

## The Effects of Mobile Phones on Diabetes and Appetite

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### Abstract

In this study we aimed to investigate the possible effects of 900 MHz GSM Radiofrequency radiations (RFRs) on ghrelin, irisin and nesfatin-1 in the blood, brain and adipose tissues of healthy and diabetic rats by using oxidative stress parameters. Twenty-four Wistar albino rats, each weighing approximately 230 g, were divided into four main groups: Sham (n:6), RF (n:6), Diabetes sham (n:6) and RF+Diabetes (n:6). While the rats in the experimental groups were exposed to 900 RFR for two hours a day, the rats in the sham group were kept under the same experimental conditions but with the RF generator turned off. TAS (Total antioxidant), TOS (Total oxidant), H<sub>2</sub>O<sub>2</sub> (hydrogen peroxide), insulin, ghrelin, nesfati-1, irisin levels in the blood, brain and adipose were determined. 900 MHz RFR significantly decreased TAS level in brain and adipose tissue of diabetic rats. It caused an increase in TOS levels in the brain and adipose of rats.

It caused an increase in H<sub>2</sub>O<sub>2</sub> levels in diabetic rats, a significant increase in irisin level in the brain of diabetic rats, and a decrease in ghrelin levels in the brain and adipose of diabetic rats. It caused a decrease in nesfatin-1 levels in the blood and adipose of diabetic rats. A decrease was observed in the blood insulin level of rats after RFR exposure. It was determined that 900 MHz RFRs caused changes in the redox balance in blood, brain and adipose tissue in diabetic and healthy rats, leading to disturbances in energy metabolism.

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### Introduction

With the developments in technology, mobile phones have become one of the indispensable tools of daily life. GSM modulated radiofrequency radiation (RFR) is used for image and sound transmission in mobile phones. The effects of these tools, which are used by people of all ages, on human health cause public concern. Especially in recent years, research on the effects of RFRs on health has increased. The results of the research also correlated RFRs with the changes in the incidence and prognosis of many diseases, especially brain tumors.

It was determined that long-term RFR exposure to pregnant women may cause some biochemical changes in their cord blood taken at birth<sup>1</sup>. It was reported that RFR causes changes

in blood sugar and insulin levels<sup>2,3</sup>. It was found that 900 MHz RF emitted from mobile phones (3 to 6 hours a day for 7 days) can cause damage to Langerhans islet cells, but not alter insulin secretion<sup>4</sup>. Moreover, in another study, it was revealed that having a higher prevalence HbA1c and type 2 diabetes mellitus in students exposed to high RF (925 MHz, 9,601 nW/cm<sup>2</sup>, 6 hours per day) was significantly higher compared to the students exposed to low RF (925 MHz, 1.909 nW/cm<sup>2</sup>, 6 hours per day)<sup>5</sup>. Altpeter et al. (1995) indicated that in Schwarzenberg, Switzerland, the incidence of diabetes in the population living within a radius close to a shortwave transmitter was higher compared to a population living far from the shortwave transmitter<sup>6</sup>. Although it was determined that long-term exposure to very low frequency electromagnetic fields may be associated with biological effects such as increased pancreatic islet size and decreased glucose level<sup>7</sup>, limited studies were conducted on the effects of exposure to RFR used in mobile communications such as 900 MHz<sup>5</sup>. Due to the increase in electromagnetic pollution and its ubiquitous nature, it is thought to be a factor of

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the increased incidence of diabetes<sup>8</sup>. The results obtained are impressive and they require further research. Stimulation of stress proteins by very low frequency or RF electromagnetic fields may explain the increases in glucose, but there are many ways electromagnetic fields can undermine our health that are currently unexplored<sup>9</sup>. However, the most prominent of these ways is the increase in free radicals or the weakening of the antioxidant defense systems.

