ACTA ZOOLOGICA BULGARICA

Acta Zool. Bulg., June 2024, 76 (2): 197-205

Terrestrial Ecology and Behaviour
Research Article

Published online 24 April 2024
DOI: https://doi.org/10.71424/azb76.2.002724

Effects of Submaximal Training on Ghrelin in Female and Male Rats with Dietary-Induced Metabolic Syndrome

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Abstract: Ghrelin is a hormone that stimulates appetite and its serum levels are inversely proportional to body weight. Decreased ghrelin levels correlate with obesity, insulin resistance and elevated blood pressure, which are components of the metabolic syndrome (MetS). The effect of physical exercise is studied mainly after an already induced MetS, the results are contradictory and it is not clear what the effect of exercise on ghrelin concentration is. There are no data for gender comparisons of changes in ghrelin due to submaximal training. In this study, we aimed to determine the effect of submaximal training on serum ghrelin concentrations during the dietary induction of MetS in female and male rats. Dietary-manipulated female and male Wistar rats (n = 32) were used. They were divided into four groups: male and female sedentary (MD and FD) and male and female trained on treadmill (MDT and FDT). For 16 weeks, the four groups received high-fat high-carbohydrate (HFHC) food and both MDT and FDT simultaneously with the diet were subjected to submaximal training (70-75% VO_{2max}). At the end of the experiment, mixed blood was collected and ghrelin concentrations were measured. The submaximal training and gender had a significant main effect on ghrelin levels, as trained dietary-manipulated rats had higher serum concentrations compared to the sedentary ($p < 0.05$) and males had higher concentrations compared to females ($p < 0.05$). Overall, applied for 16 weeks, simultaneously with the combined HFHC diet, the submaximal training had a positive effect, lowering the serum ghrelin concentration. Sexual dimorphism, characterised by higher ghrelin in male rats, was also found.

Key words: Ghrelin, metabolic syndrome, submaximal training, female and male Wistar rats

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Introduction

Ghrelin is a hormone that stimulates appetite in humans and rodents (Kojima et al. 1999, Hosoda et al. 2000, Tschöp et al. 2000). It is a peptide hormone secreted predominantly by X/A-subunits and P/D1 cells in the fundus glands, but also in small amounts by the pancreas, small intestine, kidney, liver, pituitary gland and hypothalamus (Date et al. 2000, Inui et al. 2004, Wierup et al. 2007, Stengel et al. 2012). The serum levels of ghrelin are inversely proportional to body weight: high at low body mass and in obesity fall below normal values (Otto et al. 2001, Hansen et al. 2002). Ghrelin regulates food intake and body weight and increases gastric emptying, gastrointestinal tract motility, gastric acid secretion, exocrine pancreas secretion (Kojima et al. 2007). It is reported that ghrelin concentrations are increased by various factors, some of which are starvation, anorexia, insulin-induced hypoglycaemia, leptin administration and weight reduction (Toshinai et al. 2001, Knerr et al. 2003, Guo et al. 2008, Beasley et al. 2009, Li et al. 2009, Zou et al. 2009). Studies on changes of the amount of ghrelin in obese individuals and such with normal body weight showed a decrease in ghrelin levels in obese individuals as well as an increase with weight loss (Aydin 2007). There is a suggestion that ghrelin may be involved in the pathogenesis of metabolic disorders typical for the metabolic syndrome. According to Fagerberg et al. (2003) and Tschöp et al. (2000), reduced ghrelin levels positively correlate with obesity and insulin resistance, which are components of the metabolic syndrome (MetS). A negative correlation between the concentrations of serum ghrelin and the abdominal circumference, which is a prerequisite for the development of the syndrome, has been reported also by Fagerberg et al. (2003).

The effect of different types of physical exercise on the hormone has been studied in more detail; however, the results are contradictory and it is not clear what the effect of the exercise on ghrelin concentration is. According the available data, most studies have been conducted on healthy people or such with developed obesity or MetS. The conducted experiments included mainly intact or treated with high-fat diet male rats. Only a small portion are performed on female animals on high-fat diet after ovariectomy (Vuković et al. 2014, Iwasa et al. 2018) or high-fat diet and high-fat high-sugar diet and polycystic ovary syndrome (Roberts et al. 2017, Begum et al. 2022).

