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OSTARINE DOES NOT ENHANCE THE METABOLIC EFFECT OF EXERCISE IN OBESE RATS

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Overweight and obesity are associated with severe metabolic disorders and an increased risk of cardiovascular diseases. It is a known fact that physical activity has a positive effect on metabolic parameters, and also reduces the risk of diseases such as diabetes. Some products can enhance the rate of lipolysis and help in improving fat loss. One of these are selective androgen receptor modulators (SARMs) which act as anabolic agents and are also believed to aid in fat-burning. In this study, we investigated whether 30 days of ostarine administration could potentially improve metabolic parameters using the rat model of obesity combined with exercise. We assessed the levels of biochemical and hormonal parameters in serum samples as well as insulin sensitivity indices of tissues. There were significant changes in the metabolic parameters with exercise. However, we did not find any additive effects of ostarine and exercise on most of the parameters tested. Similar results were obtained from the analysis of gene expression and the concentration of leptin and adiponectin. Our results indicated that ostarine had a lowering effect on cholesterol concentration in the serum ($P < 0.05$). Moreover, when combining ostarine and exercise, additive changes were only observed in the levels of total and HDL cholesterol. No significant change was observed in the metabolic parameters of obese rats with the use of ostarine at the dose of 0.4 mg/kg body weight. Since ostarine is known to enhance performance, further research on its effects is needed.

Key words: *ostarine, enobosarm, male obesity, selective androgen receptor modulator, physical activity, lipolysis, lipogenesis, insulin, leptin, adiponectin, testosterone*

INTRODUCTION

The number of obese people has increased in recent decades. The World Health Organization declared obesity an epidemic in 1997 (1-3). Therefore, finding solutions that will help prevent this condition has become a growing challenge for scientists worldwide. Exercise and maintaining a healthy lifestyle is one of the easiest ways that aids in preventing obesity and treating its consequences by limiting body weight (4). In the last few years, an increasing number of people are showing interest in practicing a healthy lifestyle and regular physical exercise (5). However, this increase is also correlated with increased use of doping agents or weight-loss supplements by individuals, including obese people who want to lose weight quickly (6, 7). Selective androgen receptor modulators (SARMs) are used as supplements to support muscle growth and fat loss (8, 9). SARMs are a group of substances that mimic the positive effects of anabolic agents during steroid therapies but are much safer and have a limited androgenic effect on tissues (10). An example of SARM that

has been gaining increasing popularity is enobosarm, which is also known as ostarine (11).

To date, ostarine or Gtx-024 has been the best researched. It has an anabolic effect similar to that of steroids. Due to its nonsteroidal structure, ostarine does not undergo enzymatic transformation with aromatase or 5- α reductase, and thus characterized by a better safety profile (12). Several clinical trials have been conducted in healthy elderly men and cachexia patients. It has been shown that the use of ostarine results in significant improvement in muscle strength (14, 14). Although ostarine has not been approved by any drug committees, the promising results from clinical trials have prompted the sports community to become interested in this compound (13). Since it is not available as a pharmaceutical product, there is no defined dosage, and the doses used in clinical trials are very low. However, a case report revealed that the dosage used in the sports community is several times higher than that used in the clinical trials (15). In this study, we analyzed the effect of ostarine at a dose at which it is popularly used as a performance-enhancing drug. Literature data suggest that even higher doses

have been studied and can also be used by humans. However, due to both the medical potential of ostarine and the lack of evidence of its high-dose effects, further studies are needed.

Ostarine affects the carbohydrate-lipid metabolism, intensifies lipolysis, and inhibits lipogenesis in isolated rat adipocytes (16). Clinical trials have shown that administration of ostarine caused a decrease in fat mass by an average of 0.6 kg, as well as a decrease in glucose levels, resulting in improved insulin sensitivity (17). Moreover, studies on male and female osteoporosis models demonstrated the positive effect of ostarine on bone structure and bone mineral density (18, 19). However, adverse changes, such as prostate hypertrophy or elevated serum cholesterol levels, were also found in both models.

Moreover, a previous study showed that ostarine could stimulate lipolysis and inhibit lipogenesis in isolated rat adipocytes (16). It could also induce proliferation and differentiation of muscle C2C12 and L6 cells, as well as differentiation of rat muscle cells *in vivo* (20). The major purpose of this study was to investigate the potential impact of ostarine supplementation combined with exercise on the improvement of the metabolic status of obese rats.

MATERIALS AND METHODS

Animals

All procedures were performed according to the protocol approved by the Local Ethics Committee (protocol number: 39/2019).

Male Wistar (n=7 per group) rats were kept under standard conditions (temperature 21±1°C; daily cycle: 12 hours dark/12 hours light). The animals were handled for 14 days in the first stage of the experiment, and were then introduced to running on a treadmill. After acclimatization, obesity was induced in rats by providing a high-fat diet, in which 50% of the energy was from fat (Morawski, Kcynia, Poland). Obesity induction lasted for about 8 weeks and was confirmed by changes in the biochemical parameters and body weight. Then, rats were randomly assigned to one of the four experimental groups:

- 1) Obese (DIO);
- 2) Training (DIO+T);
- 3) Ostarine treatment (DIO+OST);
- 4) Training with ostarine treatment (DIO+OST+T).

