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Investigating the effect of radiofrequency electromagnetic field exposure on molecular pathways related to insulin resistance and adipogenesis in zebrafish embryos - A pilot study without quantitative exposure metrics

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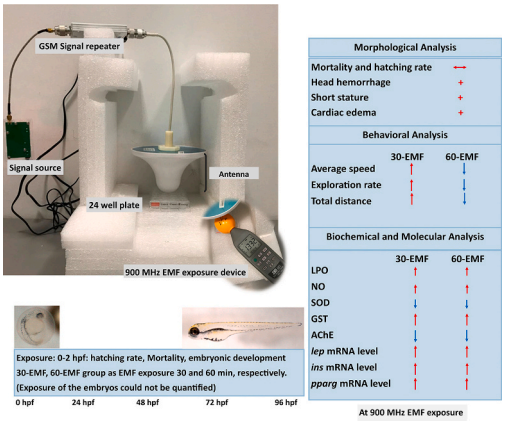
HIGHLIGHTS

- In zebrafish embryos, RF-EMF exposure (without quantitative metrics) decreased the abundance of *lepa*, *ins*, and *pparg* mRNA transcripts.
- Increased nitric oxide and disrupted oxidant-antioxidant balance were associated with the detrimental effects of RF-EMF exposure.
- RF-EMF during embryogenesis disrupted molecular pathways related to insulin resistance and adipogenesis in zebrafish.
- Molecular pathways related to insulin resistance and adipogenesis may have consequences for the fetal origins of obesity.
- RF-EMF exposure during the embryonic period reduced AChE activity in zebrafish embryos.

GRAPHICAL ABSTRACT



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ABSTRACT

In recent years, obesity has become a global problem in children and adolescents, in parallel with the rapid increase in the use of information and communication technology. Recognizing the embryonic causes of obesity may help prevent adverse adult health outcomes. In our study, we hypothesized that radiofrequency-electromagnetic field (RF-EMF) exposure during embryogenesis would affect the molecular mechanisms related to adipogenesis and insulin resistance in zebrafish. To achieve this, we set up a system that emits RF-EMF in the 900 MHz band and subjected zebrafish embryos to its RF-EMF. We created two groups in which we exposed 30 min (EMF-30) and 60 min (EMF-60) per day, and a control group that was not exposed to RF-EMF. We ended the exposure at 96 hpf and analyzed the expression of *lepa*, *ins*, and *pparg* that are involved in the

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regulation of glucose and lipid metabolism. In addition, we analyzed oxidative stress parameters, embryonic development, and locomotor activity. We found decreased mRNA transcript abundance of *lepa*, *ins*, *pparg*, and activities of superoxide dismutase and acetylcholine esterase, along with increased lipid peroxidation (LPO), nitric oxide (NO), and glutathione S-transferase (GST). Locomotor activity increased in the EMF-30 group and decreased in the EMF-60 group. Our results showed that exposure to RF-EMF during the embryonic period disrupted the molecular pathways related to insulin resistance and adipogenesis in zebrafish. However, due to limited available resources, we were not able to appropriately quantify the actual RF exposure strength of the samples. Hence the results reported here should only be seen as preliminary, and further studies employing high quality exposure apparatus and dosimetry should be carried out in future.

1. Introduction

In recent years, exposure to long-term electromagnetic fields originating from mobile phones and computers has increased simultaneously with the rapid increase in the use of information and communication technology.

Exposure to radiofrequency electromagnetic fields (RF-EMF) is quite common due to the widespread use of mobile phones. Since the absorption of RF-EMR emitted by mobile phones by body cells directly affects the living system, concerns about the possible health hazards of RF-EMR are increasing (Cucurachi et al., 2013).

Penetration of RF fields into tissues can move charged particles, such as electrons and ions of large macromolecules, and may cause harmful effects on tissues. RF fields penetrate into tissues depending on frequency, and as the frequency decreases, tissue penetration increases (Torres-Ruiz et al., 2024; Gherardini et al., 2014). RF-EMFs that cause changes in the behavior of cells and tissues can have several important effects on cellular components, including disorders of cell proliferation and differentiation, damaged DNA, chromosomal abnormalities, blood disorders, birth defects, and various mutations (Asghari et al., 2016).

Some studies reported an association between defects in DNA structure as a result of exposure to RF-EMF and metabolic diseases. As a result of long-term exposure to electromagnetic fields, disorders can be seen, such as insomnia, concentration disorders, headaches, fatigue, anxiety, depression, reproductive health problems, cancer, and heart diseases (Türkkan and Kayıhan, 2009; Yalçın and Saygın, 2016; Valberg et al., 2007; Cammaerts et al., 2011; Brillaud et al., 2007; Tripathi et al., 2022).

