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RESEARCH ARTICLE

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Adverse effects of 900, 1800 and 2100 MHz radiofrequency radiation emitted from mobile phones on bone and skeletal muscle

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ABSTRACT

The goal of this study was to biomechanically and morphologically research both the impact of mobile phone like radiofrequency radiations (RFR) on the tibia and the effects on skeletal muscle through oxidative stress parameters. Fifty-six rats (200–250 g) were put into groups: healthy sham (n = 7), healthy RFR (900, 1800, 2100 MHz) (n = 21), diabetic sham (n = 7) and diabetic RFR (900, 1800, 2100 MHz) (n = 21). Over a month, each group spent two hours/day in a Plexiglas carousel. The rats in the experimental group were exposed to RFR, but the sham groups were not. At the end of the experiment, the right tibia bones and skeletal muscle tissue were removed. The three-point bending test and radiological evaluations were performed on the bones, and CAT, GSH, MDA, and IMA in muscles were measured. There were differences in biomechanics properties and radiological evaluations between the groups ($p < .05$). In the measurements in the muscle tissues, significant differences were statistically found ($p < .05$). The average whole-body SAR values for GSM 900, 1800 and 2100 MHz were 0.026, 0.164, and 0.173 W/kg. RFRs emitted from mobile phone may cause adverse effects on tibia and skeletal muscle health, though further studies are needed.

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Introduction

Cellular devices emit non-ionizing radiofrequency radiation (RFR) in the 900, 1800 and 2100 MHz frequency bands. This radiation is frequency modulated, with channel rotation frequency of 217 Hz, and belongs to the radiation of the pulsed mode (Yakymenko et al. 2016). Radiofrequency can affect living things in two ways, either thermal or non-thermal. The Specific Absorption Rate (SAR) is a dosimetric value implemented to discern how much RF is absorbed into the biological system. However, a growing body of evidence has revealed that RFR at frequencies emitted by mobile phones causes non-thermal effects (Bektas 2021; Dasdag and Akdag 2016; Hardell and Sage 2008; Lai 2018; Yakymenko et al. 2016). According to the research, RFR may pose a non-thermal threat in the way it causes disordered transitions through ion channels on cell membranes. It is also known to trigger ROS production and damage related to oxidative stress (Bektas 2021).

In 2008, the World Cancer Report published by The International Agency for Research on Cancer (IARC) declared that brain tumors may be connected to the RFR emanating from mobile phones (Scarfi 2014).

Many local or systemic effects affect bone and skeletal muscle health. Diabetes is one of these factors (Dial et al. 2020; Murray and Coleman 2019). Moreover, excessive production of ROS and associated oxidative stress influence the bone and skeletal muscle in both a physiological and pathological manner (Bredahl et al. 2021; Reis and Ramos 2021). It is clear that oxidative stress is a primary mechanism through which RFR interacts with living cells (Dasdag and Akdag 2016; Yakymenko et al. 2016).

Exposure to ionizing radiation is known to harm bone tissue (Soares et al. 2019). However, although there are many studies on the effects of non-ionized RFRs on different tissues and organs, few studies discuss how they affect bone and muscle. It has been reported that deviations of bone morphogenetic proteins (BMP) in newborn babies can be traced back to GSM-like RFR which interferes with gene expression during early gestation (Pyrpasopoulou et al. 2004). The contradictions between the observable outcomes of the studies on electromagnetic fields (EMF) also prove to be fascinating. It has been stated that electromagnetic waves of much lower frequency than RFRs emitted from mobile

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phones are stimulating in bone healing (Ciombor and Aaron 2005; Haddad et al. 2007; Walker et al. 2007). There are also studies showing that RFRs accelerate healing in bone fractures (Durgun et al. 2016). On the other hand, human and animal studies have reported that RFRs and mobile phone usage negatively affect bone mineralization (Atay et al. 2009; Cidem et al. 2012; Scarfi 2014; Yıldız et al. 2006). Another study revealed that 900 MHz RFR emitted from mobile phone appears not to have a direct impact on macrometric parameters within the rats' bones; however, it does alter the bone mineralization process and the intensity by which bone turnover occurs, thus influencing the mechanical strength of bones (Sieroń-Stołtny et al. 2015).