The effects of different aerobic trainings with varying intensity have been also studied. However, few studies have explored the effect of aerobic training on ghrelin levels in individuals with the metabolic syndrome and, overall, physical exercises have been administered after an already induced MetS. Some authors found no change due to the applied physical regime (Kraemer et al. 2004, Manshouri et al. 2008), some found a decrease in ghrelin levels (Kraemer et al. 2004, Broom et al. 2009), while others found that as a result of the physical training ghrelin increased (Leidy et al. 2004).

The effect of submaximal training on serum ghrelin levels in dietary-induced metabolic syndrome with high-fat high-carbohydrate (HFHC) diet has not been studied yet. In the available data, there is lack of information on the effect of submaximal training, administered simultaneously with a high-fat high-carbohydrate diet in rats, in the course of the dietary induction of MetS on ghrelin levels and potential sex differences.

Therefore, the main aim of our study was to determine the effect of the submaximal training on ghrelin levels during the induction of metabolic syndrome in female and male rats.

Materials and Methods

All experimental protocols were approved by the Bulgarian Agency for Food Safety (BAFS resolution No.119/18.06.2015) and were in compliance with the ethical standards of the Medical University of Plovdiv (resolution of the University Ethic Committee No.4/21.09.2017). All experimental procedures were performed in accordance with the recommendations of the European Commission for the Protection and Welfare of Laboratory Animals in accordance with the requirements of the Helsinki Declaration on Ethics in Science, Legislative Acts in the Republic of Bulgaria for Scientific Research and Experimentation with animals as well as for the protection and welfare of experimental animals. Sixteen female and sixteen male Wistar rats with an initial body weight of 160-180 g were used in the study. They were respectively divided into four groups: male and female dietary-manipulated sedentary (MD (n = 8) and FD (n = 8)) and male and female dietary-manipulated trained on treadmill rats (MDT (n = 8) and FDT (n = 8)). They were further raised in independent metabolic cages at a temperature of 20 ± 2º C, controlled humidity, 12:12 h light-dark photoperiod and access to water and food *ad libitum*. During the experiment, all rats had free access to combined high-fat high-carbohydrate food with energy content of 30% fats, 57% carbohydrates and 13% proteins for a period of 16 weeks. The composition of the combined HFHC diet was 13.45 g proteins, 14.4 g fats and 61.6 g carbohydrates per 100 g. This food was prepared by adding of lard and powdered sugar to standard rat chow. The total energy density of the combined HFHC food was 4298 $kcal.kg^{-1}$. In order to avoid any possible gastrointestinal disturbances, the rats were gradually fed and switched from a standard rat chow to a combined high-fat high-carbohydrate diet. For the first three days, they received HFHC and standard food in proportion 25% : 75%, the following three days in proportion 50% : 50%, followed by three days on 75% : 25% and from the 10^{th} day of the experiment, they were subjected only to HFHC diet. Transitional diets were also prepared by adding lard and powdered sugar to standard pellets. Simultaneously with the administration of the HFHC diet, the rats from MDT and FDT groups were subjected to a submaximal training on a treadmill for small experimental animals EXER-3R-Treadmill (Columbus Instruments, Columbus, OH, USA) at 25 $m.min^{-1}$, 5° slope (about 70-75% VO_{2max}), 5 days a week for 16 weeks. On the first day the duration of the training was 20 min and gradually increased by 5 min every second day. At the end of the second week, it reached 40 min and this load was maintained until the end of the experiment. Once a week the body weight of each animal was determined using a digital scale. The food was temporarily withdrawn an hour before and returned to the metabolic cages after the measurement. The abdominal circumference of the experimental animals was determined once a month. It was measured with a tape in the widest part of the abdominal area (Angelova et al. 2015).

