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Effects of Extremely Low Frequency Magnetic Fields on Blood Coagulation in Mice: An Initial Study

D. Vallejo,[1,*] M. Torre,[2] P. Sanz,[3] and M. L. Picazo[3]

[1]Department of Physiology, [2]Department of Analytical Chemistry, and
[3]Department of Animal Biology, University of Alcalá,
Alcalá de Henares, Madrid, Spain

ABSTRACT

OF1 mice were chronically exposed to a 50-Hz sinusoidal East–West magnetic field 15 μT (rms), in order to evaluate the blood coagulation variations related to the effect of this nonionizing radiation. Mating and pregnancy of ancestors (first generation), and birth, lactation, and development of second-generation female mice until adulthood took place in the experimental field. A global blood coagulation study of both control and exposed 14- to 15-week-old and 50- to 52-week-old, second-generation females was carried out. Plasma calcium content was determined by atomic absorption spectrophotometry. Different steps of blood coagulation were studied by thromboelastography (TEG) in whole blood (WB), platelet-rich plasma (PRP), and platelet-poor plasma (PPP). A significant decrease (approximately 34.5%) of calcium

*Correspondence: Dolores Vallejo, Departamento de Fisiología, Edificio de Ciencias, Universidad de Alcalá, E-28871 Alcalá de Henares, Madrid, Spain; Fax: 34-91-8855080; E-mail: dolores.vallejo@uah.es.

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concentration was detected with aging; however, no change was induced by medium-term or long-term exposure to extremely low frequency magnetic field (ELF-MF). Medium-term exposure could not be related to noticeable changes in global coagulation. However, a great deterioration of fibrin clot formation in mature exposed female mice was detected as a result of the long-term exposure that was strengthened by aging. These deficiencies seemed to be compensated by the discrete, although statistically not significant, decrease of platelet counts and the significant decrease of blood cells' mean corpuscular volume associated to ELF-MF exposure of 50-Hz, 15 μT. Consequently, whole blood TEG values of mature exposed female mice were similar to those from the young control group. In view of the obtained results, further studies on variations associated with ELF-MF exposure in different coagulation parameters will be necessary.

Key Words: Extremely low frequency magnetic fields; Plasma calcium; Blood coagulation; Medium-term and long-term exposures; OF1 mice.

INTRODUCTION

The hemostasis, orientated on preserving the stability and the volume of blood, is characterized by a great complexity in mammals. Cellular elements (especially red blood cells and platelets), numerous coagulation and fibrinolysis factors, as well as plasma calcium are involved in the hemostasis process.

Many aspects of the biological effects of extremely low frequency magnetic fields (ELF-MF) on humans, diverse animal and vegetal organisms, as well as on cultured or isolated cells have been thoroughly studied over the last decade (Adey, 1993; Galvanovskis et al., 1996; Goodman et al., 1995; Kheifets et al., 1997; Lisi et al., 2000; McCann et al., 1997; Mishima, 1988; Norton and Rovetti, 1988; Picazo et al., 1999; Walleczek, 1992; Zecca et al., 1998). However, reports about changes in blood coagulation and fibrinolysis by exposure to ELF-MF are very scarce and rather fragmentary (Gorczynska, 1986; Gorczynska and Wegrzynowicz, 1983; Kazimierska, 2001; Kuksinskii, 1978; Temur'iants and Mikhailov, 1985).

Thus, the purpose of this study was to find out whether medium-term and/or long-term exposures to sinusoidal magnetic fields (rms) of 50 Hz, 15 μT could be associated with modifications in plasma calcium content and/or in global blood coagulation of OF1 mice.

MATERIALS AND METHODS

Animals

OF1 mice, purchased from IFFA CREDO, were maintained at a daily mean temperature of 22 ± 2°C, under natural light–dark cycles, with feed (standard chow from PANLAB, Spain) and water ad libitum. They were randomly alloted into control (C) and exposed (E) groups and housed in standard macrolon cages with methacrylate plastic covers. The study was carried out using the second generation of OF1 female mice.

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Experimental Design

Animals were exposed to a 50-Hz sinusoidal magnetic field of 15 μT (rms), generated in a system of Helmholtz coils of 0.375 m radius and oriented to East–West direction, as previously reported (Picazo et al., 1995a). Moreover, the local geomagnetic field in the laboratory was tested as recently described by us (Vallejo et al., 2001).