Obesity is caused by the accumulation of excess fatty tissue mass and often results in excess body weight. Genetic factors play a 25–40 % role in the formation of obesity. Genetic factors determine the susceptibility to obesity, but an "obesogenic" environment is also necessary for its development. Adipose tissue is an endocrine organ that produces adipocytokines, defined as biologically active molecules, and protein hormones with pleiotropic functions involved in the regulation of appetite, insulin sensitivity, inflammation, cell proliferation, and energy metabolism. In obesity, lipid accumulation causes dysregulation of adipokine production, which strongly contributes to the onset of obesity-related diseases (Wright and Aronne, 2012; Nigro et al., 2014). The hormones leptin and insulin regulate energy homeostasis, which is dependent on nutritional behavior and is managed by the hypothalamus (Belgardt et al., 2009; Kim et al., 2000). Peroxisome proliferator-activated receptor γ (PPARγ) is involved in adipocyte differentiation, regulation of glucose metabolism, and storage of fatty acids. PPARγ agonists contribute to increased insulin resistance by opposing the effect of TNF-α on adipocytes (Tyagi et al., 2011). The transcriptional function of PPARγ is essential for embryonic development, and inhibition of the transcriptional activity of PPARγ has been shown to cause severe adipocyte hypertrophy, insulin resistance, and hepatic steatosis (Guo et al., 2020).

The genes involved in embryonic development play an important role in adipocyte development and fat distribution. Obesity has been suggested to have a developmental genetic origin. Significant differences have been reported in the expression of multiple genes involved in embryonic development and pattern specification among adipocytes. Some of these developmental genes exhibited expression changes that are closely related to the level of obesity and lipid distribution (Gesta et al., 2006).

Lipid metabolism, adipose tissue biology, pancreas structure, and glucose homeostasis in zebrafish are similar to those in mammals (Maddison and Chen, 2017). The endocrine system of zebrafish develops within the first 5 days. Accordingly, zebrafish have been suggested to be an efficient model for understanding the mechanisms of obesity and developing treatment strategies (Oka et al., 2010).

Zebrafish embryos are widely used in metabolic, systemic, genetic, and toxicology research as they have 70 % orthology with humans and reproduce by external fertilization (Choi et al., 2021). Based on the hypothesis that RF-EMF exposure during embryogenesis will affect the molecular mechanisms related to adipogenesis and insulin resistance, we chose the zebrafish embryo as a model organism to investigate this mechanism due to the advantages of its external fertilization feature and transparent structure. Given the relationship between metabolic disorders, increased ROS production, obesity, and insulin resistance, we evaluated the oxidant-antioxidant status and the mRNA transcript abundance of lepa, ins, and pparγ genes coding for leptin, insulin, and peroxisome proliferator-activated receptor γ, respectively. Finally, we conducted locomotor activity and morphological examinations to assess the impact of RF-EMF on the normal embryonic development of zebrafish.

2. Methods

2.1. Animals and treatment

Wild-type AB/AB strain zebrafish were kept in aquarium system (Zebtec Tecniplast, Italy) at 27 ± 1 °C under a light/dark cycle of 14/10 h and apparently in disease-free conditions. Aquarium system water pH was kept at approximately 7.2, and reverse osmosis water containing 0.018 mg/L Instant OceanTM salt was used. Animal husbandry and seeding were performed in accordance with protocols approved by the University of Marmara Institutional Animal Care and Use Committee. They were fed commercial flake fish food complemented with live artemia twice a day. Adult fish were matched at a ratio of 2 (female)/1 (male), and embryos were obtained. Then, embryos were selected according to their development and morphology using a stereomicroscope (Zeiss Discovery V8, Germany) and included in the experiment (Westerfield, 1995). All experiments were performed in a blinded manner. By numbering the embryo groups in petri dishes and eppendorfs containing homogenates instead of writing the group names, the researchers performing the analysis were kept unaware of the procedures applied. Identity of the groups was only released after completion of the analysis. Since the zebrafish embryos used were <5 days old, no ethical approval was required for this study in accordance with the protocol specified by Louhimies (2002).

2.2. RF-EMF exposure

We created three different groups in the study. Since the gastrula and

blastula stages in zebrafish embryos may be completed at 6 hpf, we started the first exposure at 0–2 hpf in our study (Piccinetti et al., 2018; Dasgupta et al., 2020). The control group was kept away from the exposure system during the exposure period. However, while the system was off, the control group was kept in the system in the same position for the duration of exposure, and the background RF value was determined by measuring the RF level <0.1 V/m (RF OFF) during this period. We applied RF-EMF exposure once every day from 0 to 2 hpf to 96 hpf, 30 min/day to the EMF-30 group, and 60 min/day to the EMF-60 group. Embryos were kept in the incubator at 28 °C during periods of no exposure. In the close near field of an antenna the SAR induced in a biological sample does not depend on the antenna input power but on the RF current distribution along the radiating elements of the antenna (more related to the magnetic field component than to the electric field component). While the measured value when the system is on is 12–18 V/m, the measured value when the system is off is 0.1 V/m. The measurement pertains to the value emanating from the antenna, not the radiofrequency value that the embryos encounter.

The RF value coming out of the system was measured by measuring the device when the RF was on and off. However, the RF-EMF value to which the embryos were exposed could not be measured.

A TES-593 electro-smog meter was used to measure the amount of RF-EMF generated. In the exposure system, there is no distance between the well plates containing the embryos and the antenna (Indoor omni mushroom antenna). We added 1 mL of E3 to each well, and there are 20 embryos in each well. The height of the E3 solution is approximately 5 mm. The distance between the E3 solution's top and the antenna is approximately 8 mm. In order to apply approximately the same level of RF to each well, an antenna was placed at the middle point of eight wells, and the system was operated.