This study sought to analyze the effects of RFRs in the frequency bands (900, 1800 and 2100 MHz) used by mobile phone operators for data exchange on the bone and skeletal muscles of diabetic and healthy rats.

Materials and methods

Animal care

All phases of the research presented here were undertaken in Van Yuzuncu Yil University's Experimental Research and Application Center. The guidelines of that university's Animal Experiments Local Ethics Committee were applied in designing and performing all steps of the study (Protocol No: 2021/05-10). The experiment was run under blind condition.

The animals used in this research included 56 healthy male Wistar Albino rats, which were 8 to 10 weeks old and weighed 200–250 grams. They were provided by the Experimental Animal Research Center at which the research was performed. 28 healthy and 28 diabetic rats were separated into eight different groups, each one composed of seven rats. Each group was kept together in its own stainless-steel cage. They were maintained in a laboratory setting with a 12 hour light/dark cycle and ad libitum pellet feed (with 21% crude protein; Purina, Istanbul, Turkey) and water under optimized conditions of temperature of 22 ± 2°C and a humidity level of 60 ± 5%.

Animal groupings and protocol

As stated above, there were a total of four groups of rats in the experiment, each consisting of seven rats. In the healthy sham group, seven healthy rats were held in a Plexiglas carousel for two hours each day for a period of 30 days. During this time, the RFR generator was turned off. The 21 rats in the healthy RFR groups also spent two hours each day in the Plexiglas carousel where they were exposed to RFR (900, 1800, 2100 MHz, GSM-modulated) over the course of 30 days. In the diabetes sham group, seven rats with diabetes were held in the Plexiglas carousel for 2 hours daily for 30 days with the generator turned off. And the 21 diabetic rats of the Diabetes + RFR group were exposed to RFR (900, 1800, 2100 MHz, GSM-modulated) for 2 hours daily for 30 days.

Experimental model of diabetes

In the creation of animal models of type 1 diabetes mellitus in laboratory settings, streptozotocin (STZ) is commonly used as an antibiotic capable of destroying pancreatic islet β-cells (Furman 2015). For the induction of diabetes in the present experimental model, the animals in Groups 3 and 4 went without food for one night. Subsequently, each rat in these groups received a single intraperitoneal injection of STZ at a dose of 45 mg/kg that was dissolved in 0.1 M phosphate-citrate buffer. Similarly, equivalent volume of sterile citrate buffer solution was injected into the control rats. After 72 hours had passed, blood was sampled from each of the rats in these groups to confirm the clinical presence of diabetes. An IME-DC glucometer was used to evaluate the animals' blood glucose levels and all values of >250 mg/dL were accepted as evidence of the presence of diabetes (Öntürk and Özbek 2007).

Exposure and field measurements

The GSM signal generators (900, 1800 and 2100 PM10 type Everest Comp., Adapazari, Turkey) used on the rats in the experiment are able to produce RF levels comparable to that of mobile phones (900, 1800 and 2100 MHz). The power emitted (omnidirectional on the plane perpendicular to the antenna axis) by the generator remained stable throughout the period that the rats were being exposed. The signal generator antenna was essentially identical to that of a mobile phone. The Plexiglas carousel in which the rats were station was completely surrounded by a type of electromagnetic absorbent material in order to prevent external electromagnetic fields from interfering with the experiment. The TES 593 field probe measured both the electrical field and the density of power (Shenzhen, Guangdong, P.R.C). The power density and electric field values were measured respectively as 0.039 mW/cm^2, 13.47 V/m at 900 MHz, 0.04 mW/cm^2, 22.42 V/m at 1800 MHz frequency and 0.038 mW/cm^2, 11.55 V/m at 2,100 MHz frequency. All rats belonging to the exposure groups were subjected to RFR for two hours each day

(5 days a week) for one month. The rats in the sham groups were put through the same conditions, with the only difference being that the generator was deactivated. The antenna of the generator was stationed within the center of the Plexiglas carousel to provide ideal exposure conditions. The distance of the antenna from the head of the rats was 1 cm.