The experiment administered lasted for 30 days. The rats in the DIO+OST and DIO+OST+T groups were treated with ostarine 0.4mg/kg of body weight, whereas the rats in the DIO and DIO+T groups were treated with the vehicle (ostarine dissolved in ethanol:PEG300 (10:90, v:v)). Ostarine or vehicle injections were performed subcutaneously. The DIO+T and DIO+OST+T groups were subjected to forced physical activity - the animals ran on a rodent treadmill (cat no.47300, UGO Basile S.R.L. Italy) at a speed of 20 m/min for 30 minutes every day for 30 days.

Tissue and blood collection

The rats were fasted overnight (12 hours) and then decapitated using a small animal guillotine. The blood was

collected into standard 11-ml polystyrene probes. To obtain the serum, the blood was centrifuged at 3500×g for 10 minutes, and the resulting serum was carefully removed from probes and transferred into Eppendorf tubes. The serum was divided into aliquots and frozen at a temperature of -20°C. The tissues were dissected and then quickly frozen using liquid nitrogen. The frozen tissue samples were then transferred to an ultrafreezer (-80°C).

HOMA IR, QUICKI, and McAuley

Homeostatic model assessment of insulin resistance (HOMA-IR) (21), insulin sensitivity check index (QUICKI) (22), and McAuley's (McA) index (McA) (23) were calculated from fasting glucose (G_0), insulin (I_0), and triglycerides (TG_0) levels using the following formulas:

$HOMA-IR = (G_0 \times I_0) / 22.5$; $QUICKI = 1 / [\log(G_0) + \log(I_0)]$;
 $McA = \exp[2.63 - 0.28 \ln(I_0) - 0.31 \ln(TG_0)]$.

Intraperitoneal glucose tolerance test

Intraperitoneal glucose tolerance test (ipGTT) was performed 4 days before the end of the experiment. Five animals from each experimental group were randomly selected and included in this test. Rats were fasted 4 h before starting the procedure. Fasting blood glucose was determined 5 minutes before the injection of glucose (time-0 glucose) into the tail ends using a Roche AccuCheck Active glucometer (Roche, Mannheim, Germany). The test was started by injecting an aqueous glucose solution (2 g/kg) into the peritoneum. The changes in the blood glucose concentrations were measured 5, 15, 30, 60, and 90 minutes after the administration of glucose.

Enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA)

Commercially available ELISA kits were used to determine the concentration of leptin, adiponectin (BioVendor, Brno, Czech Republic) and testosterone (LDN, Nordhorn, Germany) in blood serum. A commercial RIA test was used to assess insulin concentration in blood serum (Merck, Rahway, NJ, USA). The procedures were carried out in accordance with the manufacturers' instructions.

Biochemical analysis

Biochemical parameters in the serum were determined using commercially available colorimetric and enzymatic kits. The concentrations of glucose, triglycerides, cholesterol, high-density lipoprotein (HDL cholesterol), and low-density lipoprotein (LDL cholesterol) were measured using a Pointe Scientific kits (Canton, MI, USA). The concentration of nonesterified fatty acids (NEFA) was determined using an enzymatic test kit from Wako (Oxoid, Dardilly, France). The optical density of samples was measured using a Synergy 2 microplate reader (Biotek, Winooski, VT, USA).

Table 1. Sequence of primers.

Target	Forward primer (5'>3')	Reverse primer (5'>3')	Product (bp)
<i>GAPDH</i>	CTGCACCACCAACTGCTTAG	TGATGGCATGGACTGTGG	92
<i>Adiponectin</i>	TGGTCACAATGGGATACCG	CCCTTAGGACCAAGAACACCT	93
<i>Leptin</i>	CCAGGATCAATGACATTTTACA	AATGAAGTCCAAACCGGTGA	68

Real-time PCR

Total RNA was isolated from visceral adipose tissue using the Extrazol reagent as per the manufacturer's instructions (DNA Gdansk, Poland). cDNA was synthesized using 1 µg of total RNA and a high-capacity cDNA reverse transcription kit (Life Technologies, Grand Island, NY, USA). Real-time PCR was performed using a Quant Studio 12K Flex™ system with GAPDH as the reference gene. All the primer sequences used in PCR are listed in *Table 1*.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism Software (GraphPad Software, Inc., USA). The results are presented as mean ± standard error of the mean (SEM). The effect of the applied treatment was analyzed using one-way analysis of variance with Tukey's *post hoc* test.

