Indian Journal of Experimental Biology
Vol. 63, February 2025, pp. 134-141
DOI: 10.56042/ijeb.v63i02.10247

# Exercise and metformin effect on glucose homeostasis and gastrointestinal hormone levels in rats

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*Received 06 April 2024; revised 09 September 2024*

This study aimed to determine the effects of exercise and metformin on glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), insulin (INS), ghrelin (GHRL), and blood glucose levels. Forty-two male Wistar rats were divided into 6 groups as control (CONT), exercise only (EXE), metformin 100 mg/kg (Met100), metformin 200 mg/kg (Met200), metformin 100mg+exercise (Met100+EXE), and metformin 200mg+exercise (Met200+EXE). Metformin was administered intraperitoneally. Rats were subjected to an incremental exercise protocol. A 12-week study was conducted, including an adaptation period for exercise. At the end of the study, serum samples were obtained from the rats, and the levels of GLP-1, GIP, insulin, and ghrelin were determined using the ELISA method. The blood glucose levels of the Met200 group were lower than CONT, EXE, and combination groups. INS levels of the metformin and combination groups were lower than CONT group. GLP-1 level of EXE was higher than all groups. GIP levels of EXE and combination groups were higher than CONT and Met100 groups. The ghrelin levels of the exercise and Met200 group were higher than CONT group. As a result, it was determined that exercise and metformin had significant effects on glucose homeostasis and caused significant results on GIP, GLP-1, INS, and GHRL.

**Keywords**: Metabolism, Glucose, Diabetes, Incretin hormones

Metabolic disorders resulting from impaired glucose homeostasis, such as glucose intolerance and insulin resistance, increase the risk of developing diabetes, cardiovascular and kidney diseases[1]. Lifestyle changes and medication use are important to improve blood glucose and insulin levels and reduce the risk of developing chronic diseases. According to the International Diabetes Federation Diabetes Atla), the global prevalence of diabetes among individuals aged 20-79 was 10.5% in 2021, reaching 537 million people. It is projected to increase to 643 million by 2030 and is expected to reach 783 million by 2045, with a prevalence rate of 12.2[2]. Today, various studies are being carried out to reduce the risk of developing diabetes, minimize the severity of the damages caused by the disease, and provide recovery. As a result of these studies, new treatment methods are being developed[3].

Metformin is a drug in the biguanide group used in the treatment of type 2 diabetes mellitus T2DM, and exerts its effect mainly by inhibiting gluconeogenesis and glycogenolysis and reducing hepatic glucose output. Additionally, it is effective in the treatment of T2DM by increasing peripheral glucose uptake, reducing intestinal glucose absorption, altering the gut microbiome, and regulating the secretion of certain intestinal hormones[4]. Oral glucose intake leads to the release of incretin hormones, namely glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), from enteroendocrine cells in the intestine[5]. The contribution of incretin hormones to insulin secretion varies depending on the dose of glucose administered, typically ranging from approximately 25% to 75%[6].

GIP is released from enteroendocrine K cells in response to food intake and enhances insulin secretion in pancreatic cells in a glucose-dependent manner. Additionally, it supports the development and survival of pancreatic beta cells and contributes to the stimulation of adipogenesis[7]. GLP-1 is a product of the glucagon gene. It is released from enteroendocrine L cells into circulation shortly after nutrient intake[7]. GLP-1 reduces food intake and inhibits gastric emptying, thereby slowing the passage of nutrients into the intestine. Both GLP-1 and GIP stimulate insulin secretion by activating specific G protein-coupled receptors expressed on pancreatic β cells. GLP-1 also stimulates proinsulin gene expression,

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promoting the replenishment of insulin stores[8]. It also inhibits glucagon secretion[7]. Circulating incretins are rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4). The serum half-life of GIP is approximately 7.3 min and that of GLP-1 is 2 min[7].

Ghrelin, primarily released from the stomach, is a 28 amino acid peptide hormone that binds to the growth hormone secretagogue receptor (GHSR)[9]. Involved in maintaining energy balance, stimulating appetite and energy intake[10]. It also plays a role in regulating GLP-1 and insulin secretion[11].

The role of exercise is also important in preventing and treating diabetes and many other chronic diseases[12]. In addition, exercise, when combined with antihyperglycemic drugs, enhances the glucose-lowering effect of the drugs[13]. Exercise increases the levels of GLUT4 (Glucose Transporter Protein 4), which is a glucose transporter, facilitating the entry of glucose into cells and enhancing glucose metabolism within the cells[14]. At the same time, it also increases insulin levels, thereby reducing plasma glucose levels[13].