Evaluations of specific absorption rate (SAR)

The environment for the simulation was established to match the experimental setup as much as possible. The Faraday cage, the applicator antenna, and the rats were modeled in a 3D electromagnetic field solver using CST (CST AG, Darmstadt, Germany). Thermal and electromagnetic modelling within the CST is based on the finite-integration technique, which is a combined method of integral and differential solvers acting on space and time.

Radiological evaluations

After the rats were euthanized, radiological imaging was performed to determine the values of the lateral cortex, the medial cortex, and the medulla for the right tibia bones that had been removed from the rats. The following bone parameters were measured: Anterior cortex thickness: It is the width of the cortex measured from the anterior midpoint of the length of the tibia (proximal-distal). Posterior cortex thickness: It is the width of the cortex measured from the posterior midpoint of the length of the tibia (proximal-distal). Medulla Width Measurement: The width of the medulla measured from the midpoint of the length of the tibia (proximal-distal) (between the posterior border of the anterior cortex and the anterior border of the posterior cortex).

Biomechanical testing

The three-point bending (3PB) test is a frequently-applied methods for the evaluation of loading conditions in fracture mechanics. In this research, the 3PB test was applied to samples obtained from the experimentally created groups using the BESMAK BMT-100E universal testing device. Tibia diameters were taken as 4 mm. In all tests, the loading speed was 2 mm/s and the distance between the two end supports was adjusted and fixed as 14.5 mm according to tibia lengths. Before starting the tests, preloading of 20 N was applied to prevent soft tissue blanks on the bone surfaces.

Preparation of skeletal muscle tissue homogenates

At the end of the experiment, under ketamine (Ketasol, Richter Pharma AG, Vienna, Austria) and bacillazine (Rompin, Istanbul, Turkey) anesthesia, the rats were killed by cervical dislocation and the skeletal muscles were removed immediately. 0.5 g part of the muscle was used for the preparation of a homogenate. Phosphate buffer containing 5 mM pH: 7.4, 458 mM sucrose was prepared. 9.5 ml of buffer was added to the tissue sample taken into a plastic tube without a conical bottom. Samples were homogenized for 20 minutes at maximum speed with the TissueLyser LT (QIAGEN, The Netherlands). Homogenates were centrifuged at 8000 g for 60 minutes without waiting. The filtrate was taken into another tube. Then measurements were made.

Determination of catalase activity

Utilizing hydrogen peroxide as the substrate, catalase activities of the sample materials were quantified based on the Aebi method as follows. For replicates of two tubes, 2.8 mL of 30 mM H_2O_2 was added to the blank tube with an additional 0.2 mL of phosphate buffer being supplied after. For the sample tube, 2.8 mL of 30 mM H_2O_2 was added, and that was followed by the addition of 0.2 mL of enzyme. These tubes were vortexed to ensure thorough mixing and then the absorbance values reflecting the catalase activities were read twice at 240 nm at intervals of 30s (Aebi 1984).

Determination of reduced glutathione (GSH)

GSH levels were evaluated based on the yellow color that arose upon reacting erythrocyte sulfhydryl groups with 5,5′-dithiobis (2-nitrobenzoic acid). This process was conducted by spectrophotometer using blood samples with EDTA within 24 hours at 412 nm (Beutler et al. 1963).

Determination of malondialdehyde (MDA)

MDA is a product of peroxidation that occurs when fatty acids react with free radicals. MDA values were obtained based on observing the color that became visible upon the application of thiobarbituric acid (Gutteridge 1994).