We can say that exposure to RFR leads to excessive ROS production in living cells by looking at the results of previous studies<sup>10</sup>. Free radicals can cause harmful effects through direct damage due to oxidation of biological macromolecules<sup>11</sup>. ROS is an intrinsic part of cellular signaling cascades. For example, hydrogen peroxide functions as a second messenger in both insulin signaling and growth factor-induced signaling cascades<sup>12</sup>. So, these free radicals are also required for some metabolic processes<sup>13</sup>. The peripheral peptide, which was identified as an appetite enhancer in mammals, is the ghrelin hormone<sup>14</sup>. Ghrelin is a stomach-derived hormone that affects various processes related to eating, body weight and blood sugar regulation as well as stimulating the secretion of growth hormone<sup>15</sup>. It was reported that ghrelin can inhibit or stimulate insulin secretion in humans and rats by depending on the experimental conditions<sup>16</sup>.

Recent studies have shown that Nesfatin-1 has a regulatory role in glucose homeostasis<sup>17</sup>. It was reported in the literature that Nesfatin-1 reduces food intake and regulates energy homeostasis<sup>18,19</sup>. Data obtained from peripheral administration of Nesfatin-1 to mice were found to be similar to daily administration of subcutaneous insulin to patients with diabetes<sup>20</sup>. The determination of Nesfatin-1, which has antihyperglycemic and anorexigenic effects in beta cells containing insulin, showed its relationship with glucose metabolism.

Irisin, involved in energy homeostasis, is a promising regulator of glucose metabolism<sup>21</sup>. Irisin contributes to normoglycemia through functions in muscle, liver and adipose tissue. In addition to skeletal and cardiac muscle, irisin has also been detected in the brain (neurons and neuroglia)<sup>21</sup>.

In this study, we investigated the effect of RFR on blood brain and adipose tissues, nesfatin-1, ghrelin, irisin, blood sugar and insulin levels of

healthy and diabetic rats, through ROS production and redox balance.

## Materials and methods

This experimental study was carried out in the Experimental Research and Application Center of Van Yuzuncu Yil University. All stages of the study were designed and performed according to the guidelines of the Animal Experiments Local Ethics Committee of Van Yuzuncu Yil University (Protocol No: Date:2021/05-10).

Twenty-four Wistar albino rats were divided into 4 main groups: Healthy Sham (n:6), RF (n:6), Diabetes sham (n:6) and RF+Diabetes (n:6). All rats were kept in a plexiglass carousel for two hours a day for one month. While the rats in the experimental groups were exposed to 900 RFR for two hours a day, the rats in the sham group were kept under the same experimental conditions but with the RF generator turned off.

### Induction of experimental diabetes

Streptozotocin (STZ) is an antibiotic that produces pancreatic islet  $\beta$ -cell destruction and is widely used experimentally to produce a model of type 1 diabetes mellitus (T1DM)<sup>22</sup>. To induce experimental diabetes, the rats were fasted overnight (groups 3 and 4) and then 45 mg/kg of STZ was administered i.p. at a single dose. Blood samples were taken from rats 72 hours after STZ application for diabetes control. Blood glucose levels were measured by a glucometer (IME-DC). Blood glucose levels above 250 mg/dl were considered diabetic<sup>23</sup>.

### Exposure and field measurements

A GSM signal generator (900 PM10 type Everest Comp., Adapazari, Turkey), which produces 900 MHz band RF waveform identical to the one in mobile phones was used in the study to expose the rats. Emitted power (omnidirectional on the plane perpendicular to the antenna axis) of the generator was fixed during the exposure. The antenna of the generator was equivalent to that of a typical mobile phone. The rats were confined in a Plexiglas carousel and exposed to 900 MHz RF exposure emitted from the generator. The carousel was surrounded with electromagnetic absorber material backed by metal to isolate outdoor electromagnetic fields from the test set-up during the study duration of one month. Experimental set-up is illustrated in Figure 1.

Power density and the electrical field were measured by field probe TES 593 (Shenzhen, Guangdong, P.R.C). The rats in the exposure groups were exposed to RFR 2 h per day (5 days a week) for one month. For the sham groups, the rats were placed in the carousel and the same procedure was applied to the rats except that the generator was turned off, i.e., no RF signal was present. The antenna of the generator was placed at 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 (Figure 1). All rats were kept under identical conditions for one month with free access to food and water.