RESULTS

Obesity induction

The effectiveness of obesity induction was verified in the first stage of the experiment, by determining the changes in body weight and basic metabolic parameters such as glucose, triglycerides, cholesterol, and NEFA in rats fed with the high-fat diet (HFD) in comparison to the animals fed with a standard feed. The analysis showed an increase in all the investigated parameters in obese rats compared to nonobese animals (body weight, $P<0.01$; glucose, $P<0.01$; triglycerides, $P<0.01$; cholesterol, $P<0.01$; and NEFA, $P<0.05$), which confirmed the effectiveness of obesity induction. The results of the analysis are presented in *Table 2*.

Body weight, ipGTT, HOMA IR, QUICKI, and McA index

Changes in the body weight of rats were monitored during the experiment (*Fig. 1A*). It was noted that training (DIO+T), as well

Table 2. Metabolic characterization of the model compared to nonobese rats.

Parameter	Nonobese n=7	Obese n=7
Body mass [mg/dl]	302.7±7.948	364.3±3.939**
Glucose [mg/dl]	95.00±4.11	115.5±4.36**
Triglycerides [mg/dl]	145.9±5.167	201.6±11.31**
Cholesterol [mg/dl]	84.04±4.65	128.1±4.69**
NEFA [mmol/l]	0.631±0.02	0.749±0.03*
Testosterone [ng/dl]	5.145±1.053	0.73±0.172**

Data are presented as mean ±SEM. Statistically significant changes in groups are marked * $P<0.05$ and ** $P<0.01$.

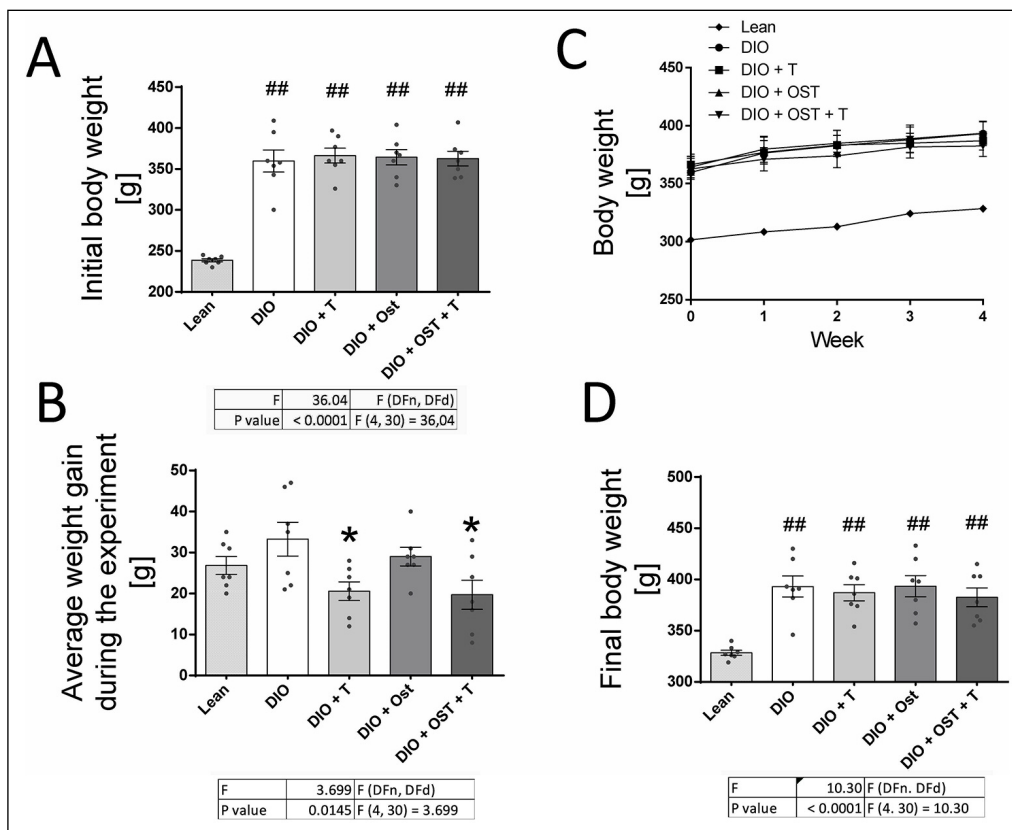


Fig. 1. (A) Initial body weight of rats before training and ostarine supplementation. Effect of training and ostarine supplementation on (B) body weight of rats during the experiment, (C) average weight gain, and (D) final body weight. Values are presented as mean ±SEM. Statistically significant changes in groups are marked compared to the Lean (# $P<0.05$ and ## $P<0.01$) and to DIO rats (* $P<0.05$ and ** $P<0.01$).