900 MHz frequency GSM band electromagnetic exposure was produced using a wide band noise source with low power (BG7TBL Wide Band RF Noise Source) to apply the high-power amplifier (two-way GSM repeater/ GSM DCS signal repeater 900 MHz) to specify the 900 MHz GSM frequency band in order to generate 935 MHz–960 MHz band electromagnetic exposure homogeneously. Wide-angle omni-directional GSM antenna were used to radiate the RF-EMF to zebrafish. The RF-EMF level was confirmed by an electromagnetic field meter (TES-593 Electro-smog Meter), placed at the same level before application. The electric field strength level was 12–18 V/m when the RF power supply was on. The measuring device can display The measuring device uses an electric field sensor and is capable of converting the results to electric field (E), magnetic field (H), and power density (W). For convenience, we used electric field values as a reference. Local EM power under antenna is measured with open-ended probe, intensity and frequency spectrum shown in supplementary file: Measurement of the output signal.

2.3. Locomotor activity analysis

The locomotor activity of the zebrafish embryos was evaluated in a blinded manner. For this purpose, we placed each embryo individually in the center of the 11 cm diameter petri dish after the last RF-EMF exposure at 96 hpf, touched the tail with a pipette tip, and recorded the embryo's movement, speed, and exploration area using a camera (Fig. 1A). Then, the images were analyzed in Wondershare, Kinovea, and Toxtrac programs, respectively, and behavioral tests were completed. The camera was placed on top of the petri dish, and the distance between the petri dish and the camera was kept constant for all groups during the recording period, and the image was analyzed in the toxtract software (Cansız et al., 2024) (Fig. 1A-C).

2.4. Biochemical analysis

Zebrafish embryos were collected after RF-EMF exposure, and 50 embryos were pooled for each group for each biological replicate to form three biological replicates. Three technical replicates were performed for each biological replicate. Embryos were homogenized in physiological saline to produce 10 % (*w/v*) homogenates. After centrifuging briefly, the supernatant was separated and used to determine biochemical parameters. Total protein content was measured by the method of Lowry, and results were expressed per protein (Lowry et al., 1951). Yagi's method was used to measure malondialdehyde (MDA) levels, while the thiobarbituric acid reactive method of Miranda was used to measure nitric oxide (NO) levels (Yagi, 1998; Miranda et al., 2001). Superoxide dismutase (SOD) activity was measured by the ability of SOD activity to increase the effect of photooxidation of o-dianisidine sensitized by riboflavin, and the results were expressed in U/mg protein (Mylroie et al., 1986). Glutathione S-Transferase (GST) catalyzes the conjugation of GSH, and GST activity was determined using a spectrophotometer at 340 nm (Habig and Jakoby, 1981). Acetylcholine esterase (AChE) activity is based on the hydrolysis of acetylcholine to choline and acetic acid. AChE activity was measured using a spectrophotometer at 405 nm (Ellman et al., 1961). Biochemical analyses were performed by investigators blinded to the treatments.

2.5. RT-PCR analysis

The Rneasy Mini Kit and Qiacube (Qiagen, Germany) were used to isolate RNA from zebrafish. Single-stranded cDNA was synthesized from 1 μg of total RNA using RT2 Profiler PCR Arrays (Qiagen). The DNA Master SYBR Green kit (Qiagen, Germany) was used for PCR analyses. Quantitative RT-PCR was used for gene expression analyses using the Rotor Gene-Q Light Cycler (Qiagen, Germany), and the analyses were performed in a blinded manner. *β-actin* was used as the housekeeping gene, and the delta-delta Ct method was used to calculate relative transcript levels by normalizing the values to the housekeeping gene (Livak and Schmittgen, 2001). For each biological replicate, three technical replicates of each RT-PCR reaction were performed. Primers used for gene expression analysis are given in Table 1.

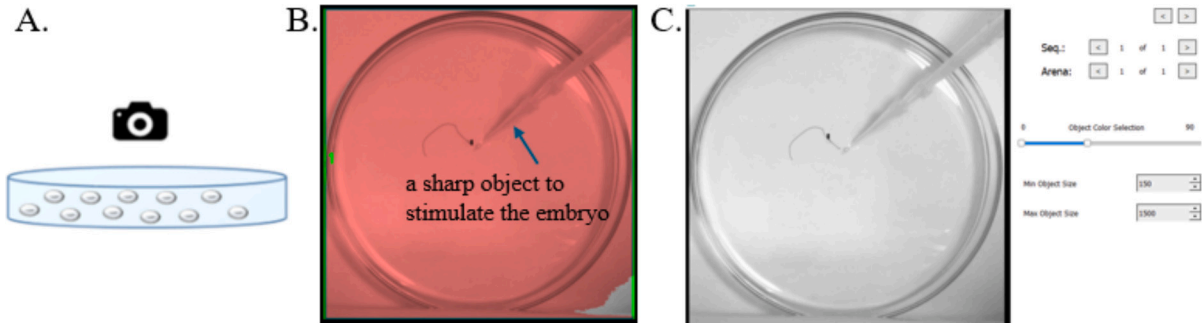

Fig. 1. (A) Locomotor activity tracking setting and Position of the camera monitoring the embryo in the petri dish (B-C) Analyzing the image after camera recording.

Table 1
Forward and reverse primers used in the study.