Figure 1. Experimental setup.

### Specific Absorption Rate Analysis

Simulation setup was created to match the experimental setup as much as possible. The applicator antenna, the Faraday cage, and the rats were modeled in 3D electromagnetic field solver using CST (CST AG, Darmstadt, Germany). Electromagnetic and thermal modelling within the CST is based on a technique called finite-integration technique which is a combined method of integral and differential solvers acting on space and time, respectively. Using IEEE/IEC 62704-1 method whole body SAR's were calculated as 0.026 W/kg. SAR at brain was calculated as 0.065 W/kg and 0.106 W/kg for 10g and 1g averaging, respectively. Maximum SAR at blood was calculated as 0.051 W/kg and 0.087 W/kg for 10g and 1g averaging, respectively. Rat voxel models and SAR distribution for 1g averaging are displayed in Figs. 2A, 2B, and Fig 2C respectively.

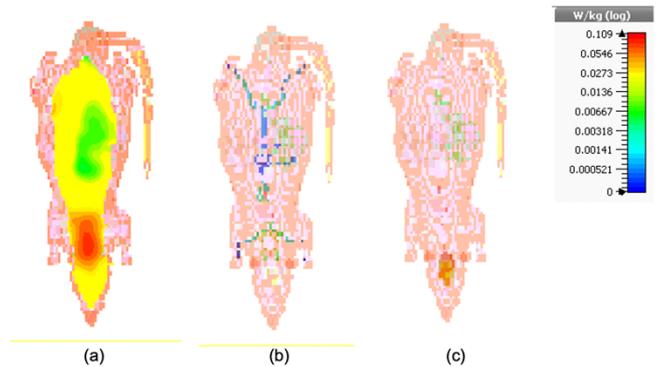


Figure 2. SAR distribution for 1 g averaging at 900 MHz, a) coronal cut, b) blood, c) brain.

### Sample collection

At the end of experiment, after the cardiac blood samples were obtained under ketamine (Ketasol, Richterphar Up, Vienna, Austria) and bacillazine (Rompin, Istanbul, Turkey) anesthesia, all rats were terminated. Blood samples were centrifuged at 4500 rpm for a period of 10 min.

### Preparation of brain and adipose tissues homogenates

At the end of treatment, the rats were euthanized by cervical dislocation, and the eyeballs were removed immediately for the preparation of a homogenate. The lens tissues were carefully washed, and their weights were recorded. The 10% homogenate was prepared in phosphate- buffered saline (PBS) and centrifuged at 10.000×g for 10 min. The supernatant was collected for further biochemical assays.

### ELISA Analyzes

Gherelin, nesfatin, irisin, insulin, TAS, TOS and H<sub>2</sub>O<sub>2</sub> concentrations in blood, brain and adipose tissues were determined using the sandwich Enzyme-Linked Immunosorbent Assay (ELISA) method. This includes Nesfatin-1 (SunRed. Biological Technology Co., Ltd., Shanghai, China, Catalog No: 201-11-1426), ghrelin (Sun Red Bio Biotech Co. Ltd Shanghai Shanghond; Cat Number: 201-1-1650) irisin (Sun Red Bio Biotech Co. Ltd Shanghai Shanghond; Cat Number: 201-1-1713), total antioxidant status (Sun Red Bio Biotech Co. Ltd Shanghai Shanghond; Cat Number: 201-11-2672), total oxidant status (Sun Red Bio Biotech Co. Ltd Shanghai Shanghond; Cat Number: 201-1-1669), H<sub>2</sub>O<sub>2</sub> (Sun Red Bio Biotech Co. Ltd Shanghai Shanghond; Cat Number: 201-11-3366), insulin (Sun Red Bio Biotech Co. Ltd.) Shanghai

Shanghond; Cat Number: 201-11-0708). Precoated ELISA kits were made in accordance with their protocols. All samples were measured on a Biotek ELx800 device at a wavelength of 450nm.

