weeks of age. Body weight was measured every 8 weeks until the end of experiment. Animals' general health conditions were checked three times a day, except on Sunday (just twice). Besides, starting from 6 weeks of age, animals were clinically examined to observe their pathological changes. Clinical controls were executed weekly for the first 4 weeks and then every 2 weeks until the end of experiment. All animals were kept under observation until spontaneous death. Animals (36 males and 36 females) were selected for our experiments. Only animals in good health conditions and without mammary tumor were enrolled for the experiments.

We conducted two experiments by exposing the animals to electromagnetic fields at different wavelengths. The first experiment was carried out in 18 male and 18 female rats exposed to extremely low frequency electromagnetic fields (50 Hz); while the second experiment was carried out in 18 male and 18 female rats exposed to radiofrequency electromagnetic fields (1.8 GHz). In the first experiment, both male and female animals were divided into three groups (6 in each group), which were exposed to ELFEMFs (50 Hz) for 19 h/die continuously (C) starting from the twelfth day of embryonic life. The first group was exposed to 1000 μT, the second group to 100 μT and the third group to 0 μT (control), respectively. Also, in the second experiment, the animals, both male and female animals were divided into three groups, which were exposed to GSM-1.8 GHz for 19 h/die continuously (C) starting from the twelfth day of embryonic life. The first group was exposed to 50 V/m, the second group to 25 V/m and the third group to 0 V/m (control), respectively.

Blood was collected from animals exposed to ELFEMFs at 84 weeks of age and from those exposed to RF-EMFs at 56 weeks of age, respectively.

Sampling

Blood was taken through contusion of the retrobulbar plexus with a silyconised glass Pasteur pipette after anesthesia with ethyl ether as previously described[14]. Blood samples (0.5 mL each) were collected into 400 μL-test-tubes containing heparin and centrifuged at 3000 rpm for 10 min at 4 ℃.

Plasma was harvested and frozen at -20 ℃ for further analysis. Blood sampling was repeated from the same animals every 3 hours around the clock. A low intensity red light was used during the night to avoid retina-hypothalamic tract stimulation. Glucose was measured immediately using a portable glucometer (Accu-Check Compact-Roche). Total cholesterol and triglycerides in plasma were determined using an automated analyzer (BM Hitachi 911 Plus, Roche, Basel, Switzerland).

Statistical Analysis

Statistical elaboration of the data was based on the average values obtained at various time points (equidistant 3 hours) for each animal group divided by sex, since the intra-group variance was not significant.

One-way analysis of variance (ANOVA) was used to determine significant differences. $P<0.05$ was considered statistically significant. Statistical Newman-Keuls (SNK) test was applied for *post-hoc* comparison. The acrophase of a rhythm was determined by an iterative curve-fitting procedure based on the single cosine procedure[13]. For each variable of each animal, a cosine wave was fitted to the data points according to the function: $Yt = A + M \times \cos(q\ t + j)$, where Yt denotes each data point in the time series, M is the mean level of rhythm, A is the amplitude, q t is the trigonometric angle (in degrees) corresponding to time t, and j is the angle displacement for acrophase. The value of u was determined by iteration. The true value of u was considered to be the one that produced the smallest sum of squares of deviations between iterated cosine functions and raw data.

RESULTS

In the first experiment, ANOVA test showed a statistically significant time effect on all blood parameters in female rats ($P<0.05$). In male rats, time showed a significant effect only on triglycerides and total cholesterol ($P<0.05$). Figure 1 shows the trend of mean values obtained from female rats exposed to different electromagnetic fields (1000 μT, 100 μT, and 0 μT) for glucose. Female rats exposed to 1000 μT showed glucose acrophase in the morning (07.52 h) whereas female rats in the second group (exposed to 100 μT) and control group maintained a physiologic acrophase level at night (01.00 h and 24.08 h). Figures 2 and 3 show the pattern of mean values for plasma triglycerides and total cholesterol in female rats exposed to different electromagnetic fields (1000 μT, 100 μT, and 0 μT). Triglycerides showed a daytime acrophase at 10.08 h, 12.08 h, and 10.36 h in the first, second, and control groups, respectively, and total cholesterol showed a night-time acrophase at 01.32 h, 23.24 h, 00.44 h in female rats of the first, second, and control groups, respectively. In male rats, time showed a significant effect only on triglycerides and total cholesterol. Figures 4-6 show the pattern of mean values for plasma glucose, triglycerides, and total cholesterol in male rats exposed to different electromagnetic fields (1000 μT, 100 μT, and 0 μT). The glycaemic circadian rhythm was lost in animals exposed to 1000 μT and 100 μT. The control group maintained a glucose acrophase level at night. Triglycerides and total cholesterol showed a night-time acrophase level in groups exposed to 1000 μT at 05.56 h and 100 μT at 05.48 h, and 1000 μT at 03.48 h, and 100 μT at 04.12 h, respectively, inverted compared with the physiological rhythm.