At the end of the experiment, after decapitation under narcosis with 30 $mg.kg^{-1}$ Thiopental i.p., mixed blood was collected for clinical and chemical analysis. Serum ghrelin concentration was determined using a Ghrelin Unacylated test kit (BioVendor Inc., Czech Republic) on Sirio S microplate reader (SEAC, Italy). Stomach specimens were taken from each animal after decapitation, fixed in 10% formalin and after paraffin inclusion, they were studied immunohistochemically with a primary antibody for ghrelin (goat polyclonal ghrelin antibody: sc-10368 – Santa Cruz Biotechnology, USA) by the ABC method with ABC Staining System (Santa Cruz Biotechnology, USA). The ghrelin antibody was diluted in PBS at a ratio 1:100. Positive reaction for ghrelin was reported in the presence of black granules in the cell cytoplasm. The specificity of immunohistochemical reactions was confirmed by negative controls, in which the specific antibodies were substituted with a buffer (PBS) or normal non-immune serum. There was no product of the respective reaction in them. Observation and photo documentation of the microscopic preparations were performed with a digital photo microscopic camera attached to a light microscope Olympus BX51 (Japan).

In order to study the main effects of the sex and training and their interactions, the results were analysed using a two-way ANOVA. When the F-ratio was significant, the between-group differences were assessed by Tukey's or Games Howell *post-hoc* tests depending on the homogeneity of the dispersions. All data were presented as an arithmetic mean ± standard error of the arithmetic mean (X ± SEM). Statistically significant difference was assumed for $p \leq 0.05$. The SPSS v 19.0 program (IBM, USA) was used to process the results.

Results

Body weight

There were no significant differences in the body weights of the experimental animals before the start of the experiment (184.75 ± 13.66 g for MD, 182.00 ± 11.98 g for MDT, 176.25 ± 14.40 g for FD, 174.38 ± 11.07 g for FDT, $p > 0.05$). Until the 10^{th} week of the experiment, the applied training had no significant effect on body weight ($p > 0.05$). From the 11^{th} week until the end of the experiment, the submaximal training had a significant main effect on the body mass. At the end of the experiment, the trained dietary manipulated rats had a lower body weight than the sedentary dietary manipulated rats ($p < 0.05$; Fig. 1). The sex had a significant main effect on the weights of the experimental animals in the course of the experiment. Male rats had a higher body weight than females ($p < 0.05$; Fig. 2). No significant interaction of sex and submaximal training on the body weight was found ($p > 0.05$).

Abdominal circumference

The applied submaximal training did not have a significant main effect on abdominal circumference of the trained animals in comparison with the sedentary rats during the experiment ($p > 0.05$). Sex had a significant main effect on abdominal circumference during the experiment. At the end of the study, male rats had higher values than females ($p < 0.001$; Fig. 3). No significant interaction of sex and submaximal training on the abdominal circumference was found ($p > 0.05$).

