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High-fat diet induced cardiac hemodynamic changes: effects of exercise and obestatin

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ABSTRACT: The obese population has been rapidly increasing because of high-fat consumption and a sedentary lifestyle. Large amounts of dietary fat intake increase the risk of cardiovascular disease. We aimed to investigate the effects of exercise and obestatin on a high-fat diet (HFD) induced cardiac hemodynamic changes. Seventy-nine Sprague-Dawley rats (200 to 250 g) were fed either control diet or HFD for 8 weeks. In the 5th week, each diet group was subgrouped as follows; control, exercise, obestatin (25µg/kg, i.p), exercise+obestatin (25µg/kg, i.p). After the end of 4 weeks of exercise (swimming exercise, 5 days a week/ 20 min day) and obestatin administration period, all animals were sacrificed. Hearts were removed for hemodynamic measurements with the Langendorff apparatus. Blood samples were collected for biochemical measurements. Data were analyzed by Graphpad Prism 6.0. and $p<0.05$ was accepted as statistically significant. Cardiac contractility and hemodynamic parameters in the HFD model have been evaluated. And the effects of chronic obestatin treatment and exercise were studied together. Obestatin ameliorated derangements in LVDP, heart rate, and blood lipid levels induced by an HFD. Also, obestatin prevents decreasing BNP levels with high-fat consumption. Obestatin treatment potentiated the beneficial effects of exercise evidenced by LVDP, heart rate, blood lipids, BNP, and AT2R1 measurements. We believe that obestatin has the potential for the maintenance of cardiac function in HFD.

KEYWORDS: Obesity; high-fat diet; Langendorff; obestatin; exercise; HDL.

1. INTRODUCTION

Dietary risk factors such as disordered food consumption and inappropriate dietary habits appear to increase morbidity and mortality rates. In particular, high sodium and low whole grain consumption are high on the list of factors affecting mortality [1]. On the other hand, the amount of fat in the diet and the optimal ratio of carbohydrate to fat in a diet remain controversial. In particular, low-carbohydrate and high-fat diets have polarized nutrition experts. The benefits of diets to the body have been controversial among the groups. High-fat diets lead to increased storage of triglycerides in ectopic tissues adjacent to adipose tissue, while increased plasma concentrations of triglycerides and lipids can lead to insulin resistance and inflammation [2]. In recent years, the number of overweight and especially obese people has greatly increased as a result of the consumption of high-fat and high-energy foods. Many experimental studies suggest that HFD causes cardiac dysfunction, including decreased cardiac output and end-diastolic volume with increased end-diastolic pressure, which may also exacerbate cardiomyopathy [3, 4]. Eight weeks of HFD feeding induces changes in cardiac electrophysiological activity, especially atrial activity, in mice [5]. Moreover, it is controversial whether the adverse effects of HFD on cardiac function are reversible [6]. Interestingly, in an experimental HFD rodent model, a high-fat diet prior to cardiac ischemia-reperfusion injury has been shown to have an attenuating effect on injury [6]. The oxidative stress that develops in HFD has been shown to damage many organs, including the heart [4]. The key regulators of cardiovascular function and metabolism that have therapeutic potential in oxidative stress resulting from a high-fat diet and their relationship to exercise are questions that need to be addressed. In this context, the effects of exercise in a sedentary lifestyle characterized by cardiovascular damage-causing HFD and the effects of modulatory targets that can be used alone or in combination are current research topics.

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Obestatin (OB) is a novel peptide derived from various central and peripheral tissues such as intestine, stomach, spleen, pancreas and cerebral cortex [7]. Some physiological effects of OB include stimulation of appetite, initiation of food intake, control of gastric motility, and modulation of glucose and lipid metabolism [8]. In a previous study, peripheral and central OB treatment was shown to reduce food intake, delay gastric emptying, and decrease weight gain [9]. More recent studies have shown that OB improves cardiac contractility and cardiac β-adrenergic response [10-12]. According to Li et al, a positive correlation was found between fasting OB levels and systolic blood pressure in spontaneously hypertensive Wistar-Kyoto rats. Consistent with this, pregnancy-induced hypertensive women were also found to have high plasma OB levels compared with normotensive pregnant women [13, 14]. Tissue or serum levels of many peptides that regulate appetite and metabolism have been shown to change with exercise but still maintain their secretion, as exercise, which has a direct effect on the expression or suppression of peptides in tissues, has an effect when used as a combined treatment. Therefore, it is important to demonstrate the relationship between OB and exercise, which has been shown to regulate both appetite and the cardiovascular system, in cardiac dysfunction caused by HFD.