Six-week-old experimental animals of both sexes (first generation) were introduced into the artificial magnetic field and, after 14 weeks of exposure, mating was induced. Pregnancy of the first generation females, birth, lactation, weaning, and development of second generation female mice (object of this study) until adulthood all took place in the experimental field. The same breeding design was applied to control groups, which nevertheless were only subjected to the magnetic field of the Earth. Thus, the only experimental difference between exposed and control females was the magnetic field applied in the case of the former (Kazimierska, 2001). In short, a total of 94 second generation mice were distributed into four groups as shown in Table 1.

Sample Collection

Bleeding of the second generation female mice was carried out. Whole blood (WB) was extracted by caudal artery puncture of anesthetized animals under ethyl–ether anesthetic, and collected into 1/10 volume of 0.38% Na_3–citrate in plastic tubes. Platelet-rich plasma (PRP) samples were obtained by centrifugation at 1500 rpm for 10 min, and platelet-poor plasma (PPP) samples by centrifugation at 2000 rpm for 15 min. Fresh samples of whole blood, platelet-rich plasma, and platelet-poor plasma were used in the thromboelastographic study. Small aliquots of platelet-poor plasma were allowed to stand at −60°C for determination of plasma calcium content.

Calcium Determination

Calcium was determined by atomic absorption spectrophotometry in a 2380 Perkin Elmer Atomic Absorption Spectrophotometer (Norwalk, CT) with an air-acetylene flame and a monoelemental hollow cathode lamp.

Calibration was accomplished with standard solutions of Ca prepared from a 1000-ppm reference solution of calcium (Carlo Erba, Milan, Italy). In view of the small volume of available platelet-poor plasma samples, it was necessary to make plasma pools from 3–5 mice. Calcium content was determined in solutions prepared by

Table 1. Distribution of mice in the four groups of study.

Group (*n*)	Treatment	Time of exposure to artificial ELF-MF (weeks)	Age (weeks old)
C1 (30)	Control	—	Young adults (14–15)
E1 (30)	Exposed	14–15	Young adults (14–15)
C2 (20)	Control	—	Mature adults (50–52)
E2 (14)	Exposed	50–52	Mature adults (50–52)

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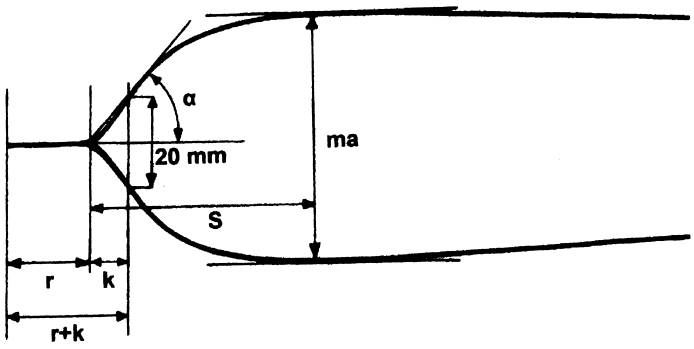

Figure 1. A representative thromboelastographic record illustrating the measured variables.

diluting aliquots (ranging from 50 to 100 μL) of each plasma pool, to 10 mL. All standard and sample solutions were prepared and duplicated in HCl 1% (v/v) and lanthanum chloride 0.4% wt/v as a suppressor of chemical interferences in the analysis.

Thromboelastography

Global clotting on recalcified WB, recalcified PRP, and recalcified PPP from each animal were studied in a Hellige Thrombelastograph Model D of two channels (Freiburg, Germany) according to the method reported by Hartert (1971).

Each thromboelastogram (TEG) was quantified by measuring the following parameters (Figure 1): r (reaction time), k (kinetic time), r + k (rate of clot formation), ma (maximal amplitude), and S (clot retractibility or syneresis), and α (growth) angle (Destaing et al., 1960; Zuckerman et al., 1981). Indices ε (viscoelasticity coefficient)

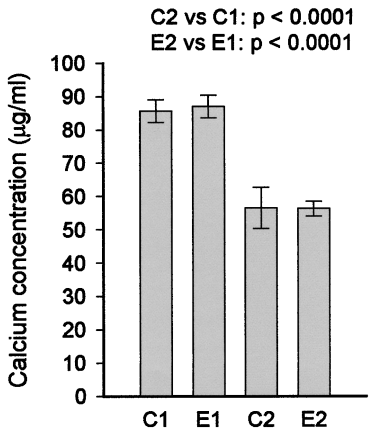

Figure 2. Variations in plasma calcium contents expressed as M ± SD. (C1, young control females; E1, young exposed females; C2, mature control females; E2, mature exposed females.)

