STUDIA UBB CHEMIA, LXI, 1, 2016 (p. 185-197)
(RECOMMENDED CITATION)

Dedicated to Professor Mircea Diudea on the Occasion of His 65th Anniversary

CHANGES IN PHYSICO-CHEMICAL CHARACTERISTICS OF HUMAN LOW DENSITY LIPOPROTEIN NANO-PARTICLES BY ELECTROMAGNETIC FIELD EXPOSURE

SOHEILA ABDI[a], DAVOUD DORRANIAN[b], GHOLAM ALI NADERI[c,*], AMIRNADER EMAMI RAZAVI[d]

ABSTRACT. Studies on the effects of electromagnetic field (EMF) exposure on cardiovascular function have provided some evidence of a possible action. Low density lipoprotein (LDL) modifications appear as an early step in the promotion and progression of atherosclerosis, the most causes of death in cardiovascular disease (CVD) patients. This study aimed to evaluate the effects of extremely low frequency (ELF) of electromagnetic fields on LDL physico-chemical modifications. LDL was separated by sequential ultracentrifugation and its susceptibility to oxidation was evaluated by continuous monitoring of conjugated dienes formation, using a spectrophotometer. LDL size and zeta potential is determined by zetasizer instrument. The results indicated that moderate ELF-EMFs of 2-4 mT can induce the susceptibility of LDL to oxidation and aggregation. Weak ELF-EMFs of 0.125-0.5 mT caused a decrease in LDL zeta potential in a time and dose dependent manner while in moderate ELF-EMFs of 1-4 mT LDL zeta potential was started to increase after an initial decrease at the first hour of exposure. LDL oxidation and aggregation are two important modifications of LDL, involved in the promotion and progression of atherosclerosis. On the other hand, alteration of the LDL surface charge can interfere with the metabolism of LDL and its interaction with other molecules. Therefore with regard to the atherogenic effects of ELF-EMFs on LDL, it can be considered as a risk factor in atherosclerosis.

Keywords: *Electromagnetic field, LDL oxidation, LDL aggregation, LDL zeta potential, LDL mean size*

[a] *Department of Physics, Safadasht Branch, Islamic Azad University, Safadasht, Tehran, Iran*
[b] *Plasma Physics Research Center, Sciences and Research Branch, Islamic Azad University, Tehran, Iran*
[c] *Cardiovascular Research Center, Isfahan Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran*
[d] *Iran National Tumor Bank, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran*
** Corresponding Author: naderi.crc@gmail.com*

INTRODUCTION

Recently, investigation on the biological effects and health implications of electric and magnetic fields becomes a subject of a public concern and private debate. However the effects of exposure to extremely low frequency (ELF) electromagnetic fields (EMFs) on human cardiovascular parameters remain undetermined. Studies indicate that the ELF-EMFs causes decrease in heart rate (HR) and increase in heart rate variability (HRV) in human subjects [1-3]. A cross sectional study on electricians revealed that long-time low-level exposure to ELF fields caused significantly frequent electrocardiogram (ECG) changes including arrhythmias, conduction disturbances, and myocardial ischemia changes [4]. However other investigations didn't find any significant changes in blood pressure and HR or cardiac arrhythmias following ELF magnetic field exposure [5-7]. Investigations indicate that local exposure of rabbits arterial sinocarotid baroreceptors to artificial static magnetic and natural geomagnetic field (GMF) will be effective in cardiovascular conditions with arterial hypertension and decreased baroreflex sensitivity [8].

Serum lipid and lipoprotein concentrations are associated with the risk of cardiovascular disease(CVD) [9-10]. A study on animals indicated that exposing to 15 Hz low-intensity pulsed magnetic fields for 8 weeks led to a significant decrease in serum triglycerides and cholesterol and increase in high density lipoprotein (HDL) levels in rabbits fed with high cholesterol diet [11]. Human studies indicated that serum lipids and lipoproteins could change under the exposure of EMFs and changes are depend on the time of exposure [12]. Among lipoproteins, low density lipoproteins (LDL) have been mostly investigated in terms of their role in atherosclerosis [13]. This is an inflammatory disease and the most cause of death in CVD patients. LDL readily enters the artery wall by crossing the endothelial membrane. Once on the arterial wall, if LDL accumulates, it is subject to a variety of modifications. LDL modification is an early step and very important event in the promotion of atherosclerosis [14]. The best known of these modifications is oxidation, both of the lipids and of the APO B [15]. LDL is also subject to other physical and chemical modifications such as aggregation [16] and glycation [17].

Investigations indicated that LDL characteristics such as particle size and its surface charge were related to the severity of CVD [18], and other related diseases like obesity metabolic syndrome and Diabetes [19].