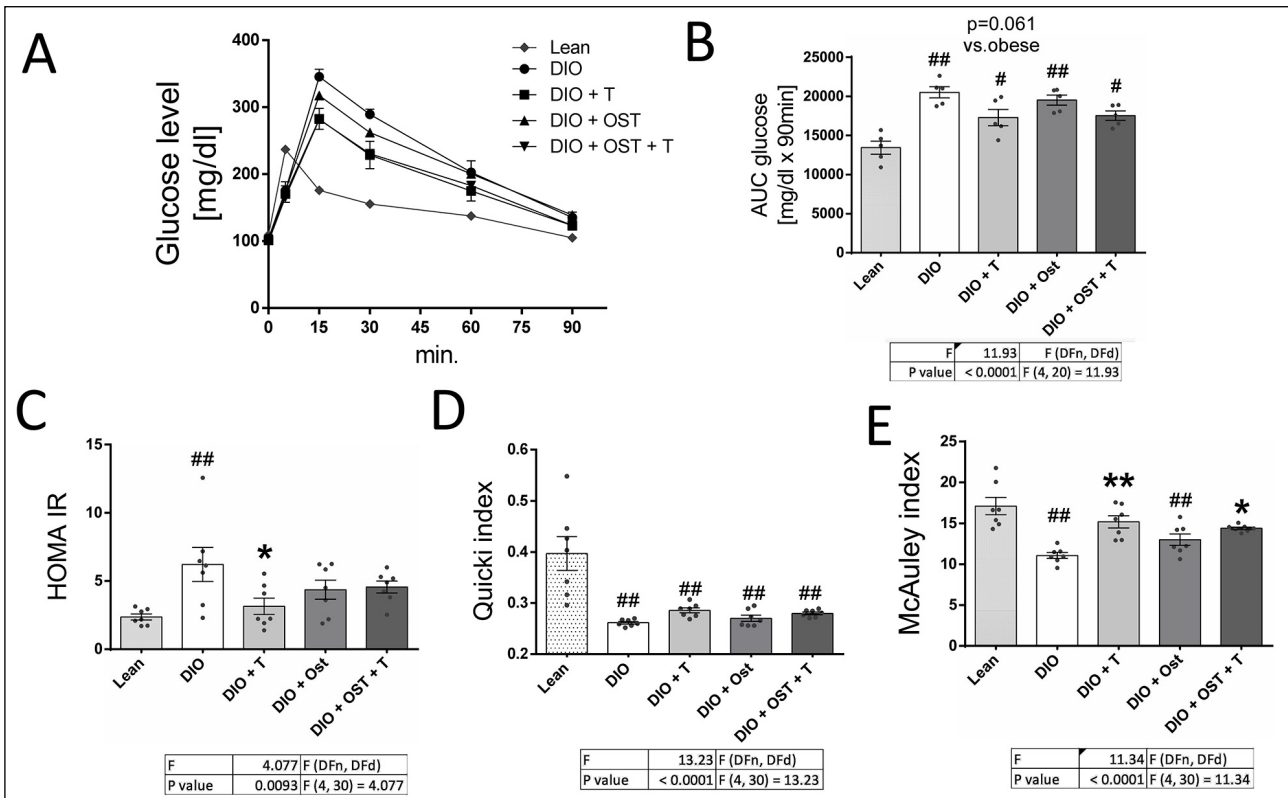


Fig. 2. Effect of treatment on (A) the rate of glucose utilization during ipGTT, (B) area under the curve for glucose, (C) HOMA-IR, (D) QUICKI, and (E) McA index. Values are presented as mean \pm SEM. Statistically significant changes in groups are marked compared to the Lean ($^{\#}P<0.05$ and $^{\#\#}P<0.01$) and to the DIO rats ($^*P<0.05$ and $^{**}P<0.01$).

as training combined with ostarine supplementation (DIO+OST+T), caused a decrease in the average weight gain compared to the DIO group (Fig. 1B; $P<0.05$). Although the average body weight gain in the Lean group of animals was slightly lower, we did not observe statistically significant changes in the average body weight gain between obese rats and the Lean group of animals. However, there were no differences in the final body weight between the investigated groups. We observed that body weight at the end of the experiment was higher in all groups of DIO rats compared to lean rats (Fig. 1D, $P<0.01$).

Next, the effect of ostarine, training, and training combined with ostarine supplementation on glucose utilization rate was investigated by ipGTT. The test revealed no differences with regard to glucose utilization in the ostarine group compared to the obese group. However, we observed an increasing trend in the glucose utilization rate in the DIO+T group (Fig. 2B, $P=0.061$) compared to the obese group. Despite this increase, the rate of glucose utilization in all DIO animal groups was lower than in the Lean group.

Moreover, It was found that rats in both DIO+T and DIO+OST+T groups showed greater insulin sensitivity to tissues, which was determined based on the HOMA-IR and McA index. A decrease in HOMA-IR was noted in the DIO+T (Fig. 2C, $P<0.01$) compared to the DIO group as well as an increase in McA index in the DIO+T (Fig. 2D and 2E, $P<0.01$) and DIO+OST+T (Fig. 2D and 2E, $P<0.05$) groups compared to the DIO group. In this groups we also observed no statistical differences in glucose sensitivity compared to lean rats, which we demonstrated in DIO and DIO+OST rats compared to lean animals (Fig. 2E, $P<0.01$). We also found that in all treated groups of animals (DIO+T, DIO+OST, and DIO+OST+T) HOMA IR was not statistically different from Lean animals. The result was slightly different in the case of the Quicki index. Although we observed a slight

increase in the DIO+T group compared to the DIO group, no statistically significant differences were found. However, unlike the HOMA IR index, we observed a statistically significant decrease in insulin sensitivity in all study groups compared to the Lean group (Fig. 2D, $P<0.01$).