There are some common mechanisms that metformin and exercise use to maintain glucose homeostasis. Both metformin and exercise are effective in increasing the levels of 5'-adenosine monophosphate-activated protein kinase (AMPK), which is one of the mechanisms for insulin-stimulated glucose uptake[15]. Mitochondrial disorders trigger the development of insulin resistance[16]. Exercise and metformin are also effective in regulating mitochondrial function[17]. They regulate plasma glucose levels by altering the composition and functional capacity of the gut microbiota[18,19]. Both exercise and metformin have common effects on the regulation of incretin hormones. They also play a role in the regulation of plasma ghrelin levels, which affects blood glucose levels[10].

The aim of this study is to investigate the effects of combined application of exercise and metformin, which have common mechanisms of action in glucose homeostasis, on GLP-1, GIP, insulin, ghrelin, and glucose levels.

## Materials and Methods

#### Animals

The rats used in the study were obtained from the Duzce University Animal Research and Application Center. A total of 42 male Wistar rats, aged 3-4 months and weighing 295±33 grams, were housed under optimal conditions of 23°C room temperature, 60±5% humidity, and a 12:12 light-dark cycle, with *ad libitum* access to food and water. Ethical approval for this study was obtained from Duzce University Animal Experiments Local Ethics Committee with code number 2022/07/04. All study methods are reported in accordance with the Animal Research: Reporting of *in vivo* Experiments (ARRIVE) guidelines.

#### Groups, substances and doses

The animals were randomly divided into six subgroups control (CONT), only exercise (EXE), metformin_100 mg/kg (Met100), metformin_200 mg/kg (Met200), metformin_100 mg/kg+exercise (Met100+EXE), and metformin_200 mg/kg+exercise (Met200+EXE). In the study, 100 and 200 mg/kg doses of metformin (Biovision Inc. 155S Milpitas Blvd, Milpitas, CA, 95035 USA) dissolved in saline were administered intraperitoneally (ip). Metformin and saline were administered 30 min before exercise. As anesthetics, 90 mg/kg ketamine hydrochloride (Keta-Control, Mefar İlaç Sanayi A.Ş., Istanbul, Turkey) and 10 mg/kg xylazine hydrochloride (Vetaxyl, VET-AGRO Sp. z o.o., ul. Lublin, Polonia) were administered intramuscularly (im). All drugs were prepared daily.

#### Treadmill exercise protocols

The animals were given exercise training in order for the rats to learn to run regularly and not to have difficulty in subsequent running-based exercises. Exercise training protocols for the treadmill was adapted from the literature[20]. The rats in the exercise groups (EXE, Met100+EXE, and Met200+EXE) underwent a 12-day training period to acclimatize to their new environment, followed by 10 weeks of exercise. The training period (15 min/day) was designed to help the rats learn how to run on a horizontal treadmill (May Time 0804, Animal Treadmill). For the training of the animals, the first 2 days (1st-2nd days), the treadmill speed was set to 2 m/min. Subsequently, from the 3rd to the 5th days, the speed was increased to 5 m/min, and in the final 5 days (6th-10th days), the speed was further increased to 8 m/min. For the main study, an exercise protocol was followed as described in the literature[20]. The rats were subjected to an exercise protocol for 10 weeks, 6 days a week, 30 min a day, between 08.00 and 10.00 AM. Each day, the rats underwent three stages of exercise: 2 m/min for 5 min, 5 m/min for 5 min, and 8 m/min for 20 min. Rats in the sedentary CONT, Met100 and Met200 groups were kept on a static treadmill for the same duration without exercise.

**Termination of the study**

Blood was collected from the hearts of the animals in the groups by cardiac puncture under ketamine/xylazine anesthesia 24 h after the last administration. The animals were then sacrificed by cervical dislocation under anesthesia. Blood samples were centrifuged at 4000 rpm for 15 min and serum was removed and stored at -80°C until analysis. Commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits from SunRed (Shanghai SunRed Biological Technology, China) were used in the study. Following the manufacturer's protocols, GHRL (Cat: 201-11-1650), INS (Cat: 201-11-0708), GLP-1 (Cat: 201-11-0720), HSL (Cat: SRB-T-84624) and GIP (Cat: SRB-T-87158) levels were measured in serum by ELISA reader (Epoch Microplate Spectrophotometer, BioTek Instruments, Inc., Winooski, VT, USA). In addition, blood glucose levels were determined with OPTIMA (OK Biotech CO., LTD., Taiwan) blood glucose meter and strips.