Determination of ischemia-modified albumin (IMA)

In order to establish the levels of IMA in the obtained samples, 50 µL of cobalt chloride solution (serum, 200 µL; cobalt chloride, 1 g/L) was added. They were

then stirred together and permitted to incubate for 10 minutes at room temperature. Fifty µL of DTT solution (1.5 g/L) was then supplemented and the samples were mixed again and left to incubate for two more minutes. Then 1 mL of NaCl solution (9.0 g/L) was added. At the same time, sample blanks were prepared by following the same protocol with the only modification being a lack of DTT. Absorbance values were determined at 470 nm for all sample mixtures and were recorded in absorbance units. For all rats, adjusted-IMA (D-IMA) levels were found by using the values obtained for IMA and albumin as follows: [D-IMA = IMA × (Albumin/Median Albumin Level of the Group)]. These D-IMA values were also recorded in absorbance units (Bar–Or et al. 2000).

Statistical analyses

Descriptive statistics for the examined features; expressed as Mean and Standard Deviation. The normality of the distribution of the data was tested with the Kolmogorov-Smirnov test, and the homogeneity of the variances was tested with the Levene test. Since the normal distribution condition could not be met, Mann Whitney U test was used for paired group comparisons and Kruskal Wallis test was used for comparisons of more than two groups. Different groups were determined by Dunn's test, which is one of the non-parametric multiple comparison tests (Post Hoc). Statistical significance level was taken as $p < .05$ and SPSS statistical software version 19.0 (SPSS Inc, Chicago, III, USA) package was used for analysis.

Results

SAR results

Whole-body SAR levels were tabulated using the IEEE/IEC 62704–1 method. The average values for 900, 1800 and 2100 MHz were 0.026, 0.164, and 0.173 W/kg respectively (Bektas 2022).

Young's modulus values of the healthy 900, 1800 and diabetic 900, 1800, 2100 groups were statistically significantly less than those of the sham groups ($p = .016$. $p = .001$). Young's modulus, maximum load, maximum stress and yield point values of the RFR 2100 group with diabetes were considerably lower than those of the healthy RFR 2100 groups ($p = .001$, $p = .040$, $p = .040$, $p = .010$). Duration and displacement values of the diabetic RFR 900 group were also noticeably less than those of the healthy 900 group ($p = .015$, $p = .001$). The displacement value of the diabetic 900 group was considerably lower than that of the diabetic sham group ($p = .015$). The diabetic, healthy sham and 900 groups possessed duration values that were much greater than those of the diabetic and healthy 2100 groups ($p = .022$. $p = .010$). Displacement values in the healthy 1800 and 2100 groups were significantly reduced compared to the healthy sham and healthy 900 group values ($p = .001$). The displacement values in the diabetic RFR groups were smaller than those of the diabetic sham group ($p = .015$) (Table 1).

Lateral cortex values in the healthy sham were higher than they were in all healthy and diabetic RFR groups ($p = .001$). Medial cortex values in all healthy and diabetic RFR groups were significantly reduced compared to those of the healthy sham. Medial cortex values

Table 1. Descriptive statistics and results of comparisons for biochemical characteristics.