Metabolic parameters and pancreas hormones

The concentration of metabolic parameters (glucose, triglycerides, NEFA, cholesterol, LDL, HDL) and hormones (insulin and glucagon) was measured in the blood serum. There were no changes observed in the concentration of glucose (Fig. 3A) or NEFA (Fig. 3C) between investigated groups. We observed only an increase in glucose and NEFA in all investigated groups compared to Lean rats ($P<0.05$). However, significant differences were noted in other lipid parameters. The level of triglycerides (TG) was lower in the DIO+T and DIO+OST+T groups (Fig. 3B, $P<0.05$). TG reduction in the DIO+T, DIO+OST and DIO+OST+T groups resulted in the concentration of this parameter not being statistically significantly different in these groups from the Lean group. Similarly, the concentration of cholesterol was lower in the DIO+T (Fig. 3D, $P<0.01$), DIO+OST ($P<0.05$), and DIO+OST+T ($P<0.01$) groups. In addition, a lower level of cholesterol was found in the DIO+OST+T group compared to the DIO+OST group ($P<0.01$). We obtained analogous results as in the case of TG when analyzing changes in cholesterol concentration compared to the Lean group. The reduction in the DIO+T, DIO+OST and DIO+OST+T groups resulted in the cholesterol concentration not being statistically significantly different in these groups from the Lean group.

We have not observed statistically significant changes in LDL cholesterol concentration between groups. However, a slight

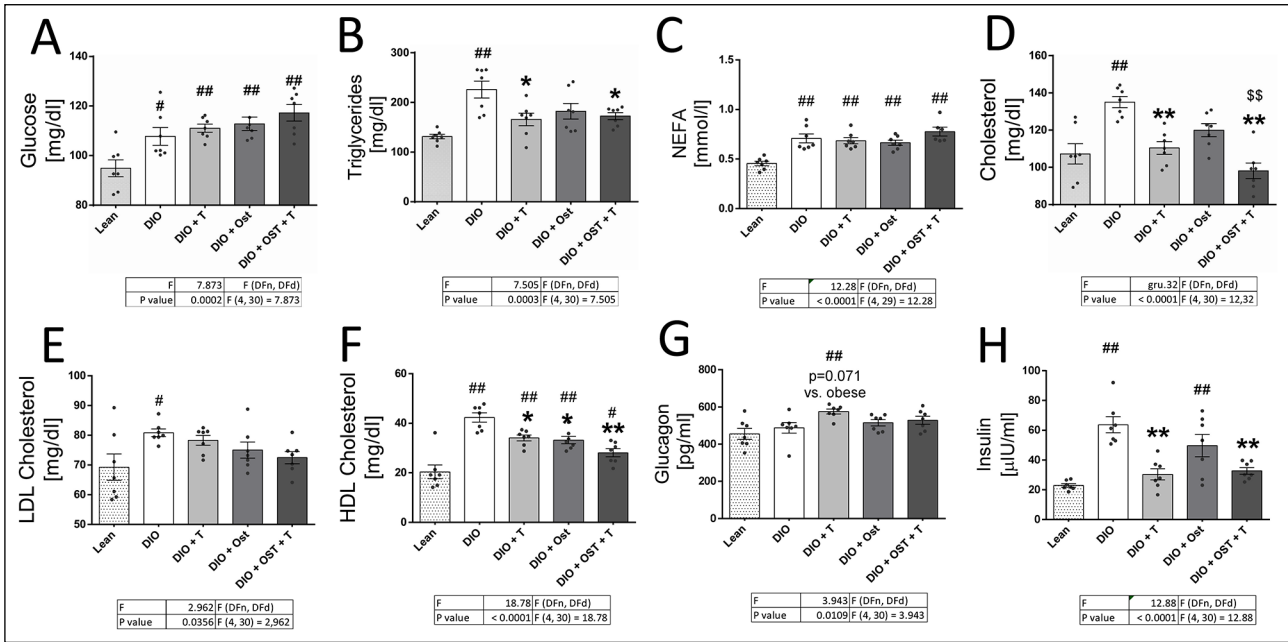


Fig. 3. Effect of ostarine supplementation and training on metabolic and hormonal parameters in blood serum in obese rats: (A) glucose, (B) triglycerides, (C) NEFA, (D) total cholesterol, (E) LDL cholesterol, (F) HDL cholesterol, (G) glucagon, and (H) insulin. Values are presented as mean \pm SEM. Statistically significant changes in groups are marked compared to the Lean ($^{\#}P<0.05$ and $^{\#\#}P<0.01$), to the DIO rats ($^*P<0.05$ and $^{**}P<0.01$) and to the DIO+OST rats ($^{SS}P<0.01$).