There are a few studies that investigated the effects of ELF magnetic field on the generation and promotion of atherosclerosis. In previous studies we investigated the effects of weak and moderate static magnetic fields on the human LDL characteristics [20].

The aim of this study is to evaluate the effects of ELF-EMFs on LDL susceptibility to oxidation and aggregation. Because of the importance of LDL size and surface charge on their metabolism and interactions with other molecules such as apo lipoproteins, receptors, and enzymes, the effect of different intensities of ELF electromagnetic fields on the LDL particles mean size and surface charge was also investigated.

RESULTS

Figure 1 shows the oxidation curves of LDL samples under the exposure of different flux densities of EMF in comparison with control. *In vitro* oxidation of LDL is mediated by copper solution (70 µg/ml LDL protein in iso-osmolar PBS with pH of 7.4, contain 10 µM CuSO4) at 37 ˚C during a time frame of 180 min.

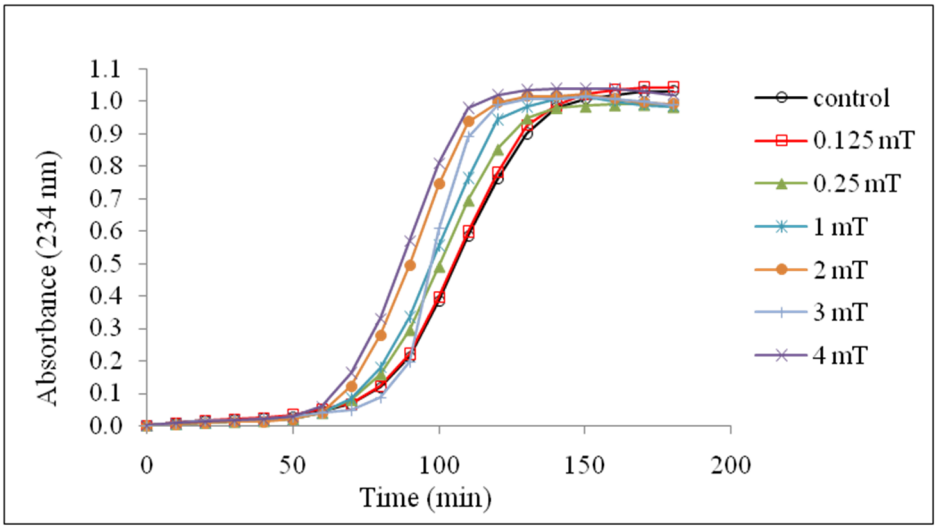

Figure 1. Effect of different electromagnetic flux densities on kinetics of copper-mediated oxidation of LDL, in comparison with the control.

Table 1 lists the parameters of LDL oxidation under the exposure at electromagnetic flux densities of 0.125, 0.25, 0.5, 1, 2, 3 and 4 mT in comparison with the control. LDL oxidation parameters were extracted from the oxidation curves and include the lag time (t_{lag}), time required for reaching half maximum dienes ($t_{1/2}$), maximum velocity (v_{max}) and maximum conjugated diene formation ($diene_{max}$). The lag time and $t_{1/2}$ of LDL oxidation decreased parallel to the increase in electromagnetic flux density (except for magnetic flux density of 3 mT)

and this decrease is significant in 2 and 4 mT ($p < 0.01$). The propagation rate or v_{max} was significantly increased to 745.8 ± 17.1, 910.7 ± 7.6 and 734 ± 8.3 nmol/min under the exposure at magnetic flux densities of 2, 3 and 4 mT, respectively; data were compared to 622.6 ± 11.2 nmol/min in control samples (p value <0.01). The production of conjugated dienes didn't show any changes under the exposure of different doses of EMF.

Table 1. Changes in LDL oxidation parameters under the exposure at different ranges of electromagnetic flux densities in comparison with the control.

Electromagnetic flux density (mT)	LDL oxidation parameters			
	Lag time (min)	$T_{1/2}$ (min)	Propagation rate (nmol/min)	Maximal diene (μmol)
Control	81.2 ± 2.6	112.3 ± 3.1	622.6 ± 11.2	35.0 ± 1.1
0.125	79.9 ± 2.1	11.4 ± 3.3	629.2 ± 12.1	35.5 ± 0.9
0.25	77.2 ± 2.9	100.4 ± 5.8	630.5 ± 14.1	33.6 ± 0.7
0.5	76.8 ± 1.9	101.5 ± 2.6	664.4 ± 19.3	34.7 ± 0.3
1	76.2 ± 2.0	98.1 ± 7.3	687.4 ± 10.8	34.4 ± 1.7
2	69.1 ± 2.6 **	91.7 ± 5.4**	745.8 ± 17.1 **	34.7 ± 0.9
3	86.2 ± 4.1	98.2 ± 8.1	910.7 ± 7.6 **	34.4 ± 0.5
4	66.4 ± 2.4 **	87.3 ± 5.1 **	734.2 ± 8.3 **	35.6 ± 0.45