**Statistical analysis**

One-Way Analysis of Variance (One-Way ANOVA) was used to compare the groups in terms of serum insulin, GLP-1, GIP, GHRL and glucose values and Tukey-Kramer Multiple Comparison Test was used to determine the different groups. Two-Way Analysis of Variance (Two-Way ANOVA) was used to compare body weights and Šídák's Multiple Comparison Test was used to determine the different groups. $P \leq 0.05$ was accepted as the statistical significance level. GraphPad Prism (version 9.0, La Jolla, California, USA) program was used for analysis. All values are reported as mean ± SD.

## Results

**Evaluation of the effects of exercise and metformin on body weight changes**

Statistically significant differences were observed in weight between groups in terms of initial body weight (IBW) and final body weight (FBW) measurements ($P<0.001$) (Fig. 1). It was determined that the mean weight measurements at the beginning of the experiment for the CONT, Met200, Met100+EXE, and Met200+EXE groups were statistically lower compared to the mean measurements at the end of the experiment ($P<0.001$).

**Evaluation of the effects of exercise and metformin on blood glucose levels**

When the groups were compared in terms of blood glucose levels, a statistically significant difference was found between the groups (*P*<0,001) (Fig. 2). Upon further detailed examination of the results, it was determined that the blood glucose levels of the Met200 group were significantly lower compared to the CONT, EXE, Met100+EXE, and Met200+EXE groups (*P* values were $P=0.01$, $P<0.001$, $P=0.020$ and $P=0.001$, respectively).

**Evaluation of the effects of exercise and metformin on insulin levels**

When comparing the groups in terms of mean INS levels, a statistically significant difference was

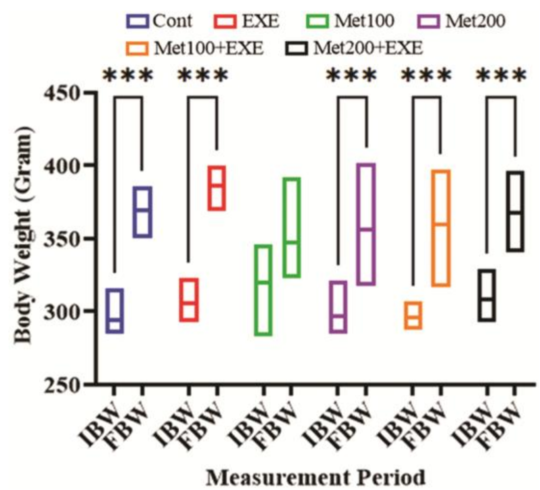


Fig. 1 — Effects of combined exercise and metformin on body weight (IBW: Initial body weight; FBW: Final body weight) (*$P<0.05$; **$P<0.01$ and ***$P<0.001$).

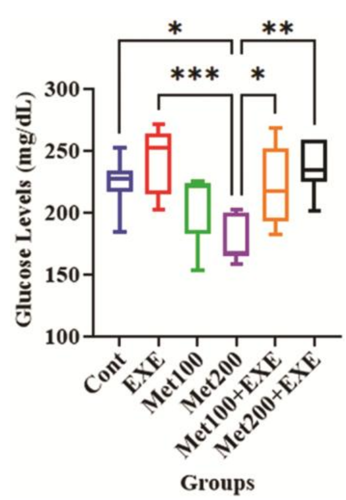


Fig. 2 — Effect of combined exercise and metformin on glocose level (*$P<0.05$; **$P<0.01$ and ***$P<0.001$).

found among the groups ($P$<0,001) (Fig. 3). Upon further examination of the results, it was observed that the INS level values of the Met100, Met200, Met100+EXE, and Met200+EXE groups were significantly lower than those of the CONT group ($P$ values were $P$=0.006, $P$=0.003, $P$=0.005 and $P$<0.001, respectively). The INS level values of the Met200 group were found to be significantly lower than those of the EXE group ($P$=0,046). Similarly, the insulin level values of the Met200+EXE group were also found to be significantly lower than those of the EXE group ($P$=0,005).