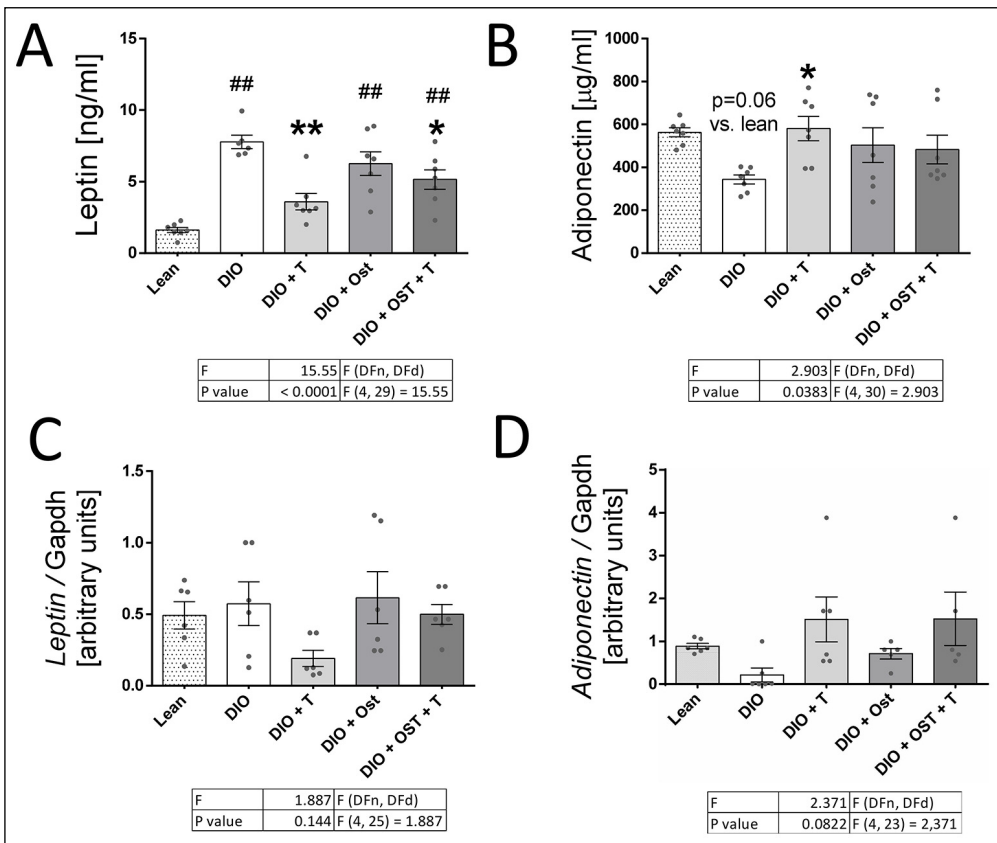


Fig. 4. Effect of training and ostarine treatment on leptin concentration (A) and adiponectin concentration in blood serum (B) and on leptin mRNA expression (C) and adiponectin mRNA expression in epididymal adipose tissue (D). Values are presented as mean \pm SEM. Statistically significant changes in groups are marked compared to the Lean ($^{\#}P<0.05$ and $^{\#\#}P<0.01$), the DIO rats ($^*P<0.05$ and $^{**}P<0.01$) and DIO+OST rats ($^{SS}P<0.01$).

reduction of LDL in DIO+T, DIO+OST, and DIO+OST+T groups resulted in we didn't note an increase of LDL in these groups compared to lean rats. This difference was observed only in the DIO group (Fig. 3E, $P<0.05$). We observed a decrease in HDL concentration in all investigated groups compared to DIO rats (Fig. 3F, DIO+T, DIO+OST - $P<0.05$ and DIO+OST+T - $P<0.01$).

There were also changes in the hormonal profile. However, no statistically significant changes were found in the groups treated only with ostarine. Glucagon concentration was higher in the DIO+T group (Fig. 3G, $P=0.071$) compared to DIO groups and compared to Lean animals (Fig. 3G, $P=0.01$). The insulin concentration was decreased in the DIO+T and DIO+OST+T