Test samples were pre-incubated at 37°C under the exposure at electromagnetic flux densities of 0.125, 0.25, 0.5, 1, 2, 3 and 4 mT for one hour and control samples were pre-incubated at the same condition without magnetic field exposing. Oxidation of LDL (70μg/ml LDL protein in iso-osmolar PBS with a pH of 7.4) was initiated by addition of 10μM CuSO4. Continuous monitoring of the formation of conjugated dienes at 234 nm was recorded at intervals of 10 min in 1 cm quartz cuvettes at 37 °C for 3 h under the exposure of applied static magnetic field. The oxidation parameters were calculated from LDL oxidation curves. Data are represented as mean ± SD obtained from 5 separate oxidation assays. *= p value < 0.05 and **= p value < 0.01.

Table 2 indicates the effect of different electromagnetic flux densities at different times of exposure on the LDL tendency to aggregation in comparison with controls. Data are represented as absorbance at 680 nm. Electromagnetic flux densities of 0.125 and 0.25 mg had no effect on the tendency of LDL particles to aggregation during the time of the experiment. The tendency of LDL to aggregation significantly increased after 3 h of incubation under the exposure at 0.5 mT ($p <0.05$) and 2 h of incubation under the exposure of 1 mg ($p <0.05$) when compared to that in control samples. Electromagnetic flux densities of 2, 3 and 4 mT caused a significant increase in the tendency of LDL particles to aggregation after the first h of exposure ($p <0.01$), and this increase is time dependent.

Table 2. The effect of electromagnetic flux densities of 0.125, 0.25, 0.5, 1, 2, 3 and 4 mT at different times of exposure (60, 120 and 180 min) on the LDL tendency to aggregation in comparison with controls.

Electromagnetic flux density (mT)	Time of exposure (min)			
	0	60	120	180
0.125	0.34 ± 0.03	0.33 ± 0.02	0.34 ± 0.03	0.35 ± 0.04
0.25	0.32 ± 0.02	0.33 ± 0.03	0.34 ± 0.02	0.35 ± 0.03
0.5	0.32 ± 0.02	0.35 ± 0.03	0.36 ± 0.04	0.38 ± 0.03 *
1	0.34 ± 0.03	0.36 ± 0.04	0.38 ± 0.04 *	0.41 ± 0.03 **
2	0.34 ± 0.03	0.40 ± 0.04 **	0.42 ± 0.02 **	0.45 ± 0.03 **
3	0.33± 0.02	0.45± 0.03 **	0.49 ± 0.03 **	0.53 ± 0.02 **
4	0.34 ± 0.03	0.48 ± 0.03 **	0.55 ± 0.04 **	0.57 ± 0.03 **

Data are expressed as mean ± SD obtained from 5 separate determinations and the results are represented as Absorbance in 680 nm. *= p value <0.05 and **= p value <0.01.

The LDL mean particle size isolated from pooled serum was 20.45 nm. No significant differences were found in the size of LDL under the exposure of different doses of electromagnetic field after the specified time in this research. The effect of different electromagnetic flux densities at different times of exposure on the LDL zeta potential is shown in Table 3. The results indicate that the zeta potential of LDL particles reduced under the exposure of 0.125 mT EMF and this reduction was significant at 180 min ($p < 0.05$).

Table 3. The effect of electromagnetic flux densities of 0.125, 0.25, 0.5, 1, 2, 3 and 4 mT at different times of exposure (60, 120 and 180 min) on the LDL zeta potential in comparison with controls.

Electromagnetic flux density (mT)	Time of exposure (min)			
	0	60	120	180
0.125	-22.4 ± 0.2	-22.2 ± 0.2	-21.2 ± 0.3	-21.3 ± 0.2 *
0.25	-22.4 ± 0.3	-21.3 ± 0.6 *	-20.5 ± 0.5 **	-19.2 ± 0.7 **
0.5	-22.9 ± 0.3	-20.2 ± 0.3 **	-19.9 ± 0.5 **	-19.5 ± 0.4 **
1	-23.0 ± 0.4	-19.4 ± 0.4 **	-19.1 ± 0.5 **	-20.8 ± 0.4 **
2	-22.9 ± 0.3	-18.7 ± 0.5 **	-19.3 ± 0.4 **	-21.4 ± 0.7
3	-22.8 ± 0.4	-18.2 ± 0.3 **	-20.4 ± 0.5 **	-22.3 ± 0.4
4	-23.0 ± 0.3	-19.2 ± 0.5 **	-21.8 ± 0.4 *	-23.1 ± 0.5

LDL zeta potentials represented as mv and the values are mean ± SD obtained from 5 separate determinations. *= p value <0.05 and **= p value <0.01.