		Sham	900	1800	2100	p#
Young Modulus (GPa)	Healthy	2.93 ± 1.82[a]	0.82 ± 0.34[b]	1.47 ± 1.08[b]	2.17 ± 0.80[ab]	0.016
	Diabetes	1.56 ± 0.59 [a]	0.64 ± 0.25[b]	0.83 ± 0.19[b]	0.59 ± 0.21[b]	0.001
	p◊	0.111	0.343	0.181	0.001	
Maximum Load (N)	Healthy	67.60 ± 19.47	74.91 ± 12.43	69.23 ± 10.69	86.53 ± 16.50	0.139
	Diabetes	47.66 ± 13.12	62.09 ± 9.99	56.87 ± 15.46	63.11 ± 17.80	0.281
	p◊	0.064	0.086	0.117	0.040	
Maximum Stress (MPa)	Healthy	67.24 ± 19.36	74.52 ± 12.37	68.87 ± 10.63	86.08 ± 16.41	0.139
	Diabetes	47.41 ± 13.04	61.76 ± 9.94	56.57 ± 15.38	62.77 ± 17.70	0.281
	p◊	0.064	0.086	0.117	0.040	
Duration (s)	Healthy	106.40 ± 25.96[a]	109.74 ± 35.68[a]	82.79 ± 28.93[ab]	57.41 ± 29.33[b]	0.010
	Diabetes	125.29 ± 43.67[a]	62.32 ± 16.89[a]	87.19 ± 37.51[ab]	58.52 ± 14.84[b]	0.022
	p◊	0.367	0.015	0.815	0.936	
Yield Point (MPa)	Healthy	36.21 ± 29.94	54.26 ± 25.00	59.14 ± 20.18	68.74 ± 22.35	0.163
	Diabetes	26.73 ± 24.16	39.86 ± 25.91	31.67 ± 33.01	22.36 ± 27.96	0.763
	p◊	0.559	0.355	0.092	0.010	
Displacement (mm)	Healthy	3.01 ± .85[a]	3.11 ± .92[a]	2.09 ± 1.03[b]	1.21 ± 0.40[c]	0.001
	Diabetes	3.67 ± 1.22[a]	1.16 ± .27[c]	1.67 ± 1.43[b]	1.45 ± 0.38[b]	0.015
	p◊	0.298	0.001	0.499	0.308	

a. b. c. →: Different lower cases in the same row represent statistically significant difference among the groups ($p < 0.05$).
#Comparison of RFR frequency groups in healthy and diabetes groups.
◊Comparison of diabetes and healthy groups in each RFR frequency group
Note: No lettering was made for the features that did not find significant difference between groups.
Sample size of each treatment group was 7.

in the diabetic 2100 group were also reduced when compared to the healthy and diabetic, sham, and 900 RFR groups (Table 2).The width of the medulla of the healthy and diabetic RFR groups were considerably less than those of the healthy sham (Table 2).

GSH levels in the skeletal muscles of all healthy and diabetic RFR groups were lower than in the healthy and diabetic sham groups (p = .001, p = .001). The GSH levels of all diabetic rats were much decreased from the GSH levels of the healthy rats in their group (p = .004, p = .001, p = .001, p = .001) (Table 3).

When compared to the healthy sham group, CAT levels in the skeletal muscles of all healthy RFR groups were lower (p = .001). Except for the RFR 900 group, CAT levels in the skeletal muscles of all diabetic groups were lower than those in the healthy groups (p = .002, p = .009, p = .002) (Table 3).

IMA levels in skeletal muscles of all healthy RFR groups were greater than those of the healthy sham (p = .001), and the increase in IMA level increased as the frequency of the RFR increased. The levels of IMA in the rats with diabetes were reduced from those of the healthy rats in their group (p = .001, p = .001, p = .002, p = .001) (Table 3).

MDA levels in skeletal muscles of all healthy RFR groups were also greater than those in the healthy sham (p = .001). The MDA levels of all diabetic rats were lower than those of the healthy rats in their group (p = .034, p = .002, p = .002, p = .002) (Table 3).

Table 2. Descriptive statistics and comparison results for radiological characteristics.