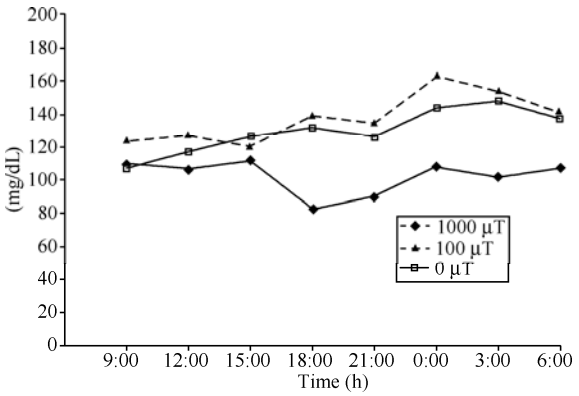

FIG. 1. Glucose mean value for female rats exposed to different electromagnetic fields (1000 μT, 100 μT, and 0 μT).

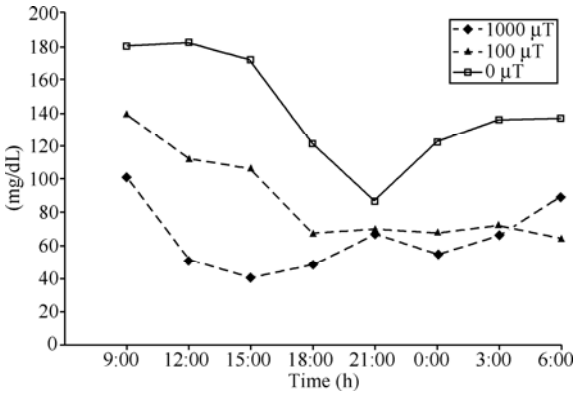

FIG. 2. Triglycerides mean value for female rats exposed to different electromagnetic fields (1000 μT, 100 μT, and 0 μT).

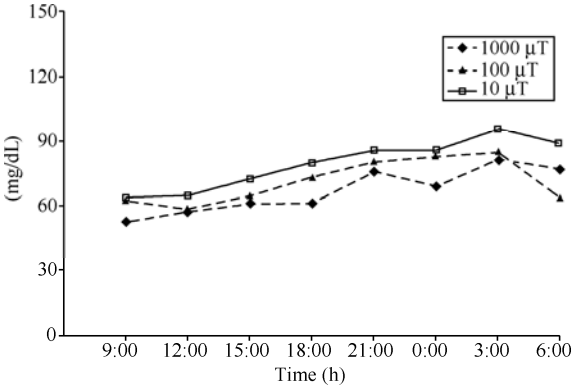

FIG. 3. Total cholesterol mean value for female rats exposed to different electromagnetic fields (1000 μT, 100 μT, and 0 μT).

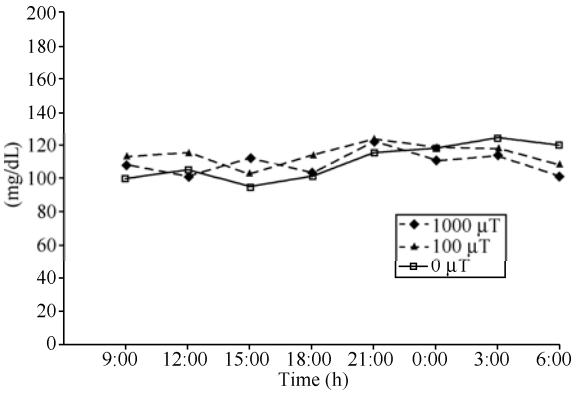

FIG. 4. Glucose mean value for male rats exposed to different electromagnetic fields (1000 μT, 100 μT, and 0 μT).

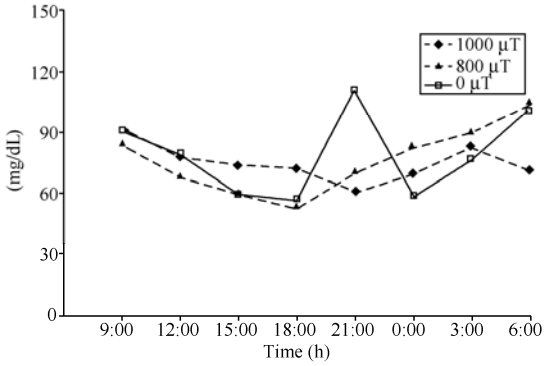

FIG. 5. Triglycerides mean value for male rats exposed to different electromagnetic fields (1000 μT, 100 μT, and 0 μT).