A significant reduction in LDL zeta potential also was seen after 60, 120 and 180 min under an exposure of 0.25, 0.5 and 1 mT ($p < 0.01$). Exposure to the electromagnetic flux densities of 2, 3 and 4 mT first lead to a significant reduction in LDL zeta potential after 60 min of incubation ($p < 0.01$). But with continuing the incubation the zeta potential started to increase, so that, there was no significant differences in LDL zeta potential after 180 min of exposure compared with control samples.

DISCUSSION

The possible cardiovascular effects of ELF-EMFs through the change in the LDL physico-chemical properties were herein investigated. LDL susceptibility to copper mediated oxidation under the exposure of ELF-EMFs with different intensities were evaluated at the first step. Our results indicated that the susceptibility of LDL to oxidation is increased under the exposure of weak intensities of EMFs in a dose dependent manner. Electromagnetic flux densities of 0.125 to 1 mT had no significant effect on the LDL susceptibility to oxidation, although can lead to a decrease in lag time and an increase in the propagation rate of LDL oxidation. Following to increase the electromagnetic flux density, the lag time was significantly decreased and reached 18.2 % reduction at 4 mT. Inverse v_{max} or propagation rate was decreased parallel to the increase of electromagnetic flux density and reached 46 % increase at 3 mT. Our previous study on the effects of static magnetic fields (SMF) on LDL susceptibility to oxidation has shown different results at magnetic flux densities of 0.25 and 0.5 mT [20]. At that investigation, weak SMFs of 0.25 and 0.5 mT opposite the moderate SMFs of 2-4 mT caused a significant decrease in the susceptibility of LDL to oxidation by increasing the lag time and decreasing the propagation rate [20].

The effect of an EMF on the living organism is a complex phenomenon. The initial mechanism is physico-chemical, but afterwards, biological effects develop. The physico-chemical action of an EMF consists in electron, ion, dipolar, macrostructural and electric polarization. Other factors may also play a role, such as molecular excitation, biochemical activation, generation of radicals, chemical bond weakening, hydration change, altered relaxation time of atom vibration, and altered spin of dipoles [21-23]. These physico-chemical changes could lead to different biological alterations that depend on the nature of the magnetic field, applied frequency, amplitude and time of exposure. The oxidative modification of LDL is a very important stage in the promotion and progression of atherosclerosis [15]. Free radical reactions are very important in the oxidation process of LDL and may require the generation of super oxide anion and

hydroxyl radicals through the Fenton reaction [24] and exert some of their deleterious effects by peroxidation of the lipids [25]. Enhanced pro-oxidant conditions and free radical formation have been suggested in different biological models as an important pathway of response induced by electromagnetic fields which modulates the turnover of oxyradicals, including induction of ROS-generating enzymes [26-28]. With regard to the effects of EMF on chemical reactions and free radicals [29-30], an increase in the free radicals production and stability may be the main cause of raising the susceptibility of LDL to oxidation in this investigation.

LDL aggregation is another lipoprotein modification with atherogenic properties. Aggregated LDL is taken up by macrophages at an increased rate, leading to foam cell formation [31]. In addition to oxidation, LDL aggregation also occurs in the arterial wall, but little is known about the mechanism responsible for this modification [32]. In this study the effects of ELF-EMFs on the tendency of LDL particles to aggregation were investigated. Electromagnetic flux densities of 0.125 and 0.25 mT had no significant effect on the tendency of LDL to aggregation in the experimental time frame. Electromagnetic flux densities of 2, 3 and 4 mT after the first hour, 1 mT after 2 h and 0.5 mT after 3 h of exposure can lead to a significant increase in LDL tendency to aggregation. In other words, the enhancing effects of ELF electromagnetic field on the LDL tendency to aggregation is a time and dose dependent process. Our previous study on the cardiovascular effects of SMF indicated the same result [20]. The surface charge of particles is an important factor in the stability of their suspension in colloids, decreasing the particles surface charge or zeta potential could lead to an increase in the tendency of particles to aggregation. The increase in the tendency of LDL to aggregation following the exposure to ELF-EMF could be the result of modifications in LDL structure or alterations in the electrostatic properties of surface molecules and LDL zeta potential.

LDL and other lipoprotein nano-particles have a distinctive electrical charge and changes in electrostatic properties directly affect the metabolism of the lipoprotein [33]. LDL zeta potential has a vital role in its structure, interaction with apolipoproteins, receptors, enzymes and finally in plasma lipid metabolism [34] and may change in different physiological and pathological conditions [35].