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and TPI (thrombodynamic potential index) were calculated based on previously mentioned parameters (Raby, 1976). Parameters r and k are associated with clot formation. The r value corresponds to the period of latency that happens following the recalcification of whole blood in the cup, platelet-rich plasma or platelet-poor plasma until the beginning of clot formation. The k parameter represents the rapidity of clot development because it is the time from the end of the r to the formation of an effective clot, that is, until 20-mm separation of the branches on the TEG is accomplished. Thus, the r + k parameter represents the rate of clot formation. Since the graphic output of the TEG advances at 2 mm/min, r, k, and r + k are measured in millimeters. The α angle represents the rate of increase in viscoelasticity; it is measured in degrees of arc from the point of branch divergence to the maximum line tangential to the curve. The ma value, defined as the greatest transverse amplitude of the graph (in mm), represents the final clot stiffness. This parameter is a direct function of the maximum dynamic properties of fibrin and platelets. The S parameter, which corresponds to the distance (in mm) between the amplitude of 1 mm and the maximal amplitude in the curve, informs us about of the clot retractibility, and is proportional to the fibrinogen concentration.

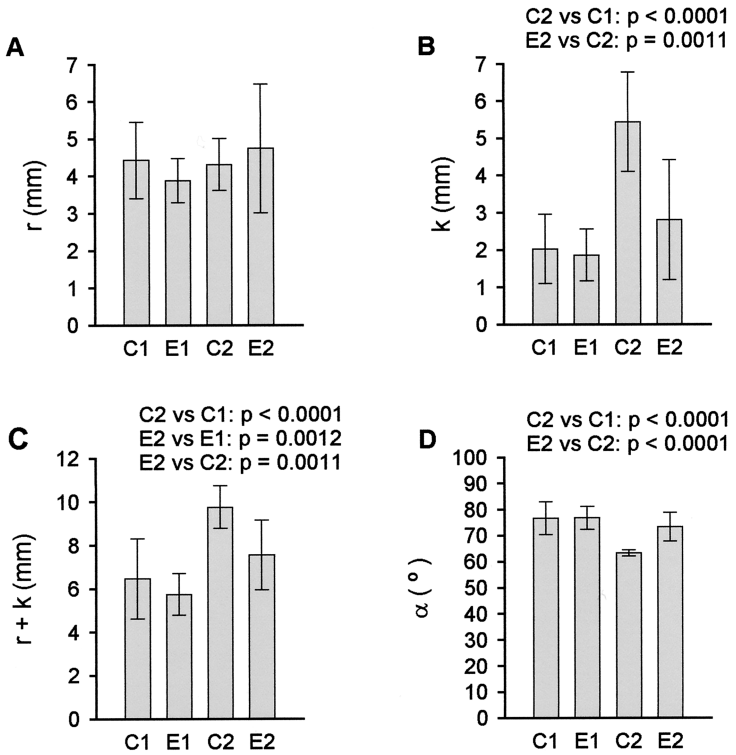

Figure 3. Whole blood TEG tracings expressed as M ± SD. (A) Reaction time, r; (B) Kinetic time, k; (C) Rate of clot formation, r + k; (D) Rate of viscoelasticity increase, α. (C1, young control females; E1, young exposed females; C2, mature control females; E2, mature exposed females.)

Finally, the viscoelasticity coefficient (ε), calculated by the expression

$$\varepsilon = \frac{100\ ma}{100 - ma}$$

shows proportionality with ma and is necessary for calculating the TPI value. The thrombodynamic potential index was established by Raby (1976) and relates the structural characteristics with the time of clot formation. Although the physiological meaning of this index is debatable, its fidelity and practical value have been repeatedly demonstrated. TPI is expressed as:

$$TPI = \frac{\varepsilon}{k}$$

Statistical Analysis

Results were processed by using GraphPad InStat (GraphPad Software, San Diego, CA). All results were expressed as mean (M) ± standard deviation (SD). The statistical