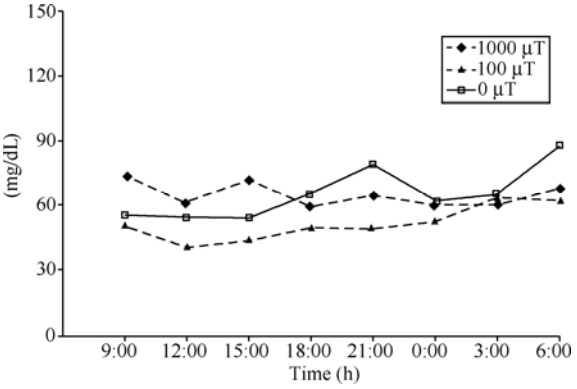

FIG. 6. Total cholesterol mean value for male rats exposed to different electromagnetic fields (1000 μT, 100 μT, and 0 μT).

In the second experiment, ANOVA test showed a statistically significant time effect on triglycerides in male rats ($P<0.05$) and glucose in female rats ($P<0.05$). Figures 7-9 and 10-12 show the pattern of mean values for blood glucose level, triglycerides, and total cholesterol in female and male rats exposed to different electromagnetic fields (50 V/m, 25 V/m, and 0 V/m), respectively. In male rats, triglycerides showed a physiologic diurnal acrophase level in all three groups at 08.12 h, 07.24 h, and at 09.00 h, respectively. Glucose showed a night-acrophase level at 24.32 h (25 V/m). ANOVA test showed a statistically significant sex effect only on total cholesterol ($P<0.05$).

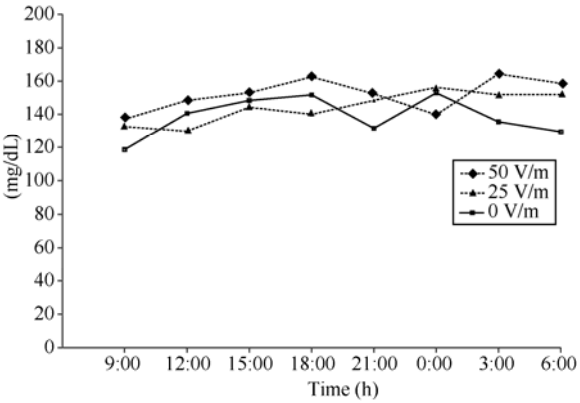

FIG. 7. Glucose mean value for female rats exposed to different electromagnetic fields (50 V/m, 25 V/m, and 0 V/m).

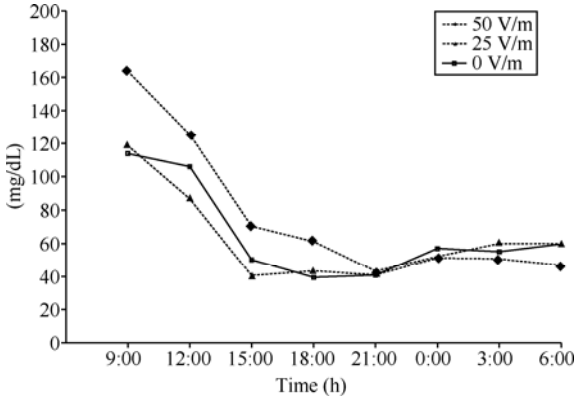

FIG. 8. Triglycerides mean value for female rats exposed to different electromagnetic fields (50 V/m, 25 V/m, and 0 V/m).

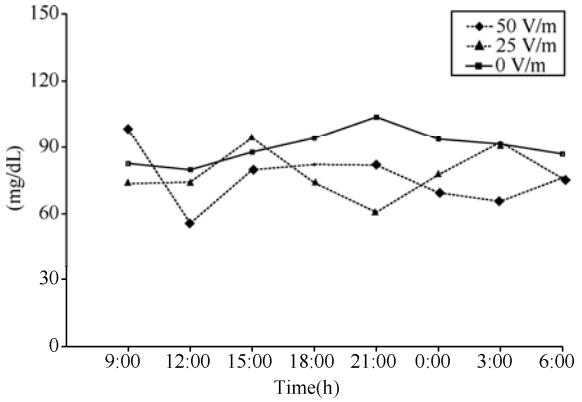

FIG. 9. Total cholesterol mean value for female rats exposed to different electromagnetic fields (50 V/m, 25 V/m, and 0 V/m).