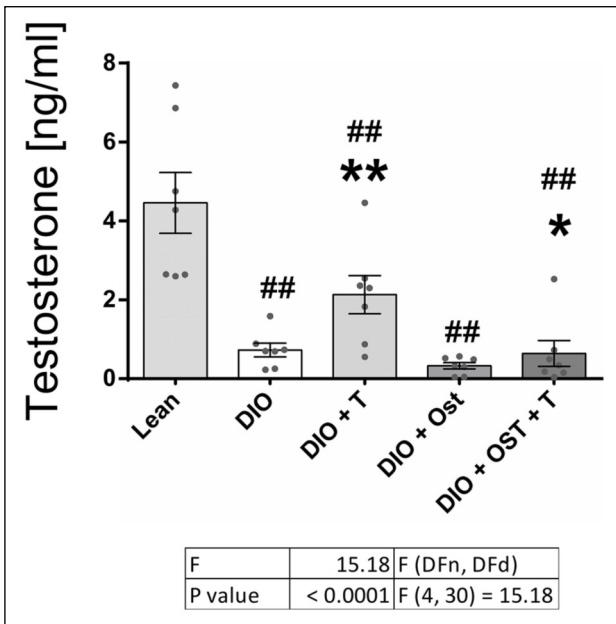


Fig. 5. Effect of training and ostarine treatment on testosterone concentration in blood serum. Values are presented as mean \pm SEM. Statistically significant changes in groups are marked compared to the Lean ($^{\#}P<0.05$ and $^{\#\#}P<0.01$), to the DIO rats ($^{\#}P<0.05$) and to the DIO+T rats ($^{\#}P<0.05$ and $^{\#\#}P<0.01$).

groups (Fig. 3H, $P<0.01$) compared to DIO rats. The statistically significant differences compared to lean rats was observed only in DIO and DIO+OST groups (Fig. 3H, $P<0.01$).

Leptin and adiponectin

The analysis of the effect of ostarine treatment and training on mRNA expression and secretion of leptin and adiponectin in serum revealed a decrease in leptin concentration in the DIO+T (Fig. 4A, $P<0.01$) and DIO+T+OST (Fig. 4A, $P<0.05$) groups compared to DIO rats. Unlike the other groups, the DIO+T group did not differ statistically in terms of leptin concentration from Lean rats. The concentration of this adipokine in the remaining groups was statistically significantly higher compared to Lean animals (Fig. 4A, $P<0.01$). We found also that the level of adiponectin was found to be higher only in the DIO+T group (Fig. 4B, $P<0.05$) compared to DIO, where, we also noted a downward trend in adiponectin concentration compared to the Lean group (Fig. 4B, $P=0.06$). We didn't observe statistically significant differences between DIO+OST and DIO+OST+T compared to Lean animals in the concentration of adiponectin. There were no statistically significant differences in mRNA expression.

Testosterone

The rats subjected to training showed a higher concentration of testosterone compared to the control (Fig. 5, $P<0.05$). The group receiving ostarine DIO+OST showed a statistically lower concentration of testosterone compared to the running group DIO+T (Fig. 5, $P<0.01$). Ostarine also lowered the concentration of testosterone in the group DIO+T+OST (Fig. 5, $P<0.05$). Despite the observed increase in testosterone concentration, its concentration was statistically significantly lower in all study groups compared to the Lean animal group.

DISCUSSION

Our study showed that ostarine did not enhance the metabolic effect of exercise in obese rats. Based on the previous research, which proved that ostarine modulates the *in vitro* metabolism of fat tissues, increasing lipolysis (16) as well as stimulates the differentiation of L6 and C2C12 cells *in vitro* and muscle cells in healthy rats *in vivo* (20). It can be concluded that ostarine has the potential to support/enhance weight loss through exercise. To demonstrate this, we conducted an *in vivo* experiment on obese rats that were subjected to forced physical training, ostarine supplementation, and training combined with ostarine supplementation, to examine whether the administration of this substance supports weight loss spontaneously or if it weight loss improves with exercise in rats. Moreover, the effect of different kinds of training or exercise *i.e.* low-volume training internal improves metabolic status by an effect on the liver and cardiometabolic health (24). We decided to combine the potential effects of both to investigate to increase the effectiveness of training and weight loss. We found that 30 days of training, ostarine supplementation, as well as training combined with ostarine supplementation caused metabolic improvement. However, no additive effect of training and ostarine supplementation was observed compared to training alone.

Research on SARMs, including ostarine, has been going on for many years; however, data on its possible use are scarce. Ostarine has shown promising results in Phase I and II clinical trials, including an increase in total lean body mass, improvement in functional performance, and a decrease in total tissue percent fat in cancer cachexia (12, 25). However, to date, ostarine has not been authorized legally on the pharmaceutical market (26). In the first stage of our research, we found that exercise combined with ostarine supplementation caused a decrease in weight gain in obese rats. The results of our research seem to complement those obtained by other researchers who showed that the administration of ostarine increased lean body mass. The effect of long-term administration of ostarine on body weight remains unclear. Hoffmann *et al.* examined ovariectomized rats injected with ostarine for 13 weeks. However, their results showed no effect of ostarine on the body weight of these animals (27). The comparison of the effect of ostarine supplementation using these models allows for the conclusion that ostarine does not affect the body weight of obese animals, regardless of sex. Though in the cited study, the rats did not receive HFD, ovariectomy caused an increase in the body mass of the animals, as seen in the charts presented by the authors (27).