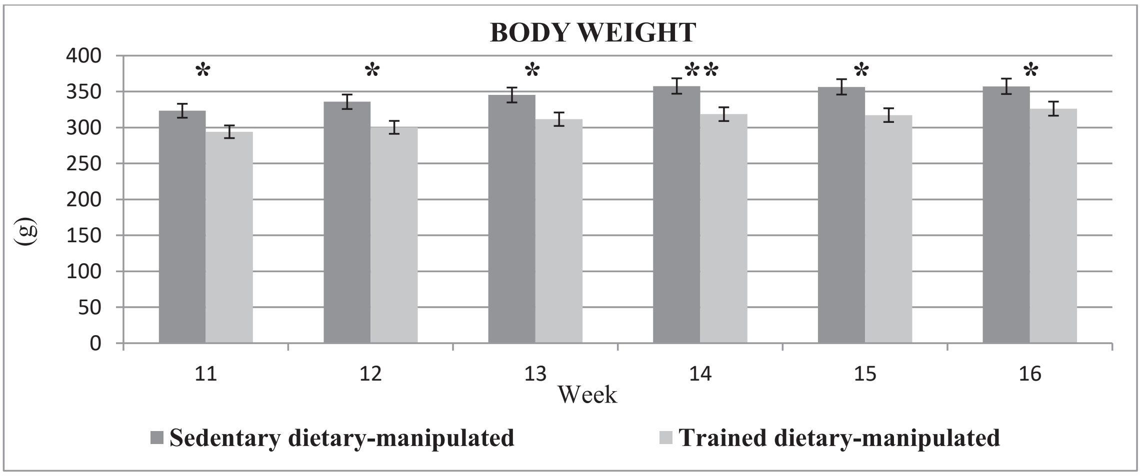

Fig. 1. Body weight (g) of sedentary and trained dietary-manipulated animals from the 11th week to the end of the experiment. *$p < 0.05$, **$p < 0.01$, sedentary vs trained.

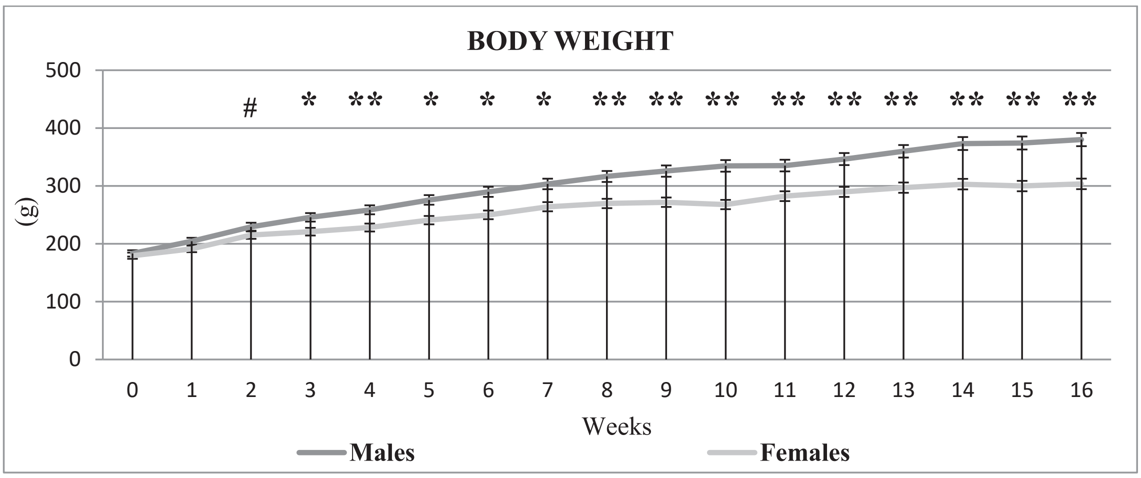

Fig. 2. Body weight (g) of male and female rats in the course of the experiment. #$p<0.05$, *$p< 0.01$, **$p < 0.001$, male vs female rats.