Size of LDL nano-particles is another physical characteristic of LDL that is very important in relation to CVD. Studies have indicated that individuals with predominantly small LDL particles have greater cardiovascular risk than those with predominantly large LDL [36]. In this study the effects of ELF-EMFs on LDL particles mean size and surface charge were evaluated *in vitro*. Our results showed that an ELF electromagnetic flux density of 0.125, 0.25 and 0.5 mT causes a decrease in LDL surface negative charge in a time and dose dependent manner. LDL zeta potential at electromagnetic flux densities of 1-4 mT, decreases at the first hour of exposure and then started to increase. Investigations indicated

that magnetic exposure reduced the zeta potential and diffusivity of nonmagnetic colloids [37]. It is not clear what is the reason for the decay in LDL zeta potential following the exposure to electromagnetic field in this study. It may be related to the physico-chemical alterations in LDL surface molecules including proteins and fatty acids and their interaction, that lead to decrease in negative charge density on the LDL surface. Investigations indicate that lipid peroxidation can lead to an increase in negative charge of LDL and HDL particles [38]. Thus, the increase of LDL zeta potential under the exposure at electromagnetic flux density of 1-4 mT, after the expected initial decay in LDL surface charge at the first hour of exposing, may be the result of lipid peroxidation induced by the production and stabilization of free radicals. It has been demonstrated that LDL tendency to aggregation is inversely related with the susceptibility of LDL to oxidation [39].

It should be considered that the degree of electromagnetic effect, in part, depends on the particle size and ions in the medium. Since different people have different size of LDL nano-particles and because LDL size distribution is associated with the risk of CVD and atherosclerosis, the effects of EMF on lipoprotein nano-particles physico-chemical characteristics and metabolism, and therefore its possible interaction with CVD may be different among individuals. However, further *in vivo* and *in vitro* studies are needed to demonstrate the adverse effects of ELF electromagnetic field as a risk factor in CVD.

CONCLUSIONS

Moderate ELF-EMFs can increase the susceptibility of LDL to oxidation through the decrease in the lag time an increase in the propagation rate. The tendency of LDL to aggregation also is increased by ELF-EMF. LDL oxidation and aggregation are two important modifications of LDL involved in the promotion and progression of atherosclerosis. On the other hand, ELF-EMFs can alter the LDL surface charge and this alteration may interfere with the metabolism of LDL and its interaction with other molecules such as apolipoproteins, enzymes and receptors. If these atherogenic effects of ELF-EMF have been confirmed *in vivo,* it can be considered as a risk factor in CAD.

EXPERIMENTAL SECTION

Serum preparation and LDL separation. A pooled serum was prepared from 12 h fasting blood samples of 25 donors. In order of separation of LDL fraction including IDL (1.006 g/cm^3< ρ <1.063 g/cm^3), 5.9 ml of serum samples were poured into 8.9 ml polyallomer ultracentrifuge tubes (Optiseal, part number 361623, Beckman/Coulter, Fullerton, CA, USA) and a discontinuous density

gradient was made by overlaying the serum samples with 3 ml of Solution A [NaCl: 0.195 mM, NaOH: 0.62 mM, 0.01% ethylene diamine tetraacetic acid disodium salt (EDTA-Na_2), d = 1.006 g/ml]. The tubes were centrifuged in a Beckman Coulter optima L-100 XP ultracentrifuge equipped with a type 90Ti fixed angle rotor, at 60000 rpm (462666 g) for 6 h at 16 °C, acceleration: "5" and deceleration: "7". After centrifugation, the very low density lipoprotein (VLDL) fraction (the white layer of the supernatant) accompanied by 3 ml of the upper layer solution in tubes was removed and residual content of tube was mixed with a solution B [containing 24.8 g sodium bromide (NaBr) in 100 ml of solution A, d=1.182 g/cm^3]. The tubes were centrifuged at 60000 rpm for 12 h at 16 °C, acceleration: "9" and deceleration: "7". After centrifugation, the LDL fraction appears as a yellow-orange band at the supernatant [40].

LDL dialysis: The obtained LDL was carefully placed in special dialysis tubes (D6191-25EA, Sigma, St Louis, MO, USA) and dialyzed against iso-osmolar phosphate buffer solution (PBS) with a pH of 7.4 for 24 h at 4 ˚C under nitrogen gas and the dialysis buffer was exchanged three times [41]. After dialyzing, in order to reduce freeze-thawing effects including physical alteration and aggregation of LDL, 10% w/v sucrose was added to LDL [Rumsey et al., 1994]. The LDL protein content was determined according to a modified Lowry method and then LDL was aliquot and stored at -70 ˚C until further analysis [42].