### Data Analysis

A computer program (SPSS 11.5, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data were analyzed by Kruskal–Wallis one-way analysis of variance (ANOVA) and Benforroni corrected Mann–Whitney U-tests. The number of specimens were low, which is why nonparametric tests, instead of parametric tests, were used for comparisons. In all hypothesis tests, a criterion level of  $p < 0.05$  was used.

### Results

	Blood sugar (mg/dL)	P	Weight (g)	P
Healthy Sham (start)	101,5	0.42	237,5	0.065
Healthy Sham (end)	93,5	3	261	
Healthy RF (start)	107	0.22	261.5	0.004
Healthy RF (end)	111	7	296	
Diabetes Sham (start)	366	0.87	281,5	0.045
Diabetes Sham (end)	368	3	265	
Diabetes RF (start)	321	0.41	278	0.109
Diabetes RF (end)	331	0	263	

**Table 1.** Body weight and fasting blood sugars of the experimental groups.

	Healthy Sham (Group 1)	Healthy + RF (Group 2)	Diabetes Sham (Group 3)	Diabetes + RF (Group 4)	P
Ghrelin pg/ml	Blood	1538,636 <sup>a</sup>	1533,383 <sup>ab</sup>	1370,145 <sup>bc</sup>	0.077
	Brain	3841,563 <sup>a</sup>	3573,509 <sup>b</sup>	3421,896 <sup>ab</sup>	0.023
	Adipose	4602,16 <sup>a</sup>	4436,067 <sup>ab</sup>	3867,801 <sup>a</sup>	0.033
Nesfatin-1 ng/L	Blood	1467,560 <sup>a</sup>	640,811 <sup>b</sup>	445,620 <sup>c</sup>	0.001
	Brain	1890,663 <sup>a</sup>	1791,339 <sup>a</sup>	1684,338 <sup>a</sup>	0.258
	Adipose	1440,122 <sup>a</sup>	1358,171 <sup>a</sup>	923,421 <sup>b</sup>	0.00
Irisin ng/ml	Blood	0,079 <sup>a</sup>	0,858 <sup>a</sup>	2,987 <sup>a</sup>	0.232
	Brain	12,273 <sup>a</sup>	12,294 <sup>a</sup>	25,483 <sup>b</sup>	0.001
	Adipose	14,845 <sup>a</sup>	16,048 <sup>a</sup>	17,055 <sup>a</sup>	0.264
TAS U/ml	Blood	4,348 <sup>a</sup>	3,925 <sup>a</sup>	3,334 <sup>a</sup>	0.047
	Brain	10,540 <sup>a</sup>	10,270 <sup>a</sup>	7,375 <sup>a</sup>	0.027
	Adipose	18,006 <sup>a</sup>	17,781 <sup>a</sup>	9,464 <sup>b</sup>	0.004
TOS nmol/ml	Blood	0,423 <sup>a</sup>	0,471 <sup>ab</sup>	0,667 <sup>b</sup>	0.009
	Brain	0,231 <sup>a</sup>	1,382 <sup>b</sup>	3,719 <sup>c</sup>	0.000
	Adipose	2,180 <sup>a</sup>	2,881 <sup>b</sup>	4,080 <sup>c</sup>	0.004
H <sub>2</sub> O <sub>2</sub> ng/ml	Blood	96,331 <sup>a</sup>	128,780 <sup>a</sup>	193,472 <sup>b</sup>	0.018
	Brain	378,191 <sup>a</sup>	416,163 <sup>a</sup>	726,259 <sup>b</sup>	0.000
	Adipose	694,655 <sup>a</sup>	883,415 <sup>ab</sup>	754,393 <sup>a</sup>	0.016
İnsülin IU/L	Blood	5,856 <sup>a</sup>	2,618 <sup>b</sup>	1,660 <sup>bc</sup>	0.001

**Table 2.** The ghrelin, nesfatin-1, irisin, TAS, TOS, H<sub>2</sub>O<sub>2</sub>, blood insulin values in the blood, adipose and brain tissues of the experimental groups.