#### Evaluation of the effects of exercise and metformin on GLP-1 levels

When the groups were compared in terms of mean serum GLP-1 level, a statistically significant difference was found between the groups ($P$<0,001) (Fig. 4). When the results were analyzed in more detail, it was found that the GLP-1 level values of CONT, Met100, Met200, Met100+EXE and Met200+ EXE groups were significantly lower than the EXE group ($P$ values; $P$=0.02, $P$=0.006, $P$=0.006, $P$<0.001 and $P$<0.001, respectively).

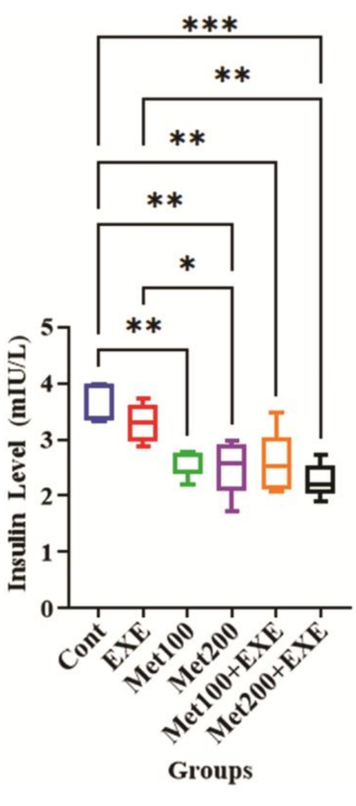


Fig. 3 — Effect of combined exercise and metformin on insulin level (*$P$<0.05; **$P$<0.01 and ***$P$<0.001).

#### Evaluation of the effects of exercise and metformin on GIP levels

In terms of GIP level, there was a statistically significant difference observed between the groups ($P$<0,001) (Fig. 5). Upon further examination of the

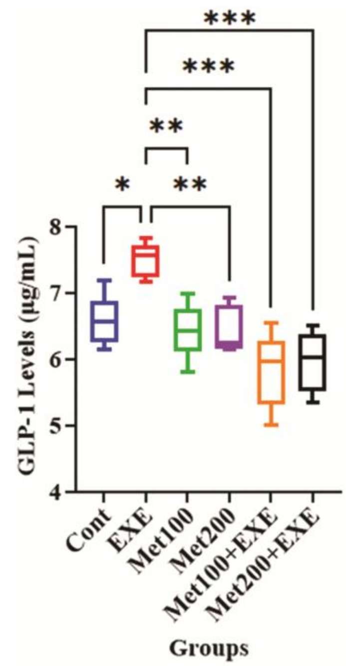


Fig. 4 — Effect of combined exercise and metformin on GLP-1 level (*$P$<0.05; **$P$<0.01 and ***$P$<0.001).

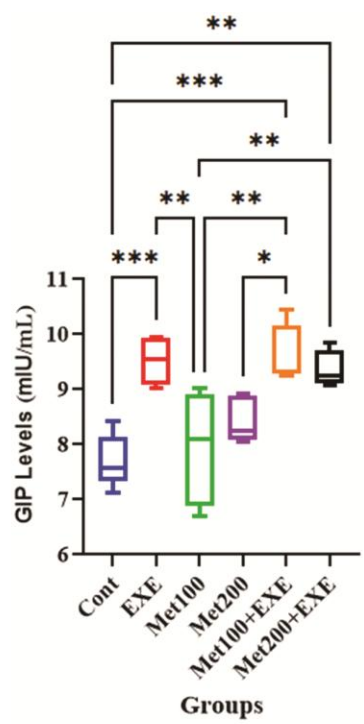


Fig. 5 — Effect of combined exercise and metformin on GIP level (*$P$<0.05; **$P$<0.01 and ***$P$<0.001).

results, it was found that the GIP level values of the EXE, Met100+EXE, and Met200+EXE groups were significantly higher than those of the CONT group (*P* values were *P*<0.001, *P*<0.001 and *P*=0.006, respectively). Similarly, it was observed that the GIP level values of the EXE, Met100+EXE, and Met200+EXE groups were significantly higher than those of the Met100 group (*P* values were *P*=0.003, *P*=0.001 and *P*=0.007, respectively). The GIP level values of the Met100+EXE group were found to be significantly higher than those of the Met200 group (*P*=0,030).

#### Evaluation of the effects of exercise and metformin on GHRL levels

When comparing the groups in terms of GHRL level, a statistically significant difference was determined among the groups (*P*=0,002) (Fig. 6). Upon further detailed analysis, it was found that the GHRL level values of the EXE group were significantly higher than those of the CONT and Met100+EXE groups (*P* values were *P*=0.004 and *P*=0.010, respectively). Similarly, the GHRL levels of the Met200 group were found to be significantly higher than those of the CONT group (*P*=0,030).