Evaluation of the susceptibility of LDL to oxidation: Continuous monitoring of the formation of conjugated dienes was accomplished by using a spectrophotometer (UV 3100, Shimadzu, Kyoto, Japan) based on the technique proposed by Esterbauer et al. [43] at the wavelength of 234 nm. After thawing, LDL was adjusted to 70 µg protein/ml in iso-osmolar PBS buffer with a PH of 7.4. Test samples were pre-incubated at 37 ˚C under the exposure of different static magnetic flux densities of 0.125, 0.25, 0.5, 1, 2, 3 and 4 mT for one hour and control samples were pre-incubated at the same condition without magnetic field exposing. The oxidative modification of LDL was initiated by addition of freshly prepared 10 µM $CuSO_4$. The kinetics of LDL oxidation were monitored every 10 min by measuring its absorbance at 234 nm for 3 h. The lag time (t_{lag}), the time period until the conjugated dienes began to increase, was determined graphically by the intercept of the tangents to the slow and fast increase of the diene absorption. The other LDL oxidation parameters are the time required for reaching half maximum dienes ($t_{1/2}$), and the maximum velocity (v_{max}) of lipid peroxidation. Every 0.01 unit change in absorbance per min equals to velocity of diene production of 0.03389. The maximum diene concentration ($diene_{max}$) is another parameter of LDL oxidation, and every 1 unit increase in absorption in 234 nm is equal to the production of 33.9 µM dienes. The conversion of absorption into concentrations is based on a molar absorptivity of ε_{234} = 29500 $L.mol^{-1}.cm^{-1}$ [43].

Evaluation of the LDL tendency to aggregation: In order to determine the effect of EMF on LDL aggregation, LDL samples (200 µg of protein/mL in iso-osmolar phosphate buffer with a pH of 7.4) were exposed to different electromagnetic flux densities of 0.125, 0.25, 0.5, 1, 2, 3 and 4 mT for 1, 2 and 3 h. At the end of exposure time, the tendency of LDL to aggregation was measured by LDL vortexing for a period of 60 s at 25 ˚C and monitoring the changes in absorbance at 680 nm in comparison with control samples [39].

Determination of the LDL mean particle size: A zetasizer nano ZS instrument (Malvern, Worcestershire, UK) equipped with a 532 nm green laser beam was used for determination of LDL particles mean size [44]. The scattered light was collected by detector at an angle of 173˚ using NIBS (Non-Invasive Back-Scatter) technology and directed to a correlator. The data were analyzed by zetasizer software (DTS, nano series, version 5.02, Malvern, Worcestershire, UK) and size information was reported as the Z-average by intensity [26]. All measurements were performed at 25˚C, in duplicate with automatic duration measurements.

In order to measure LDL mean particle size by dynamic light scattering (DLS) methodology, isolated LDL samples were mixed gently with 1 ml of phosphate buffer, 0.2 M, pH 7.4 containing 0.1% (w/v) EDTA-Na_2 as dispersant at a final protein concentration of 200 µg/ml. The mixture was then passed through a syringe filter (Millipore cellulose acetate membrane, 30 mm, 0.2 µm pore size), prior to injection into a disposable polystyrene cell (Malvern, Worcestershire, UK) in order to remove dust particles and was then subjected to size determination. Viscosity and refractive index (RI) of water as the dispersant were applied to standard operating protocol (SOP) prior to size determination. The accuracy of size measurements was examined using standard size nano particles (Gold Nanoparticles, 20 nm, 0.01% (w/v) aqueous solution, Nanocs Inc, New York, NY, USA) under the same experimental conditions and the results were matched to the diameter quoted by the manufacturer. The within-assay coefficient of variation (CV %) for 10 measurements was 1.4% and between-assay CV for 10 measurements was 2.9%.

Zeta potential measurement: The LDL particles zeta potential was also detected by Zetasizer nano ZS instrument. The zeta potential was calculated by determining the electrophoretic mobility and then applying the Henry equation ($U_E = 2\varepsilon z f(ka)/3\eta$) where z = zeta potential, U_E = electrophoretic mobility, ε = dielectric constant, η = viscosity and f(ka) = Henry's function. In aqueous solutions f (ka) is 1.5 and is referred to as the Smoluchowski approximation, dielectric constant and viscosity also in considered 78.5 and 0.8872 cP respectively. The electrophoretic mobility was obtained by performing an electrophoresis experiment on the sample and measuring the velocity of the particles using Laser Doppler