\*Statistical difference is observed between groups labelled by different letters ( $p < 0.05$ ), whereas no significant difference was observed between groups labelled by the same letter ( $p > 0.05$ ).

There was no significant change in blood glucose and weight gain in the 900 MHz RFR groups (Table 1). While there was a significant

difference between the groups in ghrelin levels in brain and adipose tissue ( $p = 0.023$ ,  $p = 0.033$ ), there was no difference in blood ( $p = 0.077$ ). In brain and adipose, the ghrelin level of group 4 was lower than group 1. There is a significant difference in nesfatin levels between the groups in blood and adipose ( $p = 0.001$ ,  $p = 0.00$ ). In the blood, nesfatin levels of group 2 are lower than group 1, and group 4 compared to groups 1 and 2. Nesfatin levels of groups 3 and 4 in adipose are lower than groups 1 and 2 (Table 2).

There was a significant difference between the groups only in the level of irisin in the brain (0.001). The irisin levels of groups 3 and 4 are quite low compared to groups 1 and 2. There was a significant difference in insulin levels in blood between the groups ( $p = 0.001$ ). The insulin level of group 2 is lower than group 1, and insulin levels of group 3 and 4 are lower than group 1 and 2 (Table 2).

TAS levels in all tissues differed between groups ( $p = 0.047$ ,  $p = 0.027$ ,  $p = 0.004$ ). TAS levels of groups 3 and 4 in adipose are quite low compared to groups 1 and 2. TAS level of group 4 in the brain is lower than all other groups. There was a significant difference between groups in TOS levels in all tissues ( $p = 0.009$ ,  $p = 0.00$ ,  $p = 0.004$ ). TOS levels of groups 3 and 4 in blood are higher than group 1. In brain and adipose, TOS levels of all groups are higher than group 1, and TOS levels of groups 3 and 4 are considerably higher than group 2. There was a significant difference between groups in H<sub>2</sub>O<sub>2</sub> levels in all tissues ( $p = 0.018$ ,  $p = 0.00$ ,  $p = 0.016$ ). While H<sub>2</sub>O<sub>2</sub> levels of groups 3 and 4 in blood and brain were higher than groups 1 and 2, H<sub>2</sub>O<sub>2</sub> levels of group 4 were higher than all other groups in all tissues (Table 2).

### Discussion

In this study, changes in nesfatin-1, irisin, ghrelin, TAS, TOS, H<sub>2</sub>O<sub>2</sub> and blood insulin levels were detected in rats exposed to RFR in diabetic and healthy groups. According to the results of the study, it can be concluded that 900 MHz RFR is effective on energy metabolism and it reveals these effects through the changes it causes in the redox balance.

While some study results indicate that RFRs accelerate weight gain<sup>24-26</sup>, some studies claim the opposite<sup>3, 27</sup>. However, it is a known fact that oxidative stress is a parameter that

affects food intake<sup>28</sup>. According to our study results, 900 MHz RFR did not cause a significant difference in the weight gain of rats.

According to the results obtained, 900 MHz RFR significantly decreased the TAS level in the brain and adipose tissue of diabetic rats. In brain and adipose, 900 MHz RFR caused an increase in TOS levels in both healthy and diabetic rats, and TOS increase only in the blood of diabetic rats; again, it caused an increase in H<sub>2</sub>O<sub>2</sub> levels in all tissues of diabetic rats. Similarly, 900 MHz RF was reported to cause oxidative damage in rat brains and changes in redox balance<sup>29</sup>. It was reported that 900 MHz RFR can cause changes in miRNA expression in the brain<sup>30</sup>. It was indicated by many studies that RFRs show their non-thermal effects via oxidative stress caused by disrupting the Redox balance<sup>10,11,13,31</sup>. Few studies stated the opposite<sup>32</sup>. Conflicting results may be due to differences in experimental setups and characteristics of the RFs used<sup>13</sup>.