## Discussion

This study investigated the effects of metformin and exercise on GLP-1, GIP, INS, GHRL, and glucose levels in rats. At the beginning and end of the study, the weights of the animals were measured. The weights of the groups at the beginning of the experiment were found to be lower compared to the end of the experiment. Indeed, Hundal *et al*. did not find a significant result for metformin-induced weight loss[21]. Contrary to our findings, Ali *et al*. reported in their study that metformin induced weight loss in both healthy and streptozotocin-induced diabetic rats[22]. Due to the conflicting results of metformin on weight loss, the Food and Drug Administration (FDA) has not approved metformin as a weight loss agent[23]. The results of our study are not consistent with studies showing that exercise leads to weight loss. Since our experiment was performed on healthy rats with normal BMI, it is likely that no weight loss was observed. It is also possible that the increase in GHRL in the exercise group may increase appetite and lead to weight gain. In the study conducted by Eltonsy *et al*., no significant difference was found in weight loss with the combination of exercise and metformin[24]. In our study as well, no significant difference was found between the combination and exercise or metformin alone.

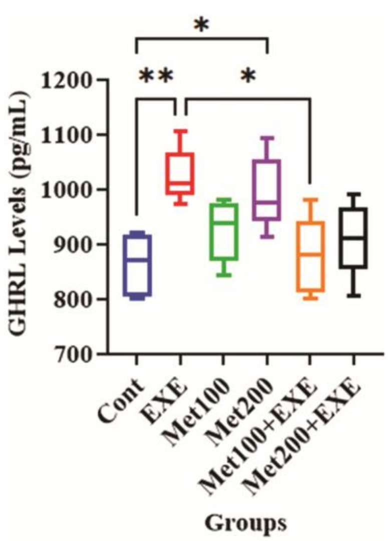


Fig. 6 — Effect of combined exercise and metformin on GHRL level (**P*<0.05 and ***P*<0.01).

In the present study, the blood glucose levels of the Met200 group were found to be lower than those of the CONT, EXE, Met100+EXE, and Met200+EXE groups. The fact that metformin decreases blood glucose levels by mechanisms such as inhibiting hepatic gluconeogenesis and activating AMPK supports the results of our study[4]. Our results are contradicted by studies reporting that exercise lowers blood glucose levels[15]. The high glucose levels observed in the EXE group are likely due to increased energy demand to meet the exercise requirements. The fact that the Met200 group had lower glucose levels than the combination group may be supported by the view that the inhibition of hepatic glucose production by metformin is blunted in the combination of metformin with exercise in the study by Hansen *et al*.[25]. Studies conducted with patients with glucose intolerance have reported that the combination of metformin and exercise results in higher plasma glucose levels compared to exercise alone[26,27]. In our study, however, while there was no significant difference between exercise alone and combination, the combination groups exhibited lower glucose levels. The differences observed among studies may stem from variations in measurement timing, duration and dosage of drug usage, type of exercise, duration, and timing of exercise sessions[24]. The results may also differ depending on whether the subjects are healthy or sick.

In the present study, INS levels were found to be low in Met100, Met200, Met100+EXE and

Met200+EXE groups. Kristensen *et al*. reported that metformin caused a decrease in plasma INS level in their study[28]. Metformin's enhancement of insulin sensitivity may lead to a decrease in plasma insulin levels. In addition, lower plasma glucose levels compared to the other groups are also likely to lead to a decrease in INS level. Some studies have indicated that metformin administration did not result in a significant change in plasma insulin levels[21,29]. According to the claim of Maida *et al*.[30] that metformin increases INS secretion by stimulating GIP and GLP-1 release, one reason for the low insulin levels in our study may be that metformin did not increase incretin hormones. In another study, it was demonstrated that exercise decreases plasma insulin levels[31]. In our study, a significant decrease in plasma insulin levels was not observed in the EXE group. This may be due to the healthy rats having normal glycemic levels. Likewise, the fact that INS level did not increase in the EXE group despite the increase in incretin hormones in our study supports this hypothesis. Similarly, INS level values in the Met200+EXE group were significantly lower than in the EXE group. Other studies have reported that combined exercise and metformin either increased or did not alter INS sensitivity[26]. On the contrary, another study reported that exercise reduced the insulin-sensitizing effect[25]. These results are consistent with our study.