Exercise offers a healthy life besides being good for the metabolic effects of these lifestyles [15]. Serum levels of anorexigenic peptide OB in male individuals, which is considered to be a promising substance in suppressing the appetite of obese people and in losing weight, change with exercise. According to Nikbakht et. al. report that exercise training might modulate fundus and intestine total OB levels via an improvement of energy source and a negative feedback action of ghrelin on this peptide [16].

Given the established cardiovascular effects of its sister hormone ghrelin, it is becoming increasingly clear that in addition to the ascribed metabolic effects of OB, it may also have important effects on the cardiovascular system [17]. There are a limited number of studies that investigated HFD induced cardiac hemodynamic changes and possible underlying mechanisms. In this study, we aimed to compare the effects of exercise and OB on cardiac hemodynamic changes induced by HFD.

2. RESULTS

2.1. Bodyweight and Lee index measurements

Treatment with OB significantly decreased body weight in OB ($p<0.01$) and combined EX+OB ($p<0.05$) group vs control group while EX only group also inhibited body weight gain in CD groups ($p<0.01$). Interestingly, HFD significantly inhibited the body weight gain in saline-treated groupv($p<0.05$) and the treatments with OB, EX or combined usage did not have different effect than their respective CD groups which points a preventative effect of OB and EX in HFD and also their combined app showed same impact ($p<0.001$, Figure 1a). In HFD (Figure 1b), OB treatment alone or combined with EX limited the weight gain ($p<0.05$) while exercise alone did not. When we evaluate the percentage of body weight changes in the CD groups, the saline group showed a negative correlation with the EX group (Fig.1e, r2= -0.700) and EX group showed a negative correlation (r2=-0.700) with EX+OB group. In the HFD groups OB negatively correlated with the saline group (Fig.1f, r2=-0.771) while exercise has no correlation (r2=-0.286) with the saline group. Moreover, in the HFD groups EX+OB group showed a negative correlation with the saline group (r2=-0.690).

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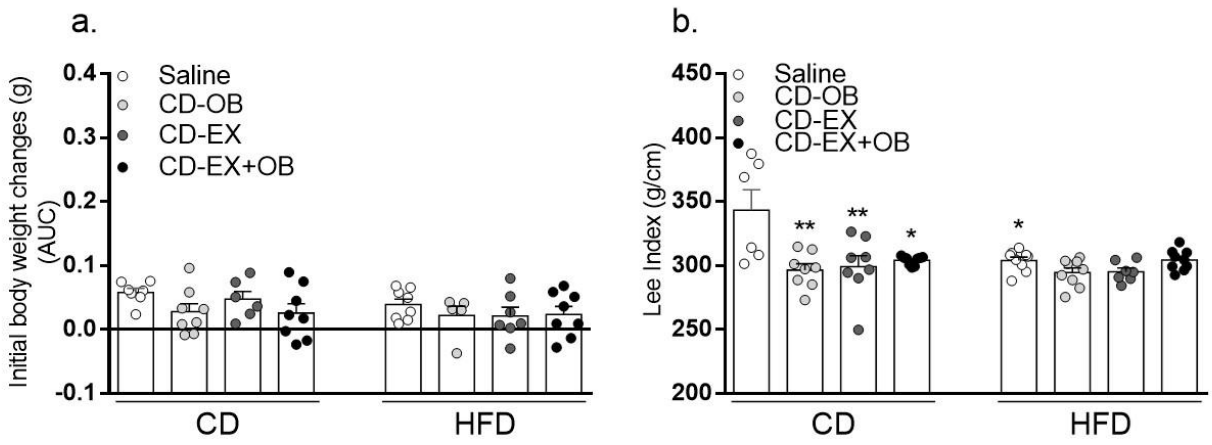

Figure 1. Initial body weight changes at 8th week (a) and Lee index measurements (b). The results are given as mean ± standard deviation. +++ p<0.001, ++ p<0.01, + p<0.05 vs HD saline groups; *** p<0.001, ** p<0.01, * p<0.05 vs CD saline groups; ### p<0.001, ## p<0.01, # p<0.05 vs respective CD groups.