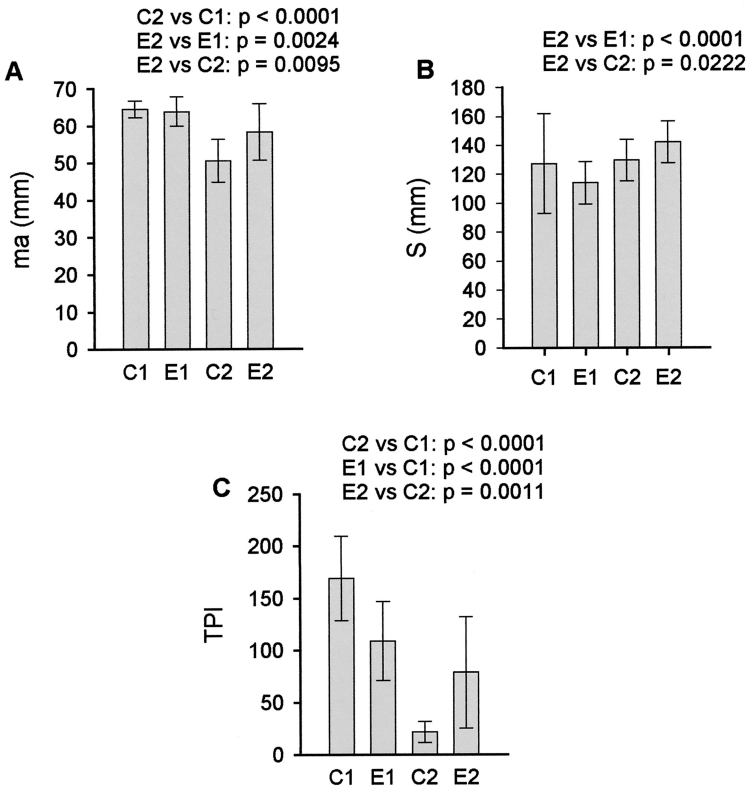

Figure 4. Whole blood TEG tracings expressed as M ± SD. (A) Final clot stiffness, ma; (B) Clot retractibility, S; (C) Thrombodynamic potential index, TPI. (C1, young control females; E1, young exposed females; C2, mature control females; E2, mature exposed females.)

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evaluation of differences were performed applying the Kruskal–Wallis and the two-tailed Mann–Whitney *U* tests (with a 95% confidence level in both tests).

RESULTS

Calcium Contents in Peripheral Blood

Values of Ca concentration in peripheral blood samples from the four different groups of studied female mice are shown in Figure 2. Comparison among the four groups as a whole by the Kruskal–Wallis test evidenced extremely significant differences ($p < 0.0001$).

At first, a sharp decrease (approximately 34.5%) of the calcium concentration in both control and exposed mature adults (50–52 weeks old) with respect to control and exposed young adults (14–15 weeks old) was observed. Thus, an extremely significant difference between Ca contents from C2 vs. C1 ($p < 0.0001$), and E2 vs. E1 ($p < 0.0001$) was detected.

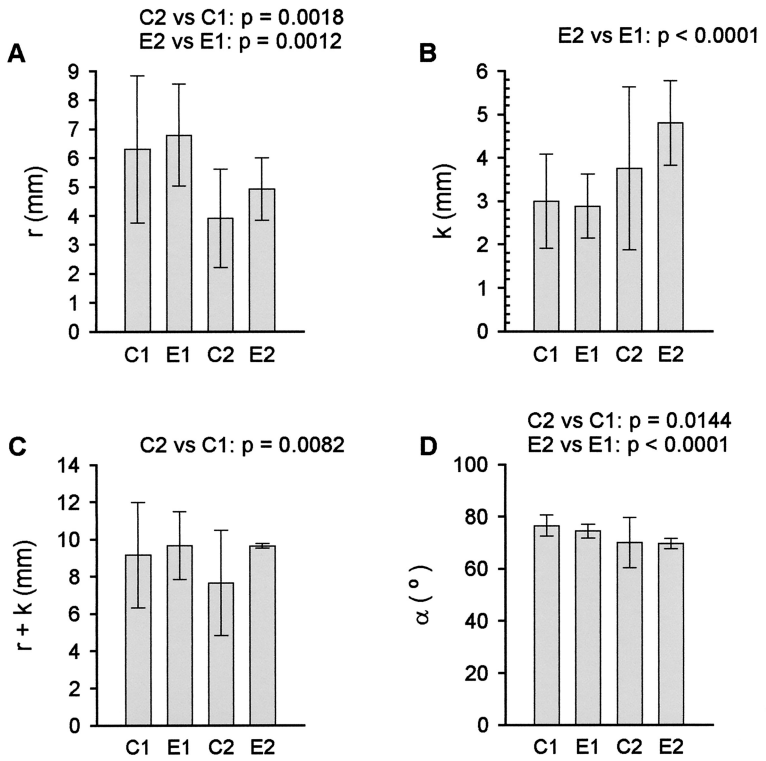

Figure 5. Platelet-rich plasma TEG tracings expressed as M ± SD. (A) Reaction time, r; (B) Kinetic time, k; (C) Rate of clot formation, r + k; (D) Rate of viscoelasticity increase, α. (C1, young control females; E1, young exposed females; C2, mature control females; E2, mature exposed females.)