		Median	Mean	Std. Dev.	Min.	Max.	p
Lateral cortex (mm)	Diabetes Sham	2.45	2.29 ^{bc}	0.48	1.63	2.94	0.001
	Healthy Sham	3.31	3.55 ^{a}	0.41	3.26	4.08	
	Diabetes 900	2.69	2.66 ^{b}	0.65	1.63	3.37	
	Healthy 900	2.45	2.56 ^{b}	0.56	1.63	3.26	
	Diabetes 1800	1.72	1.83 ^{c}	0.31	1.63	2.45	
	Healthy 1800	1.37	1.40 ^{c}	0.74	2.45	4.16	
	Diabetes 2100	1.63	1.90 ^{c}	0.42	1.63	2.45	
	Healthy 2100	1.52	1.70 ^{c}	0.37	2.45	3.37	
Medial cortex (mm)	Diabetes Sham	2.52	2.29 ^{cd}	0.56	1.15	2.58	0.001
	Healthy Sham	3.32	3.31 ^{a}	0.54	2.45	4.16	
	Diabetes 900	2.45	2.56 ^{bc}	0.62	1.63	3.26	
	Healthy 900	2.45	2.58 ^{bc}	0.87	1.63	4.08	
	Diabetes 1800	1.73	1.83 ^{de}	0.31	1.63	2.45	
	Healthy 1800	1.79	1.86 ^{de}	0.41	2.45	3.37	
	Diabetes 2100	1.63	1.59 ^{e}	0.19	1.22	1.82	
	Healthy 2100	1.88	1.98 ^{de}	0.39	2.45	3.37	
Medulla (mm)	Diabetes Sham	8.18	8.35 ^{bcd}	0.59	7.52	9.12	0.001
	Healthy Sham	9.81	9.67 ^{a}	1.30	8.20	11.90	
	Diabetes 900	7.30	7.20 ^{cd}	0.38	6.58	7.74	
	Healthy 900	7.73	7.45 ^{bcd}	1.17	6.53	9.52	
	Diabetes 1800	8.04	7.85 ^{bcd}	0.79	6.58	8.79	
	Healthy 1800	8.16	8.15 ^{bcd}	1.11	6.53	9.55	
	Diabetes 2100	7.46	7.05 ^{d}	1.00	5.47	8.04	
	Healthy 2100	8.50	8.53 ^{b}	1.02	7.35	9.79	

↓a.b.c.d.e: For each parameter, different lower cases in the same column represent statistically significant differences among the groups (p < 0.05). Sample size of each treatment group was 7.

Table 3. The results of GSH, CAT, IMA and MDA in skeletal muscle.

		Sham	900	1800	2100	p$^{\#}$
GSH	Healthy	0.07 ± 0.00^{a}	0.04 ± 0.00^{b}	0.01 ± 0.00^{c}	0.01 ± 0.00^{c}	0.001
(mmol/mL)	Diabetes	0.02 ± 0.00^{a}	0.001 ± 0.00^{b}	0.0002 ± 0.00^{c}	0.0002 ± 0.00^{c}	0.001
	p$^{\lozenge}$	0.004	0.001	0.001	0.001	
CAT	Healthy	1.06 ± 0.02^{a}	0.78 ± 0.02^{c}	0.86 ± 0.05^{b}	0.43 ± 0.02^{d}	0.001
(U/L)	Diabetes	0.83 ± 0.05	0.78 ± 0.03	0.79 ± 0.03	0.81 ± 0.03	0.191
	p$^{\lozenge}$	0.002	0.847	0.009	0.002	
IMA	Healthy	0.07 ± 0.02^{d}	0.15 ± 0.02^{c}	0.22 ± 0.02^{b}	0.88 ± 0.04^{a}	0.001
(ABSU)	Diabetes	0.001 ± 0.00	0.001 ± 0.00	0.002 ± 0.00	0.001 ± 0.00	0.904
	p$^{\lozenge}$	0.001	0.001	0.002	0.001	
MDA	Healthy	10.17 ± 0.04^{c}	12.23 ± 0.21^{b}	15.40 ± 0.59^{a}	18.29 ± 0.32^{d}	0.001
(nmol/L)	Diabetes	10.03 ± 0.24	10.12 ± 0.33	10.29 ± 0.22	10.42 ± 0.29	0.063
	p$^{\lozenge}$	0.034	0.002	0.002	0.002	

a. b. c. →: Different lower cases in the same row represent statistically significant difference among the groups (p < 0.05).
$^{\#}$Comparison of RFR frequency groups in healthy and diabetes groups.
$^{\lozenge}$Comparison of diabetes and healthy groups in each RFR frequency group
Note: No lettering was made for the features that did not find significant difference between groups.
Sample size of each treatment group was 7.