2.2. Langendorff organ bath measurements

Consumption of HFD significantly decreased heart rate compared to respective CD groups except for the EX+OB group (Figure 2a, p<0.001). Although, exercise treatment alone did not affect the BMP of CD rats, OB administration alone (p<0.05) or combined with exercise (p<0.01) significantly decreased. In the HFD groups OB and EX groups had significantly decreased heart rates while combined treatment significantly inhibited this decline (p<0.01).

LVDP (left ventricular developed pressure) measurements, which is defined as peak systolic pressure minus end-diastolic pressure, were not significantly changed due to diet regimen. In CD rats LVDP significantly decreased in EX+OB group compared to the saline group (p<0.05) and this effect was reversed in respective the HFD group (Figure 2b, p<0.001). Treatments of OB (p<0.01) and EX (p<0.001) alone in the HFD groups significantly decreased LVDP levels as compared to their respective CD groups.

In line with LVDP levels the peak rates of positive changes in left ventricular pressure which is +dP/dTmax values decreased in EX+OB group as compared to the saline group in CD likewise significantly reduced +dp/dt levels were reversed in EX+OB group with HFD (Figure 2c, p<0.05) (p<0.001).

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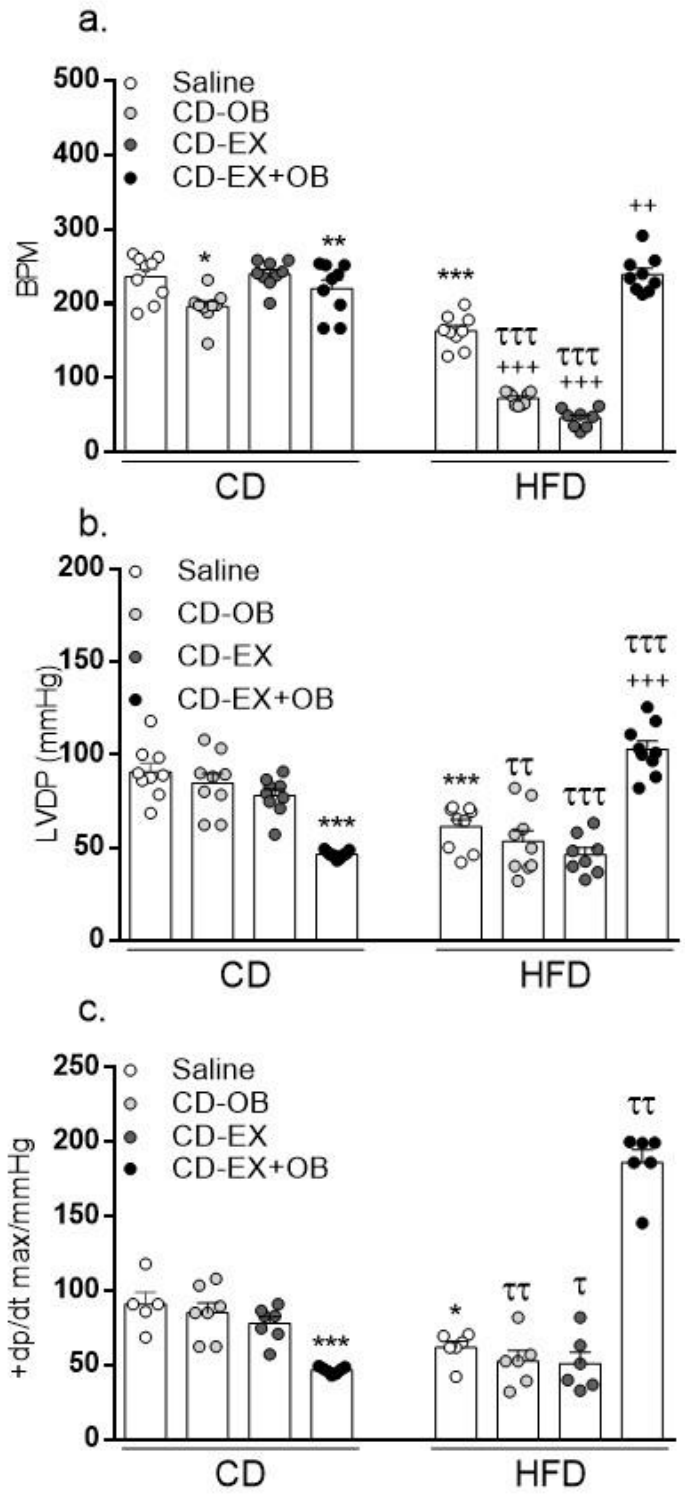