	(Forward/reverse)
β actin	5′-AAGCAGGAGTACGATGAGTCTG-3′ 5′-GGTAAACGCTTCTGGAATGAC-3′
lepa	5′-CTCCAGTGACGAAGGCAACTT-3′ 5′-GGGAAGGAGCCGGAAATGT-3′
Ins	5′-GCTCTGTTGGTCCTGTTGGT-3′ 5′-GGGCAGATTTAGGAGGAAGG-3′
pparg	5′-TATGGTGGACACGCAGACGTT-3′ 5′-GTCCATCATGTGCGGGGTTG-3′

2.6. Data analysis

Statistical analysis was performed using GraphPad Prism 9.0 in a blinded manner (GraphPad Software, San Diego, USA). The normality of the distribution was tested using the Shapiro-Wilk test. To compare the groups, a one-way ANOVA test was used, which was followed by Tukey's multiple comparison tests. The data obtained were given as the mean ± standard deviation.

3. Results

3.1. Embryonal development, hatching rate and mortality rate

RF-EMF exposure was applied to zebrafish embryos for two different periods of 30 and 60 min at 0–2, 24, 48, 72, and 96 hpf. A comparison of mortality rates and hatching rates at 24, 48, 72, and 96 hpf was made in the control, EMF-30, and EMF-60 groups, and no statistically significant difference was observed in the EMF-30 and EMF-60 groups compared to the control (Fig. 2).

To monitor their development, we took photographs of the embryos at 24, 48, 72, and 96 hpf and observed the effects of RF-EMF exposure on the development of zebrafish embryos under the light microscope. Compared to the control, head hemorrhage occurred in zebrafish embryos exposed to RF-EMF for 30 min per day. The embryos exposed to RF-EMF for 60 min per day showed short stature, cardiac edema, and head hemorrhage (Fig. 3). The mortality and hatching rates of the EMF-30 and EMF-60 groups did not differ statistically significantly from the control group (Fig. 2).

3.2. Results of locomotor activity

Average speed, exploration rate, and total distance were monitored to analyze locomotor activity in the control, EMF-30, and EMF-60 groups with the Toxtrac program.

The average speed increased in the EMF-30 group and decreased in the EMF-60 group compared to the control (Fig. 4A). Compared to the control group, the exploration rate increased in the EMF-30 group and decreased in the EMF-60 group. The exploration rate also decreased in the EMF-60 group compared to the EMF-30 group (Fig. 4B). Compared to the control group, the total distance increased in the EMF-30 group and decreased in the EMF-60 group. In the EMF-60 group, the total distance decreased compared to the EMF-30 group (Fig. 4C).

3.3. Biochemical results

LPO, NO levels, and GST activity increased in the EMF-30 and EMF-60 groups compared to the control group (Fig. 5A, C, and D). SOD activity and AChE activity decreased in the EMF-30 and EMF-60 groups compared to the control group (Fig. 5B and E).

3.4. RT-PCR analysis results

lepa mRNA transcript abundance decreased in the EMF-30 and EMF-60 groups compared to the control group. The EMF-60 group significantly decreased the lepa mRNA transcript abundance compared to the EMF-30 group (Fig. 6A). *ins* and *pparg* mRNA transcript abundance decreased significantly in the EMF-30 and EMF-60 groups compared to the control group (Fig. 6B and C).

4. Discussion

Results of our study showed that RF-EMF exposure affected the expression of genes related to insulin resistance and adipogenesis through decreased *pparg, ins*, and *lepa* mRNA transcript abundance. Oxidant-antioxidant balance was also disrupted, and locomotor activities decreased depending on RF-EMF exposure periods (30 min and 60 min).

Although there are many studies examining the effects of 900 MHz RF-EMF exposure at mobile phone frequencies during the embryonic period (Piccinetti et al., 2018; Dasgupta et al., 2020), there is no study examining its effects on insulin resistance and adipogenesis. In accordance with our findings, there are studies showing that RF-EMF exposure disrupts the oxidant-antioxidant balance, inhibiting development and causing morphological anomalies. RF-EMF exposure from mobile phones was reported to lead to a decrease in fertilization and embryonic development rates and an increase in the risk of developmental anomalies (Mahaldashtian et al., 2022).

In our study, we performed RF-EMF exposure at the embryonic stage. The blastula stage in zebrafish embryos signifies the initial stages of development, and exposure to environmental factors during this stage can result in lasting developmental and behavioral impacts. In their study on zebrafish embryos, Torres-Ruiz et al. (2024) created RF-EMF in the 700 MHz band and found a decrease in the eye size as well as yolk sac and cardiac edema.