In the current study, it was found that the GHRL levels in the EXE group were higher than those in the CONT and Met100+EXE groups. Studies conducted on healthy individuals have reported no change in plasma GHRL levels following different intensity and/or duration of exercise interventions[32]. In a study, it was reported that low-intensity exercise increased GHRL levels independently of duration compared to high-intensity exercise[33]. There are also findings showing that exercise decreases[34] does not change[35] or increases[36] GHRL levels in obese individuals. As a compensatory effect due to weight loss[37] and energy expenditure[38] resulting from exercise, total plasma GHRL levels increase. However, the lack of weight loss in the exercise group in our study contradicts these findings. The high level of GIP in the exercise group may have stimulated GHRL secretion[39]. In addition, due to the lack of studies looking at the effects of combination studies on GHRL levels, no data were found to support our conclusion that exercise increases GHRL more than combination and metformin alone. The GHRL levels in the Met200 group were found to be higher than those in the CONT group. In our study, no significant increase was found in the Met100 group, whereas a significant increase was observed in the Met200 group, which may be evidence that the results were affected by the dose. In addition, high levels of GIP in the metformin group may have stimulated GHRL secretion. Furthermore, GHRL's suppression of insulin secretion in pancreatic β-cells by attenuating cAMP formation induced by GLP-1 could be a reason for the low insulin levels observed in the metformin group.

The current study determined that the GLP-1 levels of the CONT, Met100, Met200, Met100+EXE, and Met200+EXE groups were lower than those of the EXE group. Exercise alone resulted in a greater increase in GLP-1 levels, whereas the combination with metformin resulted in a decrease in GLP-1 levels. Liu *et al*. showed that exercise increased GLP-1 levels more than metformin and combination therapy and also showed that hippocampal GLP-1 levels were higher in the exercise group[40]. Our findings are consistent with studies reporting that exercise increases GLP-1. In another study, it was found that metformin and combination therapy increased GLP-1 levels, while exercise alone did not increase GLP-1[41]. Although GLP-1 causes a decrease in food intake and weight loss, high GLP-1 levels were not effective in weight loss in our exercise group.

In the current study, the GIP levels of the EXE, Met100+EXE, and Met200+EXE groups were found to be higher than those of the CONT and Met100 groups. Studies in the literature have reported varying effects of exercise on GIP concentration, including decreases[42], no change[31] or increases[43]. Although in patients with type 2 diabetes mellitus (T2DM), exercise in combination with metformin has been reported to increase GIP concentration more than exercise alone[41] our study found that only exercise led to higher GIP levels compared to the combination. Additionally, the metformin-only group showed significantly lower GIP concentrations compared to the combination group. Some studies have reported that metformin increases GIP secretion[44]. While the literature data were generally consistent with our findings, some studies reported that metformin had no effect on GIP levels[30].

## Conclusion

The consistency of our results with many studies in the literature and the presence of supporting scientific data indicate that our study is reliable and can shed light on future research endeavors. The fact that incretins are not the main factor in the maintenance of glucose homeostasis by metformin is supported by the finding of low insulin and glucose concentrations in our study. Additionally, the effectiveness of exercise on incretin hormones has been determined. The supportive and regulatory effects of hormones on each other have also been observed in our study. The importance of exercise, which is an indispensable part of a healthy life, for glucose balance was once again demonstrated in our study. It was also observed that exercise may play a role in the treatment of metabolic disorders such as diabetes. However, in order to shed light on the conflicting results, the mechanisms of action of exercise and metformin on glucose homeostasis need to be elucidated.

## Ethics approval

Ethical approval for this study was obtained from Duzce University Animal Experiments Local Ethics Committee with code number 2022/07/04. All study methods are reported in accordance with the Animal Research: Reporting of *in vivo* Experiments (ARRIVE) guidelines.

## Author contributions

OB conceived and designed research; FO and OB performed experiments; OB analyzed data; FO and OB interpreted results of experiments; FO and OB prepared figures; FO and OB drafted manuscript; FO and OB edited and revised manuscript; FO and OB approved final version of manuscript.

## Funding statement

This project is supported by Düzce University Research Fund Project Number: 2023.04.01.1392.

## Acknowledgements

We thank Dr. Ersin Beyazcicek and Ali Gok for their help and support at Duzce University Experimental Animals Application and Research Center.

## Conflict of interest

The authors declare that there are no conflicts of interest related to this article.

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