Figure 2. Cardiac functions; BPM (a) and LVDP (b) and rate–pressure product- +dp/dt (c) in the perfused heart. The results are given as mean ± standard deviation. +++ p<0.001, ++ p<0.01, + p<0.05 vs HD saline groups; *** p<0.001, ** p<0.01, * p<0.05 vs CD saline groups; ### p<0.001, ## p<0.01, # p<0.05 vs respective CD groups.

2.3. Blood lipids

Lipid measurements, cholesterol, triglyceride, HDL and LDL were performed once at the end of 8th week. Total cholesterol levels significantly increased in the HFD as compared to CD groups (Fig.3, p<0.001,).

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In the HFD, cholesterol levels significantly reduced in OB ($p<0.05$), EX ($p<0.001$) and EX+OB ($p<0.01$) groups compared to the saline treated the HFD group.

In CD rats OB alone and combined with exercise significantly ($p<0.001$) decreased serum triglyceride levels as compared to the saline group but exercise alone had no effect. Consumption of HFD significantly raised triglyceride levels as expected and again in OB and EX+OB groups decreased ($p<0.01$) levels were determined but exercise alone still had high levels of triglyceride.

Significantly increased serum levels of HDL in the HFD groups were prevented in EX ($p<0.01$) and EX+OB ($p<0.05$) groups. In line with HDL results LDL levels significantly increased in the HFD groups and both alone treatments with OB ($p<0.01$) and EX ($p<0.001$) significantly reduced these levels.