Velocimetry (LDV). The data were analyzed by Zetasizer software DTS (nano) version 5.02 (Malvern). All measurements were performed at 25˚C, with a dielectric constant of 78.5 in duplicate. Tris buffer (1 mM, pH 7.4) was used as the dispersant and viscosity of the samples was estimated to be that of water (0.887 cP). In order to measure the LDL zeta potential, isolated LDL were mixed gently with 1 ml of dispersant in a final concentration of 70 µg/ml protein and passed through a syringe filter (cellulose acetate membrane, 30 mm, pore size: 0.2 µm) while being injected into a special folded capillary cell (DTS1060, Malvern) to exclude dust particles. To investigate the effect of electromagnetic field on zeta potential modification, LDL samples inside the cells were placed in the center of the solenoid and incubated under the exposure of different electromagnetic flux densities at 25 ˚C for different times and then subjected to zeta potential measurement after each time point. The accuracy of zeta potential measurements was examined using standard nano particles with zeta potential of 50 ± 5 mv (Malvern) under the same experimental conditions and results were matched with the zeta potential quoted by the manufacturer. The within-assays coefficient of variation (CV %) for 10 measurements was 1.9% and between-assays CV was 2.1%.

The exposure system: Experimental setup for the static magnetic field exposure was consisted of a solenoid cylinder with a diameter of 12 cm, height of 30 cm and 1200 turns [20]. The solenoid was located inside a ventilated incubator (Parsazma, Tehran, Iran). The incubator temperature was set at 37 ˚C. For LDL oxidation experiments LDL samples (inside a quartz cuvette) were put in the center of the solenoid at the middle height of it by an especial sponge holder. In order to produce suitable electromagnetic flux densities, a voltage regulator AC power supply (model: TDGC2, 220v, 50-60 Hz, Delta International Electric Co, Shanghai, China) was used to provide variable AC currents. The produced electromagnetic field at the exact site of cuvette location in the middle center of the solenoid was measured by a digital tesla meter with a three-D sensor (Holaday, Eden Prairie, MN, USA). A small ventilator was improvised at the bottom of the solenoid in order to prevent temperature rising due to the electrical current in solenoid during the experiment.

Statistical analysis: All statistical analyses were performed with the SPSS statistical software, version 16.0 (SPSS, Chicago, IL, USA). Data were expressed as mean ± standard deviations. P value <0.05 was considered statistically significant. Comparison of data between control and exposed samples was examined by non parametric two-independent samples and Mann-Whitney-Wilcoxon test. Comparison of data between different static magnetic flux densities and different exposure times was examined by non parametric test for several independent samples and Kruskal-Wallis H test.

ACKNOWLEDGMENT

This study was supported by Grant no.r 90124 from Isfahan Cardiovascular Research Institute. The authors would like to thank Dr. Maryam Boshtam for her technical support and helpful comments on the manuscript.

R E F E R E N C E S

1. M.R. Cook, C. Graham, HD. Cohen, M.M. Gerkovich, *Bioelectromagnetics*, **1992**, *13*, 261.
2. C. Graham, M.R. Cook, HD. Cohen, M.M. Gerkovich, *Bioelectromagnetics*, **1994**, *15*, 447.
3. Z. Tabor, J. Michalski, E. Rokita, *Bioelectromagnetics*, **2004**, *25*, 474.
4. R. Dănulescu, C. Goiceanu, E. Dănulescu, M. Mărgineanu, C. Croitoru, G. Bălăceanu, *J. Prev*., **2004**, *12*, 9.
5. S. Ghione, C. Del Seppia, L. Mezzasalma, M. Emdin, P. Luschi, *Bioelectro-magnetics*, **2004**, *25*, 167.
6. S. Ghione, C.D. Seppia, L. Mezzasalma, L. Bonfiglio, *Neurosci. Lett.*, **2005**, *382*, 112.
7. M.L. Sait, A.W. Wood, R.L. Kirsner, *Physiol. Measur.*, **2006**, *27*, 73.
8. J. Gmitrov, C. Ohkubo, *Bioelectromagnetics*, **2002**, *23*, 329.
9. J.E. Hokanson, M.A. Austin, *J. Cardiovasc. Risk*, **1996**, *3*, 213.
10. P. Zimetbaum, W.H. Frishman, W.L. Ooi, M.P. Derman, M. Aronson, L.I. Gidez, H.A. Eder, *Arteriosclerosis, Thromb. Vascular Biol*., **1992**, *12*, 416.
11. E. Luo, G. Shen, K. Xie, X. Wu, Q. Xu, L. Lu, X.Jing, *Bioelectromagnetics*, **2007**, *28*, 608.
12. B. Kula, A. Sobczak, R. Grabowska-Bochenek, D. Piskorska, *J. Occup. Health-Eng. Ed*.,**1999**, *41*, 177.
13. D. Steinberg, N, Engl, *J. Med*.,**1989**, 320, 915.
14. D. Steinberg, *J. Lipid Res*., **2009**, *50*, S376.
15. H. Itabe, *Clin. Rev. Allergy Immunol.*, **2009**, *37*, 4.
16. H.F. Hoff, J. O'Neil, Arteriosclerosis, *Thromb. Vascular Biol*., **1991**, *11*, 1209.
17. N. Rabbani, L. Godfrey, M. Xue, F. Shaheen, M. Geoffrion, R. Milne, P.J. Thornalley, *Diabetes*, **2011**, *60*, 1973.
18. J. Vakkilainen, G. Steiner, J.-C. Ansquer, F. Aubin, S. Rattier, C. Foucher, A. Hamsten, M.-R. Taskinen, *Circulation*, **2003**, *107*, 1733.
19. S.M. Grundy, *J. Clin. Endocrinol. Metab.,* **2004**, *89*, 2595.
20. S. Abdi, D. Dorranian, A.N. Emami Razavi, G.A. Naderi, M. Boshtam, M. Ghoran-Nevis, *Bioelectromagnetics*, **2012**, 9999*, 1.*
21. H. Berg, *Bioelectrochem. Bioenerg*., **1993**,*31*, 1.
22. D. Duda, J. Grzesik, K. Pawlicki, *J. Trace Elem. Electrolyt. Health Dis.*, **1991**, *5*, 181.