Discussion

The goal of this study was to investigate the biomechanical and morphological alterations in the right tibia bones and GSH, CAT, IMA and MDA levels in the skeletal muscles of diabetic and healthy rats exposed to RFRs at 900, 1800 and 2100 MHz frequencies.

In this study, Young's modulus values in the healthy and diabetic RFR groups, except for the healthy 2100 group, were significantly reduced from the healthy and diabetic sham groups. It is apparent that RFR considerably reduces bone flexibility. Young's modulus, max load, max stress and yield point values of the diabetic 2100 RFR group were considerably lower than those of the healthy 2100 RFR group. This result shows that 2100 MHz RFR considerably increases the decrease caused by diabetes in the endurance of bones against applied force. Moreover, the duration values in the diabetic and healthy 2100 RFR groups were noticeably less than the duration values in the sham groups. In addition, the duration value in the diabetic 900 RFR group was considerably lower than that of the healthy 900 RFR group. Therefore, 900 MHz RFR increased the damage caused by diabetes to the tibia bone more. The displacement values in the diabetic RFR groups were also reduced from those of the diabetic sham group. Moreover, displacement values in the healthy 1800 and 2100 groups were considerably lower than those of the healthy sham and healthy 900 groups This result indicates that exposure to RFR in both diabetic and healthy rats reduces bone flexibility.

Lateral cortex lengths in all healthy and diabetic RFR groups were lower than that of the healthy sham ($p = .001$). Medial cortex lengths in all healthy and diabetic RFR groups were significantly reduced compared to that of healthy sham. Medial cortex length in the diabetic 2100 group was lower than those of the healthy and diabetic sham and 900 RFR groups. Medial gap lengths in all healthy and diabetic RFR groups were considerably reduced compared to those of the healthy sham. These results may be an indication that RFR causes damage to the bone tissue or that the bone tissue cannot be nourished.

Maximum stress, maximum load, yield point, Young's modulus, displacement and duration are important intrinsic parameters that determine bone strength and are associated with the mineral and collagen structure of bone tissue (Burr 2002; Reddy et al. 2001). Changes (increase or decrease) in these parameters may indicate various threats such as increase or decrease in mineralization, increase in crystallization, denaturation in collagen, which are the precursors of different bone diseases (Burr 2002; Turner 2002). Bone morphometric features such as lateral cortex, medial cortex, and medial gap are closely related to bone biomechanics and bone components (Gürgül et al. 2008). Significant decreases in these parameters are indicative of a decrease in bone strength (Akar et al. 2022; Ferretti et al. 2001; Ito et al. 2002). The results of both morphological and biomechanical analyses obtained in this study show that RFRs reduce bone strength and are consistent with other research results (Akar et al. 2022). Similarly, it was stated that morphological and biochemical analyses of the cortical femurs of adult Wistar rats exposed to 2450 MHz RFR (2 hours/day, for 21 days, 0.079 W/kg SAR) revealed adverse changes (Akar et al. 2022). In addition, the Sprague Dawley rats that were exposed to 900 and 1800 MHz continuous wave RFR (30 min/day for 5 days/week for 4 weeks, 2 W/kg) saw a decrease in BMD levels (Yıldız et al. 2006). A human study, showed that bone mineralization was negatively affected in people regularly using mobile phones (Saraví 2011). Histological and morphological abnormalities were detected in bone, muscle and cartilage tissues of rats exposed to 1800 MHz RF (the modulation frequency was 217 Hz with a pulse width of 577 μsec and a maximum power of 2 W, for 6, 12, 24 hours/day for 20 days) during the prenatal period (Erkut et al. 2016). Another study evaluated GSM 900 and 1800 MHz RFR on Wistar rats (2 hours/day for 90 days), and revealed that as a result of radiological, biochemical, clinical, and histological evaluations, negative growth and prolongation in growth processes were detected (Nisbet et al. 2016). On the other hand, that the healing of bone fracture in Wistar Albino rats is slowed by exposure to 900 MHz RFRs (30 min/day, 5 days a week for 8 weeks, SAR: 0.008 W/kg) (Aslan et al. 2013). However, in one study, it was found that GSM 1800 MHz RFR (30 min/day, 5 days a week, 8 weeks, and 0.008 W/kg SAR) was not effective in bone fracture healing in Wistar Albino rats (Aslan et al. 2014). It has been stated that 2100 MHz RFR (3 hours/day, 7 days a week for 28 days, SAR: 0.1 W/kg) has positive effects on bone fracture healing in Wistar Albino rats (Durgun et al. 2016).