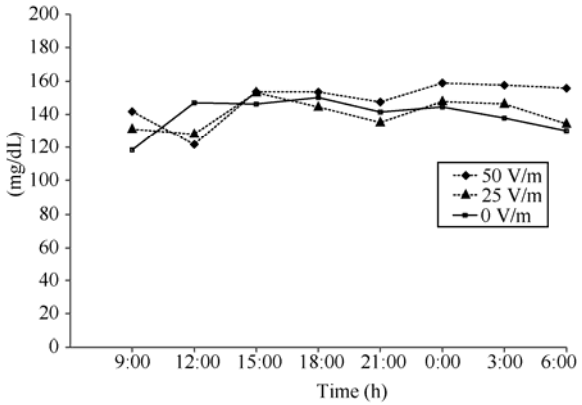

FIG. 10. Glucose mean value for male rats exposed to different electromagnetic fields (50 V/m, 25 V/m, and 0 V/m).

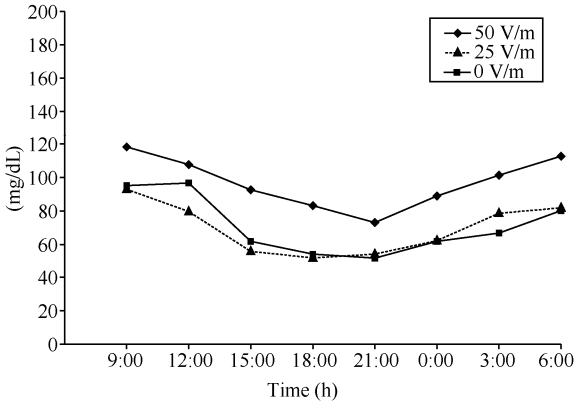

FIG. 11. Triglycerides mean value for male rats exposed to different electromagnetic fields (50 V/m, 25 V/m, and 0 V/m).

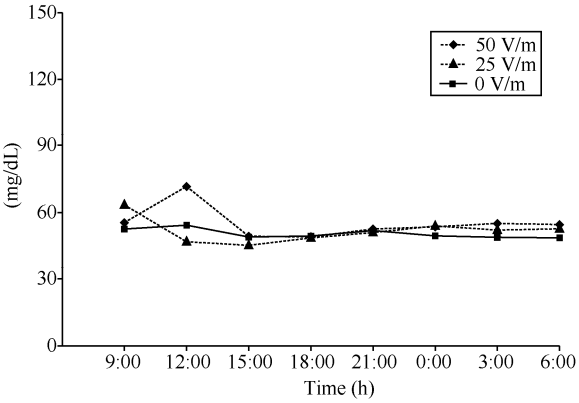

FIG. 12. Total cholesterol mean value for male rats exposed to different electromagnetic fields (50 V/m, 25 V/m, and 0 V/m).

DISCUSSION

It is hypothesized that hypothalamic nuclei can control glucose tolerance (independent of insulin) by changing the translocation of peripheral glucose transporters, which is dependent on central nervous (nervous information) and peripheral factors (membrane fluidity)[15-16]. Some studies reported that central DNA stand breaks are induced by ELFEMF both *in vivo* and *in vitro*[17]. It is therefore plausible that parameters measured with temporally defined variations (such as energetic parameters) may be influenced by incorrect feed-forwarding information

caused by ELFEMF.

The results of this study show that the circadian rhythms of parameters studied could be inverted in rats exposed to 50 Hz magnetic sinusoid fields with an intensity of 1000 μT and 100 μT, respectively. These changes are probably due to the direct current density or Eddy currents, induced in rats, which are conductive bodies. Also, in rats exposed to a higher frequency, the circadian rhythms could be inverted. The effects of fields on animal and human bodies depend on the intensity of electric and magnetic fields as well as on body size.

In male rats exposed to low and high frequency electromagnetic fields, no effect was observed on the glucose level. While in female rats, it is possible to evidence an acrophase nocturnal level independently from the frequency, which is probably due to various metabolism or metabolic levels that we found in the two sexes. For triglycerides, male rats have a night-time acrophase level if exposed to low frequency electromagnetic fields and a day-time acrophase level if exposed to high frequency electromagnetic fields, which is probably due to the different effects of electromagnetic fields on circadian rhythms of parameters studied. Our data demonstrate that there is an interaction between electromagnetic fields and circadian rhythms in Sprague-Dawley rats, which is in agreement with other investigatorstudies[2-3].

It has been demonstrated that ELFEMF may participate in the diurnal rhythm of pain threshold by acting on the system that is associated with environmental light-dark cycle[11] and might increase the risk of cancer[18]. Also, the issue of a potential association between cellular and cordless telephones and its health effects has aroused a great concern and has been discussed in several articles over the recent years[19-21].

It is well known that light exposure synchronizes to biological clock and can be used to treat sleep/wake disturbances in humans[22]. Founding this study, recovery of diurnal rhythm by electromagnetic fields could interact with the diurnal rhythm in rats, too.

In conclusion, exposure to different electromagnetic fields is responsible for the variations in circadian rhythms of parameters studied.

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