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Upon comparison of calcium concentration from the control and exposed young adults female mice (14–15 weeks old), no statistically significant differences between both groups were observed, although a very small increase in calcium in the exposed animals was seen (mean of group E1: 87.02 μg/mL against 85.66 μg/mL of group C1; p value = 0.108: not significantally different).

Unlike the behavior observed in young female mice, the peripheral blood Ca content in animals exposed during 50–52 weeks to a sinusoidal magnetic field of 50 Hz and 15 μT (rms) was slightly lower than that corresponding to the control group (mean of group E2: 56.26 μg/mL against 56.53 μg/mL of group C2; p value = 0.057: not quite significantally different).

TEG Variables

The measurements on the WB tracings of the four different groups as a whole of female mice showed statistically significant differences when applying Kruskal–Wallis test in k ($p < 0.0001$), r + k ($p < 0.0001$), α ($p < 0.0001$), ma ($p < 0.0001$), and S ($p = 0.0004$) parameters, and in TPI ($p < 0.0001$) index.

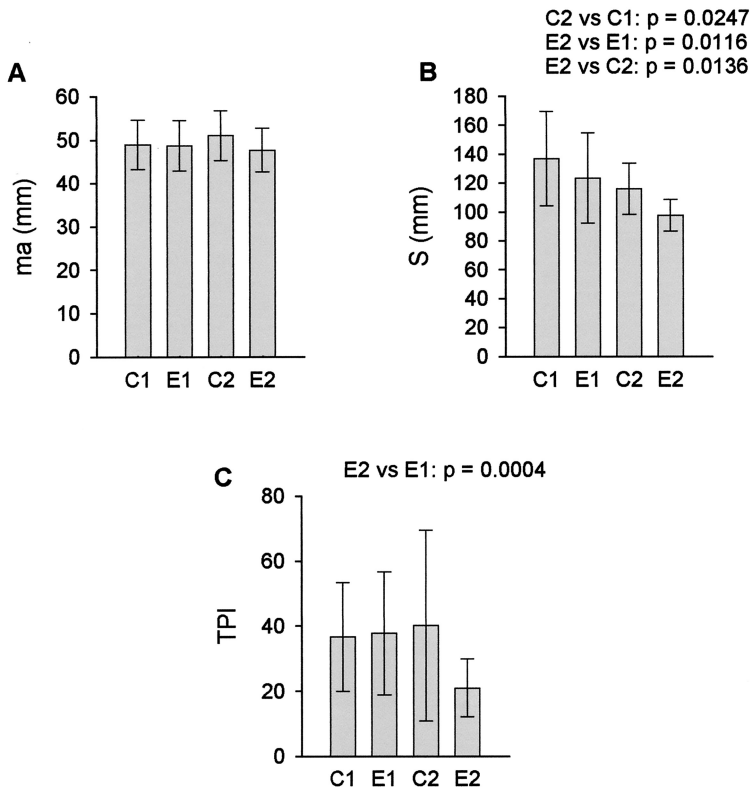

Figure 6. Platelet-rich plasma TEG tracings expressed as M ± SD. (A) Final clot stiffness, ma; (B) Clot retractibility, S; (C) Thrombodynamic potential index, TPI. (C1, young control females; E1, young exposed females; C2, mature control females; E2, mature exposed females.)

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As far as global blood clotting was concerned, the most important variations were associated with aging. Thus, Figures 3 and 4 show that when median values of the parameters evaluated in mature adults were compared with those corresponding to young adults, statistically significant enlargements of k, r + k, and S, accompanied by statistically significant decreases of α, ma, and TPI, were evident in both control and exposed animals.

Different variations in WB clotting were also associated with the different duration of exposure to the sinusoidal ELF-MF of 50 Hz and 15 μT (Figures 3 and 4). Thus, the medium-term exposure was only associated with extremely significant shortening of TPI index. However, the long-term exposure was associated with a very significant reduction in k and r + k parameters, extremely significant increase of α, significant increase of the ma parameter and the TPI index, and significant enlargement of S, relative to the corresponding control group (E2 vs. C2).

Although the study of PRP TEG tracings (Figures 5 and 6) showed smaller variations than WB-TEG, statistically significant differences in r ($p < 0.0001$), k ($p = 0.0004$), r + k ($p = 0.0091$), α ($p < 0.0001$), and S ($p = 0.0004$) parameters, and in TPI ($p = 0.0148$) index also were found by the Kruskal–Wallis test.