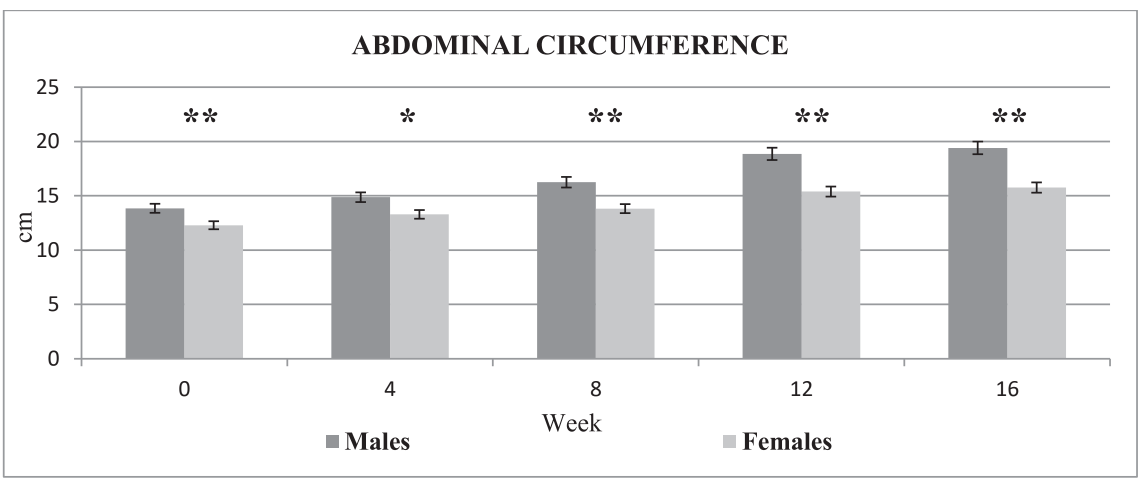

Fig. 3. Abdominal circumference (cm) of male and female animals in the course of the experiment. *$p < 0.05$, **$p < 0.001$, male vs female rats.

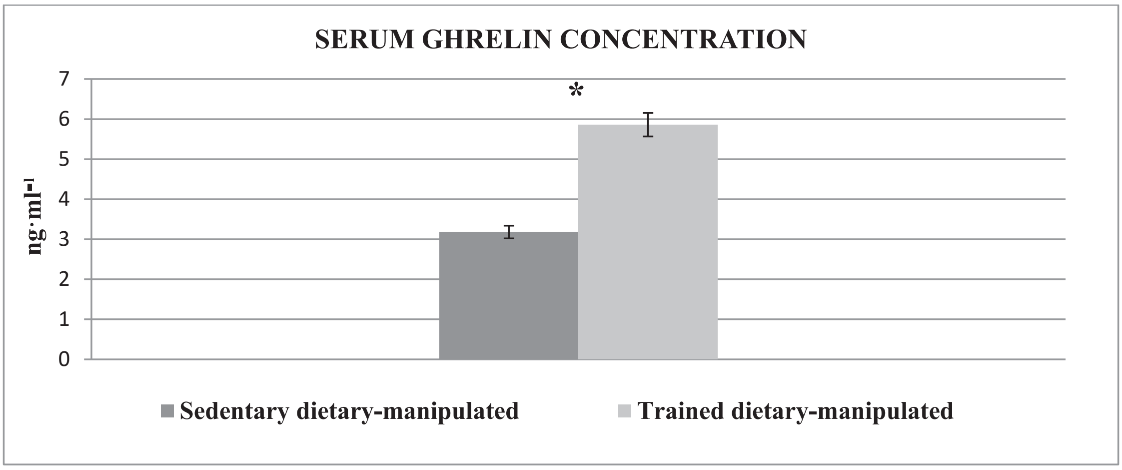

Fig. 4. Serum ghrelin concentration (ng·l^{-1}) at the end of the experiment of trained and sedentary dietary-manipulated animals. *$p < 0.001$, trained vs sedentary rats.