In addition, our study results showed that RFR lowered blood insulin levels in diabetic and healthy rats. Similarly, Mortazavi et al. (2016) reported that RFRs lower blood insulin levels<sup>4</sup>. In addition, RFRs were noted to cause changes to insulin receptors<sup>33</sup>. However, in our results, it was observed that RF did not cause a significant change in blood glucose. Conversely, It was stated that RFR exposure caused an increase blood sugar<sup>34</sup>. On the other hand; Meo et al. Wistar found that exposure to RF (15 min, three months, GSM modulated) in albino rats caused an increase in fasting blood glucose and serum insulin, and they stated that the increase in fasting blood glucose was due to insulin resistance<sup>2</sup>.

In another study, it was determined that 2.4 GHz RF causes high plasma glucose and low plasma insulin levels, and it was stated that the reason for this may be excessive ROS production or decreased antioxidant level<sup>3</sup>. There are studies expressing that disruptions in the redox balance alter the protein function and insulin effect by changing the cysteine residues<sup>35</sup>. However, the results of the study on glucose metabolism of electromagnetic fields are contradictory and still no clear result has been obtained<sup>35</sup>.

According to reported study results, irisin is involved in many physiological processes, including energy metabolism<sup>36</sup>. In addition, an

increase in irisin level was reported in cases of oxidative stress and inflammation such as morbid obesity<sup>36,37</sup>. On the other hand, it was determined that a balanced diet reduces the irisin level<sup>38</sup>. In the results obtained from our study, it was determined that 900 MHz RF caused a significant increase in irisin levels in the brain of diabetic rats. It can be thought that the increase in irisin in the brain is due to oxidative stress caused by 900 MHz RF.

According to the results obtained in our study, it was observed that RFR caused a decrease in ghrelin levels in the brain and fat in individuals with diabetes<sup>39,40</sup>.

A decrease in the number of ghrelin positive cells due to oxidative stress and changes in energy metabolism in diabetics<sup>41</sup> and a decrease in blood ghrelin levels were reported<sup>42</sup>. Therefore, changes in the amount of ghrelin caused by RFR may be associated with oxidative stress. It was suggested that changes in blood glucose trigger changes in ghrelin secretion<sup>39,40</sup>. However, on the contrary, it was indicated that oxidative stress leads to an increase in ghrelin levels<sup>43</sup>. On the other hand, in a study by Tripathi et al. (2021), it was determined that RFR exposure caused an increase in ghrelin levels<sup>44</sup>. Contradictions in study results may be due to differences in experimental setups used<sup>44</sup>.

In this study, RFR caused a significant decrease in nesfatin levels in blood and fat in diabetic rats. Nesfatin, which is related to appetite, was reported to be effective in blood glucose and insulin secretion<sup>45</sup>. In addition, a decrease in nesfatin levels in diabetics was indicated<sup>46,47</sup>. In this study, it is thought that the changes in the nesfatin redox balance in the RFR groups caused a decrease in the nesfatin levels. The results obtained are also compatible with the literature<sup>48</sup>.

Due to their high reactivity and diversity of interactions, a particular set of ROS caused by Electromagnetic fields of is quite difficult to reveal<sup>49</sup>. However, the results of our study show that 900 MHz RFR causes changes in the secretion of the hormones insulin, ghrelin, nesfatin-1 and irisin, which are effective on appetite and energy metabolism. It can be stated that these changes are induced by disturbances in the redox balance. In addition, another study reported that diabetic people may be more vulnerable to RFR than the normal population<sup>9</sup>.

## Conclusions

The results of the study reveal that 900 MHz RF is another risk factor that has the potential to contribute to the development of diabetes. Moreover, they showed that diabetic rats were more sensitive to 900 MHz RFR originating from mobile phones. People with diabetes should avoid cell phone use as much as possible. We are aware of the importance of the mobile phone industry and the benefits it brings to our lives in every field. However, our results show that they are not so innocent in terms of human health. More comprehensive molecular studies are needed to explain the changes caused by RFRs in the secretion of hormones that play an active role in energy metabolism.

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## Declaration of Interest

The authors report no conflict of interest.

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