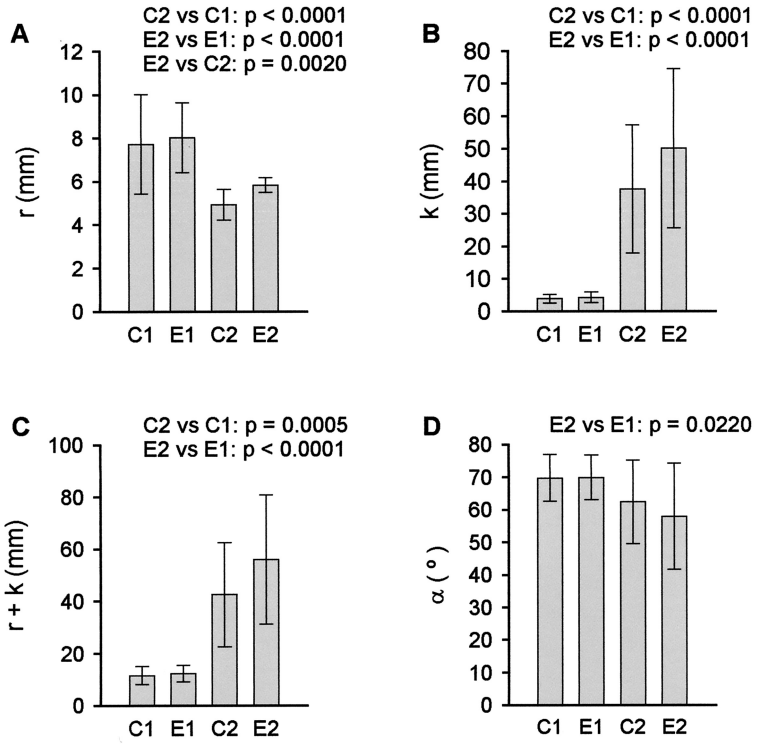

Figure 7. Platelet-poor plasma TEG tracings expressed as M ± SD. (A) Reaction time, r; (B) Kinetic time, k; (C) Rate of clot formation, r + k; (D) Rate of viscoelasticity increase, α. (C1, young control females, E1, young exposed females, C2, mature control females; E2, mature exposed females.)

As a consequence of aging, only the following changes were evident: 1) statistically very significant or significant decreases in r, r + k, α, and S, after comparison of control groups (C2 vs. C1); 2) statistically very significant or significant shortenings in r and S, very extremely significant decreases in α and TPI, and very extremely significant increases in k, after comparison of exposed groups (E2 vs. E1).

The exposure to sinusoidal ELF-MF of 50 Hz and 15 μT was only associated with a significant decrease in S (E2 vs. C2: $p = 0.0136$), once PRP tracings were analyzed (Figures 5 and 6). However, a general statistically not significant enlargement in r, k, and r + k parameters, as well as general not statistically significant decreases in α, ma, and TPI were in evidence when each exposed group (E1 or E2) was compared with the corresponding control group (C1 or C2, respectively).

Likewise, Kruskal–Wallis' test evidenced statistically significant differences among measurements on the PPP TEG tracings from the four different groups as a whole of females (Figures 7 and 8): r ($p < 0.0001$), k ($p < 0.0001$), r + k ($p < 0.0001$), α ($p = 0.0332$), ma ($p < 0.0001$), S ($p < 0.0001$), and TPI ($p < 0.0001$).

The TEG tracings again showed pronounced variations due to aging. This fact was evidenced by a significant decrease of r, α, ma, S, and TPI, and significant enlargement of k and r + k parameters.