23. B. Kula, *Bioelectrochem. Bioenerg*.,**1996**, *39*, 27.
24. D. Steinberg, *J. Biol. Chem*., **1997**, *272*, 20963.
25. K. Zwirska-Korczala, J. Jochem, M. Adamczyk-Sowa, P. Sowa, R. Polaniak, E. Birkner, M. Latocha, K. Pilc, R. Suchanek, *J. Physiol. Pharmacol*., **2005**, *56*, 101.
26. K.J. Fernie, D.M. Bird, *Evidence Environ. Res*., **2001**, *86*, 198.
27. J. Rollwitz, M. Lupke, M. Simkó, *Biochim. Biophys. Acta*, **2004**, *1674*, 231.
28. S. Roy, Y. Noda, V. Eckert, M.G. Traber, A. Mori, R. Liburdy, L. Packer, *FEBS Lett*., **1995**, *376*,164.
29. R.K. Adair, *Bioelectromagnetics*, **1999**, *20*, 255.
30. C.T.W. Eveson, B. Brocklehurst, P.J. Hore, R.K.A. McLauchlan, *Int. J. Rad. Biol*., **2000**, *76*, 1509.
31. J.W. Heinecke, A.G. Suits, M. Aviram, A. Chait, *Arterioscler., Thromb., Vasc. Biol*., **1991**, *11*, 1643.
32. D. Meyer, M. Mayans, P. Groot, K. Suckling, K. Bruckdorfer, S. Perkins, *Biochem. J*, **1995**, *310*, 417.
33. D.L. Sparks, C. Chatterjee, E. Young, J. Renwick, N.R. Pandey, *Chem. Phys. Lipids*, **2008**, *154*, 1.
34. H.I. Nishida, H. Arai, T. Nishida, *J. Biol. Chem*., **1993**, *268*, 16352.
35. M. Yano, M. Inoue, E. Maehata, T. Shiba, M. Yamakado, Y. Hirabayashi, M. Taniyama, S. Suzuki, *Clin. Chim. Acta*, **2004**, *340*, 93.
36. L. Kuller, A. Arnold, R. Tracy, J. Otvos, G. Burke, B. Psaty, D. Siscovick, D.S. Freedman, R. Kronmal, *Arterioscler., Thromb., Vasc. Biol*., **2002**, *22*, 1175.
37. K. Higashitani, H. Iseri, K. Okuhara, A. Kage, S. Hatade, *J. Coll. Interface Sci*., **1995**, *172*, 383.
38. O. Panasenko, M. Borin, O. Azizova, K. Arnol'd, *Biofizika*, **1985**, *30*, 822.
39. I. Maor, T. Hayek, R. Coleman, M. Aviram, *Arterioscler. Thromb. Vasc. Biol*., **1997**, *17*, 2995.
40. T.J. Bronzert, H.B. Brewer, *Clin. Chem.*, **1977**, *23*(11), 2089.
41. M.L. Rufail, S.C. Ramage, R. van Antwerpen, *FEBS Lett.*, **2006**, *580*(22), 5155.
42. Markwell, Mary Ann K. et al., *Anal. Biochem.*, **1978**, *87*(1), 206.
43. S.P. Gieseg, H. Esterbauer, *FEBS Lett.*, **1994**, 343(3), 188.
44. A. Emami Razavi, M. Pourfarzam, M. Ani, G.A. Naderi, *Biomarkers Med.*, **2013**, 7, 235.