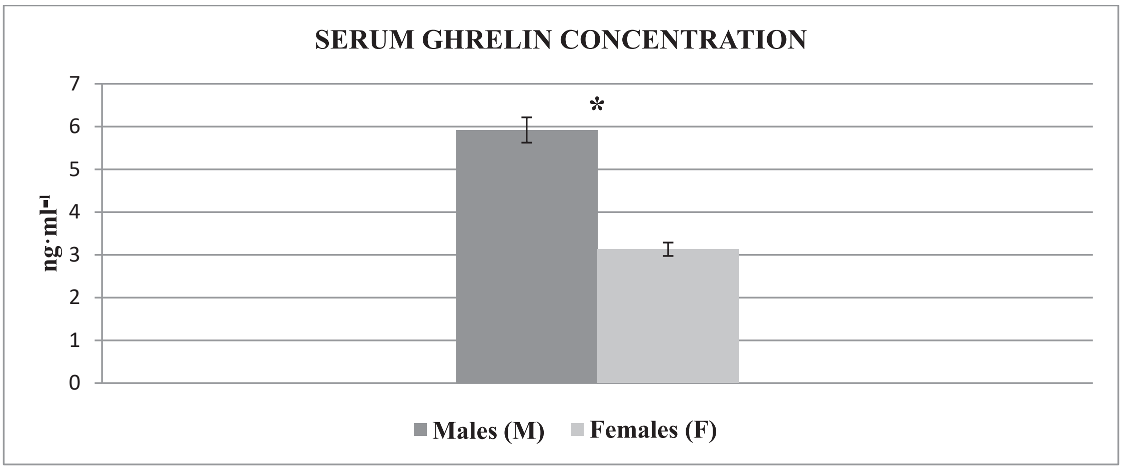

Fig. 5. Serum ghrelin concentration (ng·l^{-1}) at the end of the experiment of male and female animals. *$p < 0.001$, male vs female rats.

Ghrelin

The applied submaximal training for 16 weeks had a significant main effect on serum ghrelin: trained rats had higher concentrations in comparison with sedentary ones ($p < 0.05$; Fig. 4). Sex also had a significant main effect: male rats had higher serum ghrelin concentrations than females ($p < 0.05$; Fig. 5). No significant interaction of sex and submaximal training on serum ghrelin concentrations was found ($p > 0.05$). In both male and female sedentary dietary-manipulated groups, ghrelin-positive cells with moderate immunohistochemical activity were observed in the stomach fundus and corpus (Figs. 6, 7). In the trained dietary-manipulated animals, a great number of cells with positive ghrelin activity were detected (Figs. 8, 9). The expression was stronger than that in the sedentary rats. The results were identical for male and female rats.

Discussion

Our data show that submaximal training used in the course of dietary-induction of MetS reduces body weight of male and female rats subjected to HFHC diet, while we did not find an effect of training on abdominal circumference, when comparing the trained and the sedentary rats at the end of the experiment. These results confirm the view that adequate physical activity is a major factor for regulation and maintaining of body mass. Similarly, but using a high-fructose diet for induction of MetS and simultaneous administration of treadmill training in male rats for ten weeks, Mostarda et al. (2012) and Castro et al. (2015) found a decrease in the weight of the animals, while retaining their length.

Moreover, our data show that the dynamics of weight changes were sex-specific, with no differ-

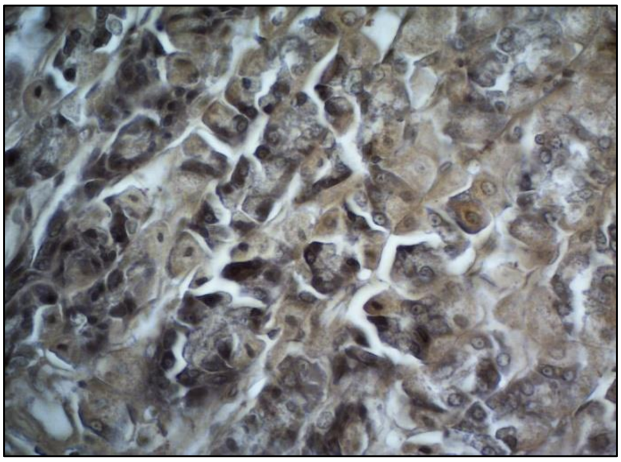

Fig. 6. Moderate immunohistochemical reaction for ghrelin in the stomach of sedentary dietary-manipulated male rats. Magnitude x400.

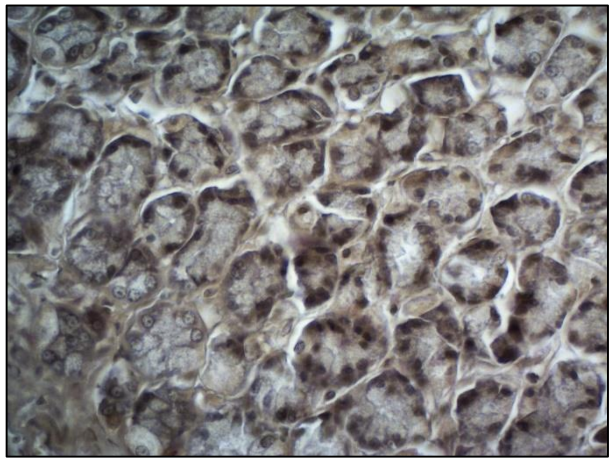

Fig. 7. Moderate immunohistochemical reaction for ghrelin in the stomach of female sedentary dietary-manipulated rats. Magnitude x400.