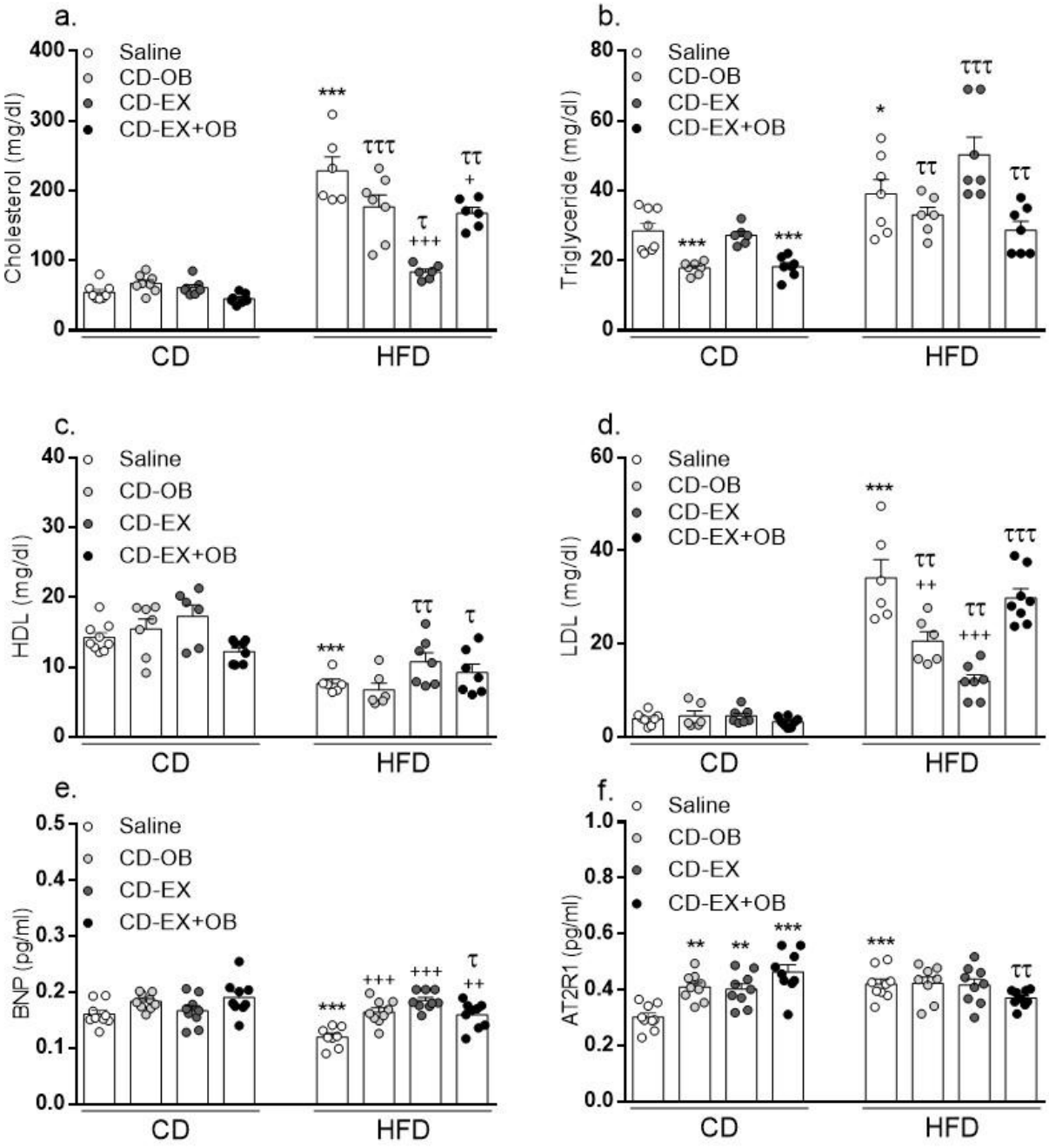

Figure 3. Serum cholesterol (a), triglyceride (b), HDL (c) and LDL (d) levels. Serum BNP (e) and AT2R1 (f) levels. The results are given as mean ± standard deviation. +++ $p<0.001$, ++ $p<0.01$, + $p<0.05$ vs HD saline groups; ααα $p<0.001$, α $p<0.05$ vs HFD+saline groups; *** $p<0.001$, ** $p<0.01$, * $p<0.05$ vs CD saline groups; ### $p<0.001$, ## $p<0.01$, # $p<0.05$ vs respective CD groups.

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2.4. AT2R1 and BNP Levels

Treatment with OB and EX alone and combine significantly increased serum AT2R1 levels in CD (Figure 3f, $p<0.01$-0.001). HFD significantly increased levels of AT2R1 and this was significantly reduced in EX+OB group ($p<0.01$).

Serum levels of BNP were not different among the CD groups but significantly decreased in the saline treated HFD group as compared to respective CD group while treatments with alone or combined OB and EX significantly increased BNP levels ($p<0.01$-001).

3. DISCUSSION

Obestatin treatment ameliorated the negative effects of a high-fat diet on cardiac hemodynamics and serum lipid parameters. It also prevented AT2R1 increase and BNP decrease due to a high-fat diet. Obestatin treatment potentiated the beneficial effects of exercise evidenced by LVDP, heart rate, blood lipids, BNP and AT2R1 measurements.

High-fat diet led to increased weight gain, which was higher in exercise groups. Previous studies showed that exercise does not alter weight gain when compared to the sedentary group in high-fat diet groups [18, 19]. In high-fat fed rats, weight gain was reduced by streptozotocin-induced diabetes, whereas obestatin did not show a significant change in diabetic rats [20]. Our study showed that in HFD groups, obestatin+exercise limited weight gain compared to exercise. Previous studies reported that obestatin inhibit food and water intake and weight gain [21]. Taken together with our findings, we suggest that obestatin may have a therapeutic role in controlling weight gain in obesity.

For example, obestatin has been reported to inhibit food and water intake, body weight gain and gastrointestinal motility and also to mediate the promotion of cell survival and prevention of apoptosis [16].

High-fat food consumption affects cardiovascular health by inducing heart failure, arrhythmias, hypertension and atherosclerosis [22, 23]. For example, a long-time high-fat diet (24 weeks) increased heart rate in mice even maternal high-fat diet altered fibrosis and hypertrophy related micro-RNA levels in cardiac cells [24, 25]. According to our study, a high-fat diet (8 weeks) with a 25 mg/kg dose of obestatin decreased heart rate. In a study, intravenous obestatin injection in different doses (10, 50 or 100 mg/kg) did not show significant effects on heart rate [26]. These findings suggest that the effects of obestatin on the heart rate may vary according to the type of diet in a dose-dependent manner.