The majority of studies on RFR interaction with components of living things suggest that RFRs lead to the activation of pathways that produce ROS and changes in antioxidant enzyme activation (Dasdag and Akdag 2016; Yakymenko et al. 2016). Also, another effect induced by RFRs is Ca^{2+} ion transitions. Numerous studies have revealed that RFRs increase the amount of intracellular Ca^{2+} and as a result, they can cause the activation of many Ca^{2+} dependent signaling pathways (Bektas 2021; Nazıroğlu et al. 2012; Yakymenko et al. 2016). A significant decrease in Ca^{2+} content in the tibia bones of rats exposed to RFRs

(900 MHz, for 28 days, SAR: 0.69 W/kg) from mobile phones was detected. It has been stated that the decrease in the Ca^{+2} content in the bones is due to either the transition of Ca^{+2} into the blood or the remodeling of the hydroxyapatite structure in the bones with the effect of RFRs emitted from Nokia 5110 Mobile Phone (Sieroń-Stołtny et al. 2015). It is also known that the regulation of Ca^{+2} ions is important for bone health and processes (Gürgül et al. 2008).

Ischemia-modified albumin (IMA) is formed by modification of albumin caused by reactive oxygen derivatives formed as a result of ischemia. IMA is also used as a marker of oxidative stress (Özsürekci et al. 2021). In this study, MDA levels in skeletal muscles of all healthy RFR groups were higher than those of the healthy sham, and the increase in MDA level increased as the frequency of the RFR increased. The MDA levels of all diabetic groups were lower than those of the healthy rats in their group. MDA and IMA are important oxidative stress markers. In this study, while CAT and GSH values in skeletal muscles of the rats exposed to RFRs were decreased, MDA and IMA values were greatly increased. This result indicates that RFRs causes oxidative stress in skeletal muscles. Similarly, oxidative stress due to significant changes in the production of reactive oxygen products in the skeletal muscles of rats exposed to EMF (40 Hz, 7 mT for 0.5 h/day for 14 days) and a decrease in protein concentrations in muscle homogenates were detected (Ciejka et al. 2010). In another study, it was determined that electromagnetic fields (50 Hz, 1mT) caused an increase in the production of reactive oxygen species, a decrease in antioxidant enzyme activation, and an increase in oxidative stress parameters such as MDA and PCO in vitro model C2C12 skeletal muscle cells (Morabito et al. 2010). The fact that increased oxidative stress is associated with muscular dystrophy and skeletal muscle degeneration has been well documented (Choi et al. 2016). In another study, it was reported that bone and muscle tissue development is adversely affected in rats exposed to RFRs (1800 MHz, 6, 12, 24 hours/day, for 20 days) during the prenatal period (Erkut et al. 2016).

Conclusion

The results of this study show that mobile phone-derived RFRs (900, 1800 and 2100 MHz) may cause adverse effects on tibia bone and skeletal muscle health, according to bone biomechanics and morphological analysis evaluations and determined skeletal muscle oxidative stress parameters. It was observed that some of these adverse effects intensify with the increase in the frequency of the exposed RFR. In addition, these results suggest that the effects of mobile phone-derived RFRs on bone and muscle tissue should be investigated further through both molecular and histological analyses.

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