In this study, we observed morphological anomalies and developmental delays in zebrafish embryos exposed to RF-EMF until 96 hpf. Tail shortening, head hemorrhage, cardiac edema, and inadequate

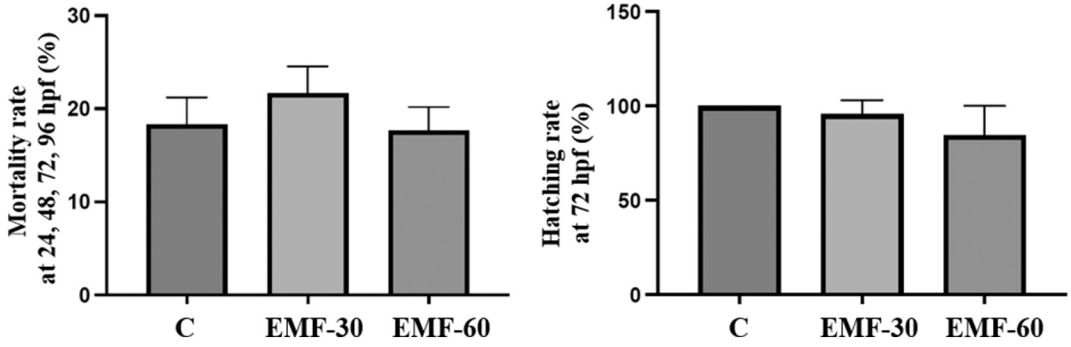

Fig. 2. Mortality and hatching rate: Comparison of mortality rates of groups at 24, 48, 72 and 96 hpf: Data are expressed as mean ± SD from the three independent experiments. EMF: Electromagnetic field.

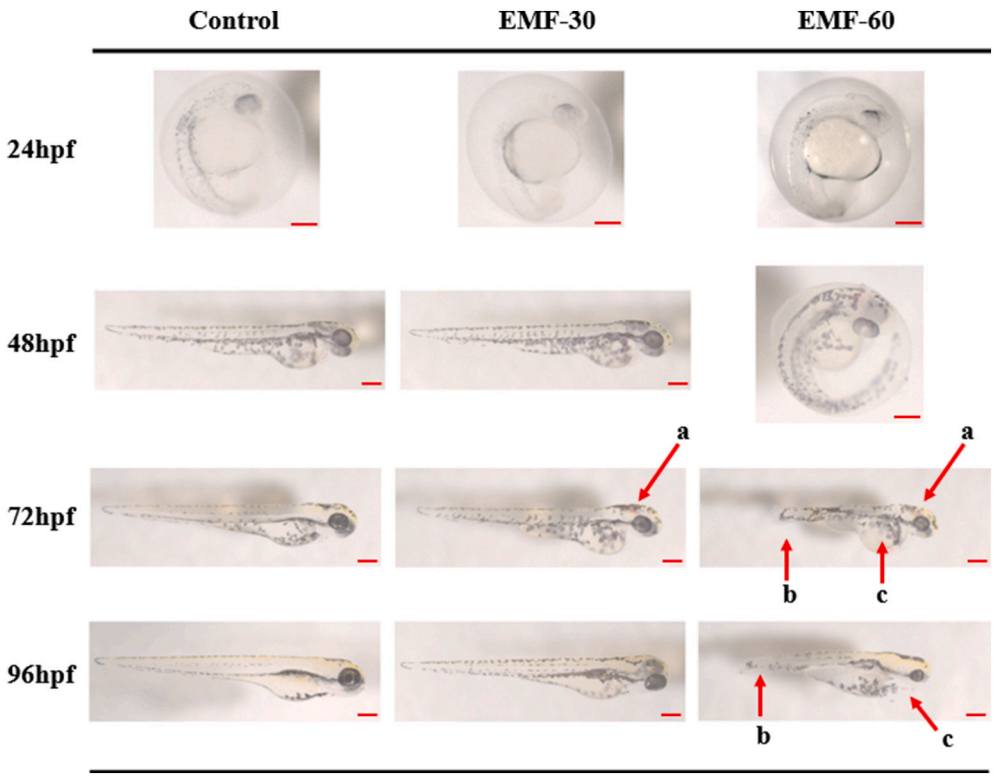

Fig. 3. Representative figures of zebrafish embryos at 24, 48, 72 and 96 hpf; a. head hemorrhage b. short stature, c. cardiac edema: A Zeiss Sterio Discovery V8 microscope was used for measurements. 6.3× magnification was used for embryos in the chorion and 3.2× magnification was used for hatching embryos. Scale bar: 500 μm.

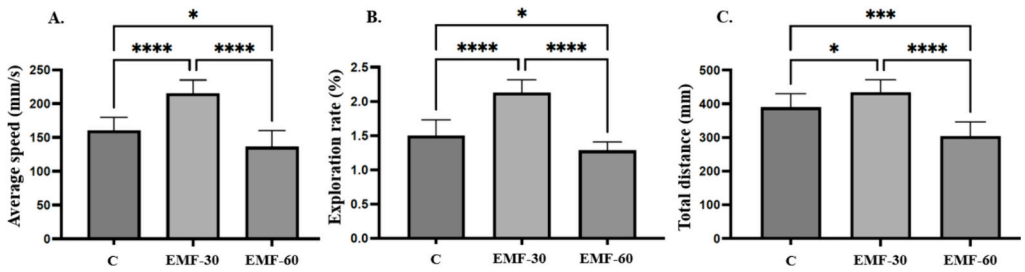

Fig. 4. (A) Average speed, (B) Exploration rate, (C)Total distance: Data are expressed as mean ± SD, $n = 10$; ****$p < 0.0001$; *** $p < 0.001$; *$p < 0.05$; EMF: Electromagnetic field; ±SD: Standard deviation.

development of eye and head structures were among the morphological anomalies detected in the embryos exposed to RF-EMF for 60 min a day. A head hemorrhage was observed in the group exposed to 30 min of RF-EMF.