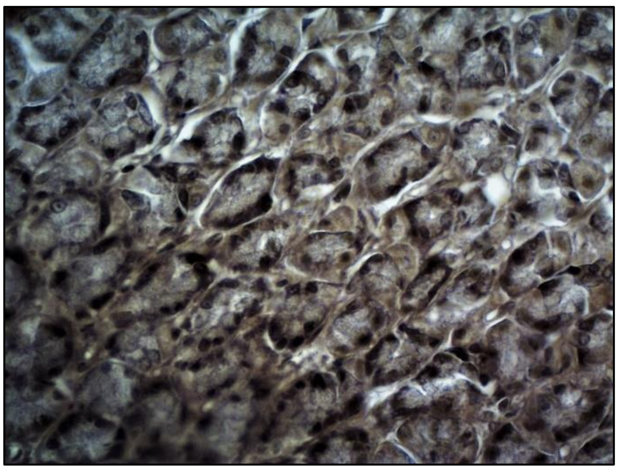

Fig. 8. Strong immunohistochemical reaction in the stomach of trained dietary-manipulated male rats subjected to HFHC diet and physical exercise. Magnitude x400.

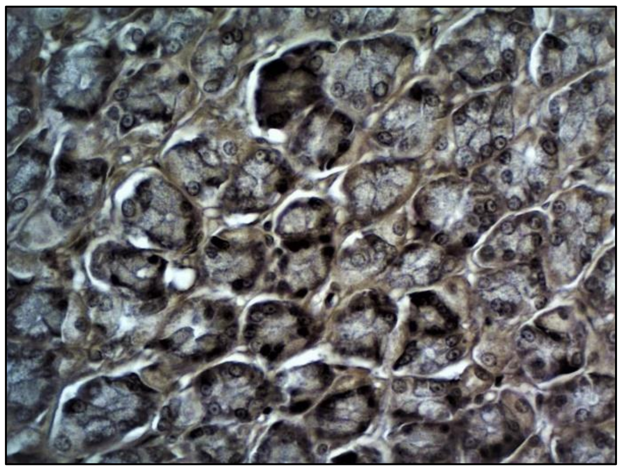

Fig. 9. Strong immunohistochemical reaction in the stomach of trained dietary-manipulated female rats subjected to HFHC diet and physical exercise. Magnitude x400.

ences in the body mass at the beginning of the experiment, while the body mass in male experimental animals was higher than that of the females after the 2nd experimental week. From the performed somatometric studies, it cannot be estimated whether these differences are a result of the submaximal training or are a result of gender differences in the growth rate. Since these differences were found in the initial stages of the MetS induction and application of the submaximal training and at the start of experiment, the abdominal circumference was higher in males than in female rats, they might rather show natural sex differences than an effect of the applied training.