Weight loss is also associated with the improvement of metabolic indicators and the improvement of the carbohydrate-lipid profile (lowering cholesterol and/or triglycerides and/or glucose concentration, changing the HDL:LDL ratio) can significantly improve the metabolism of the obese body (28). In addition, it affects the concentration of two main pancreatic hormones responsible for carbohydrate metabolism - insulin and glucagon. In this study, we examined the effect of ostarine on these parameters. As with body weight, no additive effect of ostarine was observed. The most noticeable changes observed were changes in the levels of total cholesterol and in HDL and LDL cholesterol fractions. There was a decrease in total and LDL cholesterol in all the groups compared to the control group as well as a decrease in HDL cholesterol in the group subjected to training with ostarine supplementation. These findings are partially consistent with those of other researchers and the results of the Phase II trial of ostarine (16) conducted by Komrakova *et al.* who observed lower cholesterol concentrations after ostarine treatment in ovariectomized rats. Some results from the clinical trials also indicated that the concentration of HDL cholesterol could be lowered by ostarine

treatment (29, 30). Therefore, it can be assumed that the cholesterol-lowering effect of ostarine depends both on the duration of its use and the intensity of exercise. It was observed that ostarine was safe and did not induce liver damage when used in a polypharmacy regime (31).

Moreover, in this study, we did not observe any changes in glucose concentration in the blood serum. However, there was a decrease in insulin concentration, which was accompanied by higher insulin sensitivity, in the groups subjected to exercise. On the other hand, the analysis of muscle structure, which was performed in our previous research (20), showed muscle growth after the use of ostarine. This may suggest that ostarine improved the insulin sensitivity of not only adipose tissues but also muscles (32).

We investigated the effect of ostarine on the serum levels of leptin and adiponectin, as well as the blood serum concentration as surrogate markers of the hormonal metabolism of fat tissues (33, 34). The results showed a lower concentration of leptin in the trained groups of rats, whereas a higher concentration of adiponectin in the trained group that did not receive ostarine supplementation. Our previous research on isolated rat adipocytes showed that the exposure of adipocytes to ostarine caused a reduction in the concentration of both leptin and adiponectin in the incubation medium (16). The *in vivo* study showed a similar effect in the case of leptin, whereas the serum adiponectin concentration was higher only in the DIO+T group of rats. Many previous studies showed that exercise increases adiponectin and decreases leptin concentrations (35); however, in this study, this effect was observed only in the DIO+T group. The reason for this could be the action of ostarine, which limited the release of the adiponectin from adipocytes, as observed in our *in vitro* studies. However, this aspect requires further research.

In our study, ostarine also affected the concentration of testosterone. Obesity is known to lower testosterone levels (34), and in fact obese rats had lower testosterone levels. Ostarine may lower testosterone concentrations in healthy subjects - but we did not show testosterone suppression in our study. This may be due to the already very low concentration of this hormone. It is known that training can improve testosterone levels (35) and our research has confirmed this - interestingly, ostarine lowered testosterone in rats that were running and received this SARM. This suggests the negative effect of ostarine on the hypothalamic-pituitary-gonadal axis and the reduction of the beneficial effect of training.

The use of ostarine can have negative effects, this is proved by recent reports. Ostarine has a potentially negative effect on the liver, although it should be remembered that case reports often refer to agents from an unknown source or in combination with other substances (15). Beyond its illicit use for athletic performance purposes, the use of ostarine in the clinical sector requires further research. According to data from the clinicaltrials.gov website, studies on enobosarm are still ongoing (36). The efficacy of these products has not been confirmed by FDA-approved research. Currently, no SARM is approved to treat or prevent any disease.

This study showed the positive effects of exercise on metabolic parameters that change with obesity. In the group of rats receiving ostarine administration, only changes in the levels of cholesterol and LDL were observed. However, an important finding is that we did not find any additive effects of ostarine and exercise. Based on our results, we conclude that ostarine should not be considered as a means of supporting weight reduction, while we do not question its anabolic effect in terms of increasing lean body mass.

Our study has some limitations. The basic limitation was the lack of measurements of muscle mass and fat mass in the studied animals. This perhaps explains the fact that there was no

difference in the body weight of the rats at the end of the experiment, even though the individual values of weight gain differed significantly between the training group and the group subjected to training along with ostarine supplementation. Another limitation of the study is that ostarine administration lasted only for 30 days. Although no significant changes were observed after the use of ostarine, it is essential to monitor the effects of this compound due to its widespread use. The information from the annual World Anti-Doping Agency reports and case studies about acute drug-induced liver damage after the administration of ostarine indicate an increased interest in this compound among professional athletes and recreational users.

Acknowledgements: The manuscript was financed by the National Science Centre, project number 2019/35/N/NZ7/00738 and 2016/21/B/NZ7/02748.

Conflict of interest: None declared.

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Received: November 10, 2022

Accepted: August 31, 2023

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