Piccinetti et al. (2018) exposed zebrafish embryos between 0 and 72 hpf to 100 MHz RF-EMF and demonstrated that this exposure could affect embryonic development between 24 and 72 hpf. Especially at the 48 hpf stage, they detected a decline in growth, an increase in the transcription of oxidative stress genes, the onset of apoptotic and autophagic processes, and changes in cholesterol metabolism. Dasgupta et al. (2020) applied a 3.5 GHz RF-EMF from 6 hpf to 48 hpf and stated that they did not observe any significant negative effects due to RF-EMF exposure. Piccinetti et al. (2018) stated in their study that, unlike this study (2020), there were differences in oxidative damage markers and developmental exposure groups. Researchers have suggested two reasons for this. First, Dasgupta et al. (2020) applied 3.5 GHz RF-EMF, while Piccinetti et al. (2018) applied 100 MHz RF-EMF exposure. They stated that as the frequency increased, there was a decrease in radiation penetration into the tissues, and they claimed that this was one of the reasons for the results they obtained. The other one is that while Dasgupta et al. (2020) exposed between 6 and 48 hpf, Piccinetti et al. (2018) applied RF-EMF exposure between 0 hpf and 72 hpf (Dasgupta et al., 2020). Researchers have stated that zebrafish embryos may complete the gastrula and blastula stages at 6 hpf, suggesting that exposure starting at 6 hpf may not cause any effects. In light of this information, the first exposure in our study was applied between 0 and 2 hpf.

In their exposure study on zebrafish embryos, Torres-Ruiz et al. (2024) revealed that there was no significant change in mortality and hatching rates. Our study also found no significant difference in mortality and hatching rates between the exposure groups and the control.

In our study, locomotor activity varied depending on exposure time. The group exposed to RF-EMF for 30 min (EMF-30) experienced an increase in locomotor activity, whereas the EMF-60 group experienced a decrease. Audira et al. (2018) observed rapid swimming and hyperactivity in lepa gene-silenced zebrafish embryos. In our results, decreased lepa mRNA transcript abundance was in parallel with the change in locomotor activity in the 30-min exposure group, which is compatible

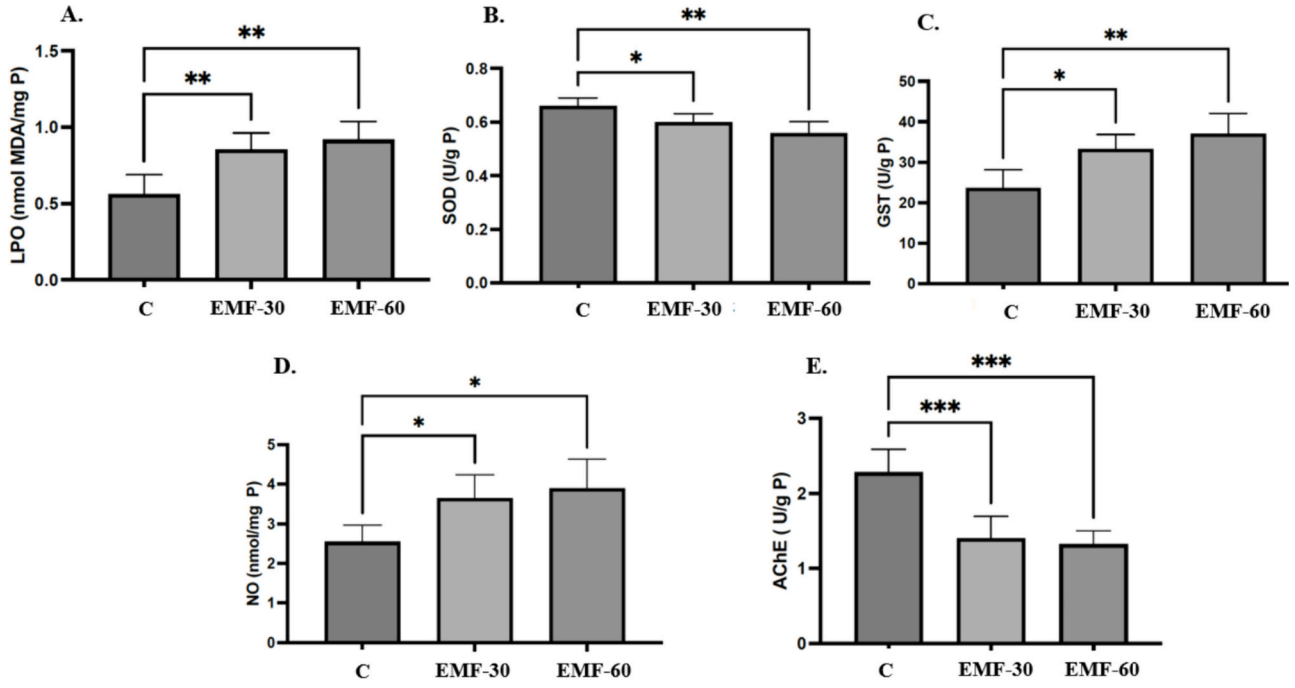

Fig. 5. (A) LPO level, (B) SOD activity, (C) GST activity, (D) NO activity, (E) AChE activity. Data are expressed as mean ± SD from the three independent experiments. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. EMF: Electromagnetic field; LPO: Lipid peroxidation; SOD: Superoxide dis mutase; GST: Glutathione *S* transferase; NO: Nitric oxide; AChE: Acetylcholinesterase ±SD: Standard deviation.