In addition, the results of our experiment show that the used submaximal training has a beneficial effect on serum ghrelin concentration. Applied simultaneously with the combined HFHC diet for 16 weeks, the submaximal workout leads to an increase in the serum ghrelin in the trained dietary-manipulated animals. Our results show that male rats had higher serum ghrelin concentrations compared to females. This data allow us to conclude that the applied submaximal training works favourably, neutralising the negative effect of the combined high-fat high-carbohydrate diet on the concentration of ghrelin and its positive effect is more pronounced in males. Our results are similar to the data reported from other studies on the effect of the physical training on the ghrelin concentration, demonstrating that exercise training increases total ghrelin levels (Kraemer et al. 2010). Fahti et al. (2010) tracked the effect of a 12-week submaximal workout in intact male Wistar rats and reported an increase in the serum ghrelin concentration. Anderson et al. (2005) found that in rats, even after a single submaximal physical activity on a treadmill, the serum ghrelin concentrations were increased. On the other hand, Mifune et al. (2015) reported that they found significantly reduced ghrelin concentrations in rats, subjected to a high-fat diet for ten weeks. Similar results were also demonstrated by Tschöp et al. (2000) and Oner-Iyidogan et al. (2007). An increase in the ghrelin concentration was observed after weight loss (Cummings et al. 2002, Hansen et al. 2002), after aerobic training (Foster-Schubert et al. 2003), even without significant weight reduction (Kadoglou et al. 2012). The adaptive change in ghrelin to reduced body mass was similar regardless of whether weight reduction was achieved through a low calorie diet, exercise or a combination of both and that change was associated with concomitant changes in leptin, adiponectin, insulin and insulin resistance as presented by Mason et al. (2015). Mifune et al. (2015) reported that in male Sprague-Dawley rats the serum ghrelin concentrations increased after a ten-week high-fat diet and simultaneously performed spontaneous physical activity (on an exercise wheel). The mechanism by which the endurance training changes the amount of ghrelin in the body is still unclear. Similar to studies conducted on humans, in male rats, spontaneous aerobic training lead to near baseline increase in the secretion and the serum levels of ghrelin and at the same time it decreased the body mass and the amount of fat (Tiryaki-Sonmez et al. 2013).

Our data also resemble and complement the data obtained from studies of changes in the ghrelin levels in humans. Foster-Schubert et al. (2003) and Mason et al. (2015) reported that in postmenopausal women with obesity, a 12-month aerobic training results in an increase in the serum ghrelin concentrations along with weight reduction. In studies, conducted by Martins et al. (2010) and Cederberg et al. (2011), a significant increase in the serum ghrelin concentration associated with a reduction in body weight, fat amount and abdominal circumference was observed in obese individuals and in patients with metabolic syndrome undergoing aerobic exercise for six months. It has been demonstrated that changes in the ghrelin serum concentration act as an adaptation mechanism in weight loss that, in turn, play a role as a compensatory signal during the body mass reduction (Doucet et al. 2007, Maclean et al. 2006).

Sixteen weeks HFHC diet leads to proliferation of ghrelin-producing cells in fundic glands of the stomach. Having in mind that gastric ghrelin is an endogenous regulator of feeding behaviour in mammals, ghrelin secretion by the stomach of the experimental male and female rats is an adaptive reaction to the diet. We could consider this as an attempt of the organism to manage with high content of lipids and carbohydrates. Ghrelin transmits a hunger signal from the periphery to the central nervous system. Taking into account all these activities, ghrelin plays important roles for maintaining growth hormone release and energy homeostasis as explained by Kojima et al. (2007). Furthermore ghrelin affects energy homeostasis and insulin action and it is involved in the regulation of energy balance and obesity too (Kraemer et al. 2010).

We found that the effect of both applications of HFHC diet and physical exercise resulted in an increase of the number of ghrelin-producing cells that expressed stronger immunohistochemical activity. It could be explained with the effect of the physical exercise because physical activity is known to regulate energy balance. Our data support the results of available studies, which have shown that ghrelin concentration increases because of aerobic workout. Most studies have been conducted after induced obesity or metabolic syndrome in rats because of a high-fat diet administration. To the best of our knowledge, this study is the first to demonstrate the positive effect of submaximal training on serum ghrelin concentrations, administered simultaneously with combined HFHC diet that leads to the development of obesity and metabolic syndrome in male and female Wistar rats along with comparing this effect between the sexes.

Conclusion

The submaximal training applied for 16 weeks works favourably, neutralising the negative effect of the combined high-fat high-carbohydrate diet. This study is the first on gender comparisons of changes in ghrelin because of physical exercise in HFHC-dietary manipulated rats. Submaximal training increases the concentration of ghrelin and its positive effect is more pronounced in males than in females.

Acknowledgments: This study is financed by the European Union-NextGene-rationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0001-C01.

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Received: 27.05.2023
Accepted: 08.11.2023