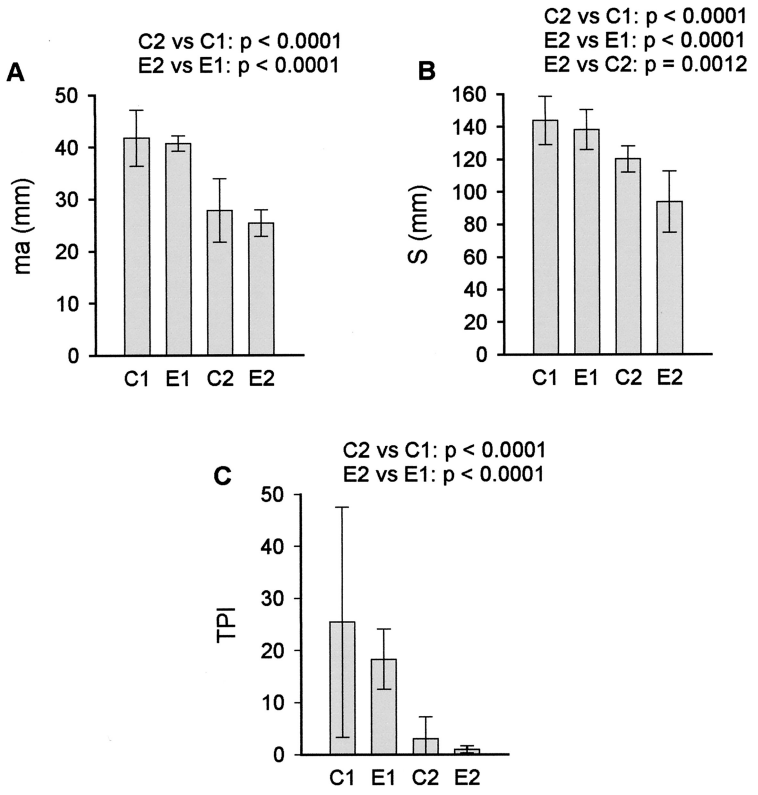

Figure 8. Platelet-poor plasma TEG tracings expressed as M ± SD. (A) Final clot stiffness, ma; (B) Clot retractibility, S; (C) Thrombodynamic potential index, TPI. (C1, young control females; E1, young exposed females; C2, mature control females; E2, mature exposed females.)

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After exposure to a sinusoidal ELF-MF of 50 Hz and 15 μT, the PPP-TEG tracings showed the same tendencies of variation as the platelet-rich plasma tracings: a general, although not statistically significant, enlargement of r, k, and r + k as well as a general, although not statistically significant, decrease of ma and TPI (Figures 7 and 8). The most pronounced variations were associated with long-term exposure, especially the r enlargement and the S decrease, which were very significant.

DISCUSSION

As is well known, the ion Ca^{2+} plays an important role in a wide variety of functions in living organisms, such as development of hemostasis (Detwiler and Feinman, 1973; Ratnoff and Potts, 1954; Rink et al., 1981; Stenflo and Ganrot, 1973), stimulation of muscle cells (Kargacin, 1994), and bone formation (Reinbold and Pollack, 1997). In this study, it can be seen that exposure to magnetic fields did not induce significant changes of plasma calcium levels either in young adult or in mature adult female mice when compared to their controls, in accordance with reports by Gorczynska (1987), Zdrojewicz et al. (1996), Bonhomme-Faivre et al. (1998), and Burchard et al. (1999). Under the same experimental conditions of this study and with the same animals of group E1 (young adults females), our group observed that the applied magnetic field did not produce significant changes in bone mass or in bone density (Vera et al., 1998), whereas a very significant decrease of calcium content in skeletal muscle from exposed females was detected (Picazo et al., 1995b). In view of these results, the significant decrease of muscle calcium content could not be associated with any grade of hypercalcemia.

On the other hand, in the present study, the concentrations of plasma calcium underwent important decreases (approximately 34.5%) with aging in both control and exposed groups, in contrast to the findings in female mice 2 and 10 months old reported by Morita et al. (1994). In spite of this pronounced plasma calcium level decrease, aged female mice did not showed signs of severe coagulopathy.

Thromboelastography is a swift and easily measurable method for monitoring clotting that allows a continuous visual evaluation of overall coagulation activity, including (if there were) clot retraction and fibrinolysis. TEG graphic record shows all phases of the coagulation process and the various phases of coagulation are geometrically well defined (Lee et al., 1979). This method has informed us about the most important variations of the global coagulation process, although further studies on changes of different parameters in both coagulation and fibrinolysis would be necessary.

In the present study, the medium-term exposure to magnetic field of 50 Hz, 15 μT could not be related to noticeable changes in global coagulation, because only TPI of whole blood had shown a very significant difference after comparison between TEG parameters of control and exposed young adult mice (groups C1 and E1, respectively).

Since platelet-poor plasma had neither red blood cells nor platelets, the PPP-TEG tracings reflected the efficacy of fibrinogen and the other soluble plasma coagulation factors.

The values of PPP parameters obtained in this study revealed a deterioration of fibrin clot formation with aging. This fact was evidenced by increases in kinetic time, and clot formation rate, as well as decreases of rate of viscoelasticity increase, final

clot stiffness, clot retractibility, and thrombodynamic potential index in mature control females (group C2) when compared to young control females (group C1).

In aged exposed female mice (group E2), the modifications were more pronounced than in group C2. Thus, platelet-poor plasma from group E2 showed a clear hypocoagulational state that must be justified by two added effects: aging and a long-term exposure to magnetic field of 50 Hz, μT. In agreement with this results, several authors (Gorczynska, 1988; Gorczynska and Wegrzynowicz, 1983; Kuksinskii, 1978; Temur'iants and Mikhailov, 1985) have reported that animal exposure to magnetic fields led to increases in prothrombin and thromboplastin times, decreased fibrinogen, and inactivation of plasma coagulation factors, all of which reflects a hypocoagulational state.