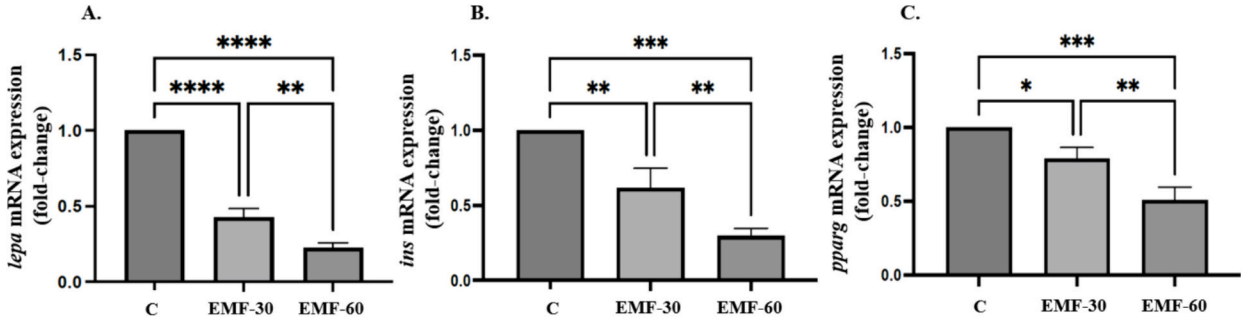

Fig. 6. (A) *lepa* mRNA transcript abundance, (B) *ins* mRNA transcript abundance, (C) *pparg* mRNA transcript abundance. Data are expressed as mean ± SD from the three independent experiments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$; EMF: Electromagnetic field; SD: Standard deviation.

with the study of Audira et al. (2018), indicating the importance of RF-EMF exposure time.

We can state that RF-EMF exposure affects locomotor activity differently depending on the duration. Leptin, which is effective in fasting-satiety metabolism and glucose metabolism, also plays a role in behavioral regulation, as leptin receptors are widely distributed in the brain and leptin affects cortisol release, dopamine pathway, serotonin synthesis, and hippocampal synaptic plasticity (Considine et al., 1996; Valleau and Sullivan, 2014). In our study, the decrease in both *lepa* mRNA transcript abundance and locomotor activity in the EMF-60 group is compatible with these data.

lepa mRNA transcript abundance was decreased in our study during 30 and 60 min of RF-EMF exposure. The secretion of the leptin hormone decreases during hunger and increases during satiety (Al-Hussaniy et al., 2021). The decrease in *lepa* mRNA transcript abundance in RF-EMF exposure groups may indicate an increase in embryonic nutritional intake requirements. In studies conducted in mouse models, susceptibility to obesity increased with leptin or leptin receptor deficiency (Friedman, 2004). In a study on adult zebrafish, researchers observed that inactivating the *lepa* gene increased appetite and susceptibility to the obesity phenotype due to the lack of neuropeptide regulation (He et al., 2021).

Wardzinski et al. (2022) conducted a study to investigate whether radiation from mobile phones and food intake were related, and 15 young men with normal weight were exposed to RF-EMF for 25 min. As a result, the calorie need increased in the group exposed to RF-EMF. Accordingly, it has been stated that RF-EMF exposure may be a potential factor contributing to the overeating underlying the obesity epidemic. However, it is important to note that, in addition to the ethical issues, as stated by Witthöft et al. (2022), the single-blinded and unbalanced experimental within-subject design, and the small sample size of this study raised questions in interpreting the results.

Insulin, which is released from pancreatic β cells, regulates glucose and lipid metabolism and adipogenesis. The mechanism underlying glucose homeostasis is controlled through insulin secretion by the pancreatic β cells. Therefore, the expression of major transcription factors in pancreatic development may dynamically alter pancreatic β cell function (Sipione et al., 2004).

Zebrafish receive their nutrition from the yolk sac during the embryonic period and get the O2 and minerals they need from the E3 medium. They continue to feed this way until 120 hpf. Therefore, decreased *ins* mRNA transcript abundance in this period may be related

to the disruption of pancreatic islet formation due to RF-EMF exposure. PPARg is a "key regulator" of adipogenesis and is also an important regulator of the cellular physiology of mature adipocytes (Tamori et al., 2002). PPARg promotes healthy organ and tissue function by preventing excessive fat accumulation in non-adipose tissues. Thus, it increases insulin sensitivity in peripheral tissues (Reaven, 1988). He et al. (2003) showed that pparg deficiency caused insulin resistance in adipose tissue and liver. In our study, *pparg* mRNA transcript abundance decreased depending on the duration of RF-EMF exposure. Spiegelman (1998) reported increased free fatty acids, oxidative stress, and decreased ability to tolerate environmental stress as a result of decreased *pparg* expression in zebrafish. In parallel with this, in our study, *pparg* mRNA transcript abundance decreased as a result of exposure, while lipid peroxidation, NO levels, and SOD activity, which are markers of oxidative stress, increased and GST activity decreased. As a result of exposure to non-ionizing electromagnetic fields, including RF-EMF, in cells, a defense mechanism is activated at the cellular level to protect the organism. Cellular and molecular responses to environmental stress factors, such as heat, ionizing radiation, and oxidation, occur with the help of antioxidant systems. In order to repair cell functions and return them to balance, antioxidant system agents are triggered by macromolecular (proteins, lipids, and DNA) damage in cells (Audira et al., 2018). The changes in oxidant-antioxidant parameters in zebrafish embryos as a result of 30 and 60 min of exposure to 900 MHz RF-EMF in our study are consistent with previous studies (Tsoy et al., 2019; Grasso et al., 2020).