Long-term (9 weeks) swimming exercise led to improvement in cardiac contractility, relaxation and systolic capacity, while shorter (3 weeks) period did not change myocardium [27, 28]. Obestatin has multiple roles in cardiac functions, it has protective effects on papillary muscle contractility from diabetic derangement, adrenergic effects to reduce contraction force of papillary muscle, and vasodilatory effect on coronary vessels [11, 29]. Four weeks of swimming with obestatin treatment exercise decreased left ventricular pressure in this study. Exercise training in the present study was not long enough to induce training bradycardia and obestatin did not change heart rate in the control diet group.

Dyslipidemia is characterized by high concentrations of triglyceride and a low concentration of high-density lipoprotein cholesterol. Low-density lipoprotein cholesterol (LDL-C) concentrations could be optimal or mildly increased [30]. Multiple studies reported the high-fat diet induces dyslipidemia like this study [31, 32]. Exercise decreased the serum triglyceride levels as shown in previous studies [18, 19]. An importing finding of this study, obestatin reduced cholesterol, triglyceride and LDL levels in the high-fat group. We consider with further studies, obestatin may be used in clinical settings of dyslipidemia.

AT2R1 has been implicated with cardiovascular pathophysiology and high-fat diet induces AT2R1 mRNA expression [33, 34]. Overexpression of AT2R1 provokes coronary vasoconstriction and fibrosis, which possibly induced myocardial ischemia [34, 35]. AT2R1 levels increased in high-fat diet groups as we expected, but this response was not affected by obestatin or exercise interventions.

BNP is mainly synthesized in ventricular myocytes for the response of myocytes stretches and pressure overload and is widely used as a biomarker for heart failure and cardiac dysfunction [36]. In our study, we demonstrated that BNP levels significantly decreased in the HFD group compared to the control diet. Obestatin or exercise, or both interventions prevent decreased BNP levels in the HFD group. These findings suggest that obestatin may have protective effects on myocardial cells.

4. CONCLUSION

In summary, we examined cardiac contractility and hemodynamics in the HFD model and, for the first time, compared the effects of chronic treatment with obestatin with those of swimming exercise in this animal model. Our results show that obestatin has protective effects on certain cardiac parameters compared with exercise. The mechanism of these protective effects modulates BNP levels. In addition, the relationship between obestatin and AT2R1 was investigated in HFD backgrounds. We believe that obestatin has the potential to maintain cardiac function in HFD. We believe that further studies on the stimulation or blockade of obestatin receptors are needed.

5. MATERIALS AND METHODS

5.1. Animals

Seventy-two Sprague-Dawley rats (weighed between 200 to 250 g) were purchased from Trakya University Experimental Animals Unit (Ethics Committee approval from TUHDYEK No:2013.06.04). All animals were housed under standard conditions (22±1 °C; 12/12h light–dark cycle; food and water access ad libitum). Rats were randomly divided into two groups: as control diet (CD, 10% calorie from fat) and high-fat diet (HFD, 40% calorie from fat) groups. Initially, diet groups were maintained under sedentary conditions for 4 weeks. After 4-weeks feeding period, each group was divided into 4 subgroups as saline, OB (OB, kaç mikrogram kaç gün, i.p.), exercise (EX) and EX+OB (n=9 for each). Subgroups maintained their respective diet protocol together with interventions for another 4 weeks as seen in Figure 4. After 8 weeks of food consumption (CD or HFD) and intervention period, animals were sacrificed under general anesthesia (Sodium pentobarbital, 65 mg/kg, i.p). Hearts were prepared for the Langendorf set up. Trunk blood was collected and centrifuged; serum samples were stored at -80°C until biochemical assays. Body weight measurements were performed on the first day of each week for 8 weeks. Body length was defined as the distance from the nose to the anus of rats (Lee index: LI=body weight (g) 1/3×1000/body length (cm)) [37]. Standard rat-chow (CD) was transformed into high-fat rat chow (HFD) as previously described [38].