Platelets, fibrinogen, and the other soluble plasma coagulation factors are contained in the platelet-rich plasma. Thus, the PRP tracings pointed out the importance of fibrinogen–platelet interactions.

There seemed to be produced a greater coagulation efficacy with aging, as judged by a significant decrease of reaction time and clot formation rate, besides discrete not significant increases of maximal amplitude, and thrombodynamic potential index in mature control female mice (group C2) when compared to young control female mice (group C1). That slight hypercoagulational tendency, observed in PRP tracings in contrast to the hypocoagulational state of PPP tracings, both detected in mature control female mice, could be explained by the elevated platelet counts registered in the same animals as previously described (Kazimierska, 2001) (1,129,353 ± 186,577 platelets/mm^3 in group C2 vs. 985,480 ± 238,449 platelets/mm^3 in group C1; Mann–Whitney U test: $p = 0.0258$). However, a simultaneous impoverishment of elastic clot properties with aging was deduced from significant decreases of rate of viscoelasticity increase and clot retractibility in mature control females (group C2) with respect to young control ones (group C1). The values of these two last parameters were very similar in both PRP and PPP tracings from group C2 because both parameters are principally related to the quality of fibrin fibers and are little affected by the increased platelet concentration.

Female mice that underwent long-term exposure to a magnetic field of 50 Hz, μT (group E2) did not maintain the slight hypercoagulability exhibited by their respective control females (group C2). This fact could be explained by the discrete decrease of platelet count associated with ELF-MF exposure (1,069,000 ± 321,677 platelets/mm^3 in group E2 vs. 1,129,353 ± 186,577 platelets/mm^3 in group C2; Mann–Whitney U test: not significant difference), reported previously by us (Vallejo et al., 2001), in agreement with those observed by Gorczynska and Wegrzynowicz (1983) after magnetic field exposure of guinea pigs. The reduced values corresponding to rate of viscoelasticity increase and clot retractibility were maintained at the same level as in PPP tracings.

The WB-TEG allowed us to know how all blood components (cellular elements and soluble factors) interact with inner blood vessels (the internal surface of TEG-vessel is similar to endothelial surface). Thus, WB tracings reflect the interactions among red blood cells, platelets, and fibrinogen to clot formation.

The WB records showed that clear hypocoagulative damage occurred with aging. In fact, significant increases of the kinetic time and clot formation rate, as well as significant decreases of rate of viscoelasticity increase, final clot stiffness, and

thrombodynamic potential index were observed in group C2 when compared to group C1. As was recently published (Vallejo et al., 2001), although the C2 females from this study only suffered a very slight and no significant decrease of red blood cells in relation to C1 (9,762,500 ± 2,321,784 RBC/mm^3 in group C2 vs. 9,970,357 ± 985,136 RBC/mm^3 in group C1; Mann–Whitney *U* test: no significant difference), they nevertheless exhibited significant decrease of mean corpuscular volume (49.4 ± 3.9 femtoliters in group C2 vs. 53.4 ± 6.7 femtoliters in group C1; Mann–Whitney *U* test: $p = 0.0371$). This hypocoagulability could probably be explained in function of diminished size of red blood cells that caused slower and more minor blood clot development in group C2 than in group C1.

However, the long-term exposure seemed to restore in great measure the thromboelastographic values of whole blood from young control mice. Both RBC count (9,846,000 ± 132,421 RBC/mm^3) and mean corpuscular volume (51.2 ± 5.9 femtoliters) of mature exposed females (group E2) remained in a intermediate position between values corresponding to young and mature control mice (C1 and C2, respectively). This fact could explain the improvement of TEG values, at least partially, although other changes in RBC that justify their increase in efficacy of clot formation could not be excluded.

In conclusion, long-term exposure to ELF-MF at 50 Hz and μT induced a loss of efficacy of fibrinogen and soluble coagulation factors to constitute the fibrin clot, that furthermore was strengthened by aging. The increase of fibrinogen–platelet interaction exhibited by mature control female mice was counteracted by long-term exposure. It was probably due to ELF-MF, which made possible only moderate increase of platelet count in mature exposed females. Nonetheless, the whole blood-TEG of mature exposed females reached almost the same values as those corresponding to young control females, in contrast to the clear hypocoagulative damage exhibited by respective control mice. This finding could only be explained by modifications induced in red blood cells as a consequence of ELF-MF long-term exposure, such as the preservation of RBC mean corpuscular volume in mature exposed females close to that corresponding to young control females.

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