Insulin resistance stimulates lipogenesis and causes weight gain. The increase in fat tissue also increases lipid peroxidation and the risk of inflammation (Manna and Jain, 2015). In our study, we found that an increase in LPO levels and a decrease in *ins* mRNA transcript abundance following RF-EMF exposure may indicate the onset of insulin resistance.

The predominant lipid damage after RF-EMF exposure refers to lipid peroxidation in cell membranes, which negatively affects membrane selective permeability and functions. Lipid peroxidation products can produce DNA adducts that can cause mutations and gene expression changes (Audira et al., 2018). In our study, decreased *lepa, ins,* and *pparg* mRNA transcript abundance were observed, along with increased lipid peroxidation as a result of RF-EMF exposure, depending on time. Decreased SOD levels in obese individuals have been suggested to be an indicator of a decrease in antioxidant response (Torkanlou et al., 2016). In systems where the antioxidant response decreases, there is an increase in ROS levels, which causes the development of obesity. In their study, Ustundağ et al. (2020) applied RF-EMF exposure to zebrafish embryos between 15 and 3000 MHz, and as a result, an increase in GST activity was observed. The increase in GST activity, which catalyzes reduced glutathione conjugation for detoxification, suggests an increase in ROS formation due to RF-EMF exposure.

NO synthesized from L-arginine is involved in many biological processes, including the oxidation of low-density lipoproteins. Morley and Flood (1994) applied NO synthase inhibitor to mice, demonstrated that this application reduced weight gain and food intake, and suggested that NO plays an important role in regulating energy balance. Sansbury and Hill (2014) demonstrated that NO bioavailability decreased in a diet-induced obese animal model, and that increased NO level had remarkable effects on obesity and insulin resistance. In light of these data, the increase in NO levels in the EMF-30 and EMF-60 groups may be associated with insulin resistance.

AChE hydrolyzes acetylcholine to form acetic acid and choline, and as a neurotransmitter, ACh is associated with locomotor activity. Alterations in AChE activity have been suggested to cause changes in locomotor activity (Singh and Chowdhuri, 2017; Chen et al., 2012). Torres-Ruiz et al. (2024) exposed zebrafish embryos to RF-EMF and revealed that there was a decrease in AChE activity and locomotor activity. In our study, which is compatible with these data, we can state that exposure times may have different effects on locomotor activity and that the decrease in AChE activity may cause hypoactivity.

In our study, increased NO levels were observed in parallel with the increase in LPO levels. Deterioration of the oxidant-antioxidant balance was induced by RF-EMF exposure, as evidenced by the alterations in GSH, GST, and SOD activities. The change in locomotor activity as a result of RF-EMF exposure varied depending on the duration of the exposure. In parallel with locomotor activity, long-term exposure decreased AChE activity. The expression of *lepa*, *ins*, and *pparg* genes, which are involved in the regulation of glucose metabolism, adipogenesis, and insulin resistance, decreased as a result of RF-EMF exposure. In light of all this information, we can state that exposure to RF-EMF during the embryonic period may disrupt the molecular pathways related to insulin resistance and adipogenesis in zebrafish, which may contribute to the fetal origins of obesity. Further studies during the embryonic period focusing on insulin resistance and adipogenesis are needed to reveal the relationship between RF-EMF and the fetal origins of obesity.

There are some limitations of our study. We used a noise source as an RF source instead of a signal generator, which could be considered a limitation. Other limitations include the lack of RF measurements absorbed inside the embryos and measuring the SAR values. The embryos were kept in the incubator during the periods when 900 MHz RF-EMF was not applied. It is not known whether the embryos were exposed to low RF-EMF originating from the incubator system during this period. The temperature increase in the well plate due to RF power absorption remained unmeasured. Therefore, we cannot make a quantitative estimate of the SAR value. We have no information about whether there is vibration when the system used is on. This is another limitation of our study. On the other hand, we believe our results would serve as a preliminary study for the implementation of more optimized RF systems in biological systems.

Consent for publication

All the authors have agreed for authorship, read and approved the manuscript, and given consent for publication.

Ethics approval

As the zebrafish embryos used were no older than 5 days old, no ethical approval was required for the protocols applied as stated by Louhimies (2002).

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CRediT authorship contribution statement

Irmak Yaldız Koç: Methodology, Investigation. **Merih Beler:** Methodology, Investigation. **İsmail Ünal:** Methodology, Investigation. **Selçuk Paker:** Validation, Methodology. **Ebru Emekli-Alturfan:** Writing – review & editing, Formal analysis, Data curation. **A. Ata Alturfan:** Methodology, Investigation. **Derya Cansız:** Writing – review & editing, Visualization, Supervision, Software, Methodology, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.176038.

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