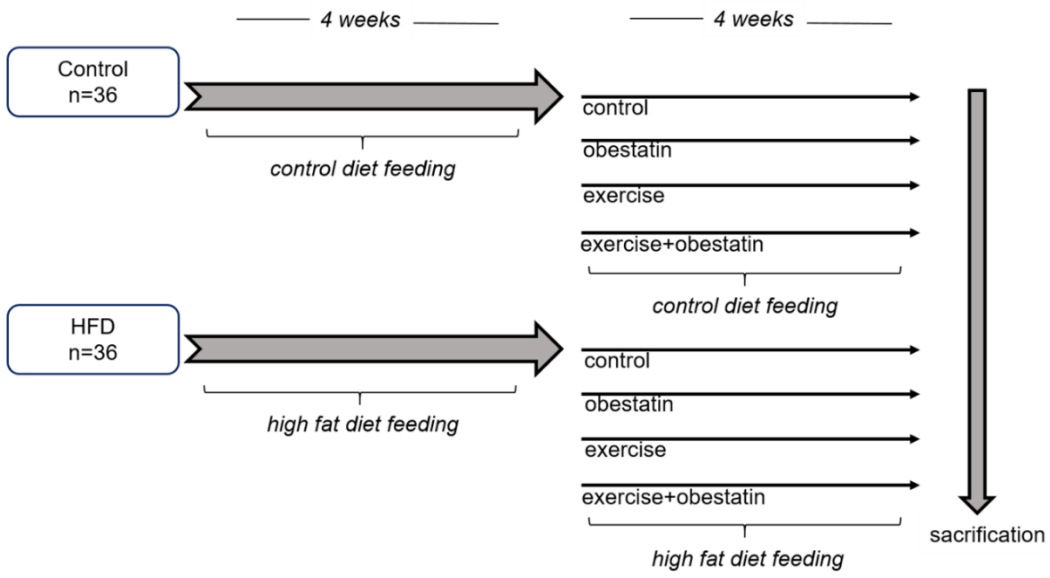

Figure 4. Experimental design of the study.

5.2. Moderate exercise protocol

Swimming exercise was conducted in a water tank (height 55 cm, diameter 58, water depth 33 cm and the temperature was 30±2 °C, five days a week/ 4 weeks/ 20 minutes) were used for swimming exercise as a regular moderate exercise model [39].

5.3. Treatments

Following 4 weeks of the feeding period, OB (cat.no.2445, Tocris, Bristol, UK) was administered intraperitoneally (i.p) at a dose of 25 µg/ml/kg to the relevant groups once a day/ five days/week for 4

weeks. Administrations were made immediately after exercise in the EX+OB group and simultaneously in the OB group (Figure 4).

5.4. Langendorff organ bath

The hearts were isolated from the rats and perfused via the ascendant aorta in a Langendorf apparatus under constant pressure (70 mmHg) with gassed (95% O2, 5% CO2) Krebs-Henseleit buffer at 37°C, as described in detail previously. A pressure transducer connected to a saline-filled balloon inserted into the left ventricle (LV) was used to assess ventricular function by measuring the ventricular pressure (mmHg) and its first derivative (dP/dt). Left ventricular end-diastolic pressure (LVEDP) was set at approximately 5-10 mmHg. After baseline recording for 15 minutes, LV developed pressure (LVDP), heart rate (HR), dp/dtmax, dp/dtmin, and LVEDP were continuously recorded for 40 minutes (BIOPAC MP36 System, Inc., USA) [40].

5.5. Serum lipid and cytokine measurements

After the experimental procedure and removal of the heart trunk, blood was drawn and centrifuged at 3000 rpm for 15 minutes, and serum samples were stored at -80°C until assayed. Lipid analysis included measurements of cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), whereas cytokine measurements included brain natriuretic peptide (BNP) and angiotensin II receptor-1 (AT2R1). Serum lipid levels were measured with an autoanalyzer. Serum BNP levels were measured with an enzyme immunoassay (EIA) using a commercial kit (RayBio catalog number: EIA-BNP-1), and serum AT2R1 levels were measured with a commercial kit (EastBiopharm catalog number: CK-E91197).

5.6. Statistics

The results are expressed as mean ± standard deviation. Normal distribution of variates were tested by Kolmogorov-Smirnov test. Intergroup comparisons were made by analyzing variance (ANOVA) and post hoc Tukey-Kramer test or Spearman correlation. A p-value lower than 0.05 was accepted as significant.

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Author contributions: G.M. and L.O. conceived of the presented idea. L.O. encouraged G.M. to investigate the cardiac hemodynamic and supervised the findings of this work. G.M. and O.P. carried out the experiment. G.M. developed the theory and performed the computations. L.O. supervised the project. All authors discussed the results and contributed to the final manuscript.

Conflict of interest statement: The authors declared the none conflicts of interest with respect to the research, authorship, and/or publication of this article.

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