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Role of Obestatin in Improvement of Obesity-Induced Metabolic and Kidney Function Changes in Exercised Rats

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Abstract

Background: Obesity is a common health problem which may be caused by feeding a high fat diet (HFD) and has caused many complications including metabolic and kidney function changes. On the other hand, obestatin is an adipokine that was found to be expressed also in the kidney and decrease body weight of obese rats. Also, moderate exercise training was suggested to improve obesity and minimize its complications, but, the exact mechanism was not well understood.

Aim: To investigate the obesity-induced metabolic and kidney function changes in exercised rats and clarify the potential role of obestatin.

Material and Methods: 48 male albino rats of local strains were randomly divided into two groups (I and II). Group I (lean group, n=24) included group IA (sedentary group, n=8), group IB (exercised group, n=8), and, group IC (obestatin treated group, n=8). Group II [obese, high fat diet (HFD)-induced obesity, n=24] was formed of group IIA (obese Sedentary, n=8), group IIB (obese exercised, n=8), and, group IIC (obese obestatin treated, n=8). At the end of experimental period, blood samples were obtained and allowed to clot at room temperature before centrifugation to obtain serum that was stored at -20 ° C for biochemical assessment.

Results: Obese groups (sedentary, exercised and obestatin treated) had a significant increase in body mass index (BMI), adiposity %, serum [glucose, insulin, C-peptide, total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), C-reactive protein (CRP), tumor necrosis factor alpha (TNFα), creatinine, urea and K^+], urinary [Na^+, total protein and albumin], homeostasis model assessment of insulin resistance index (HOMA-IR) and urine volume, with a significant decrease in serum [obestatin, high-density lipoprotein (HDL), Na^+, total protein and albumin] and urinary (creatinine and K^+) and creatinine clearance in the same groups. On the other hand, obese exercised and obese obestatin treated groups showed a significant reduction in BMI, adiposity %, serum (glucose, insulin and C-peptide, TC, TG, LDL, CRP, TNFα creatinine, urea and K^+), urinary (Na^+, total protein, albumin) and urine volume, with a significant

increase in serum (obestatin, HDL, Na^+, total protein and albumin) and urinary (creatinine and K^+) and creatinine clearance in the same groups. Within the obese sedentary and obese exercised groups, serum obestatin was negatively associated with BMI, atherogenic index, serum (glucose, insulin, TC, TNFα, creatinine and urea), HOMA-IR, and urinary Na^+, but, it was positively associated with Homeostatic Model Assessment of beta cell function (HOMA-B), urinary K^+ and creatinine clearance.

Conclusion: Moderate exercise training improved both metabolic and kidney function changes that occurred with HFD-induced obesity through its anti-inflammatory effect and increasing obestatin secretion.

Keywords: Obesity, Exercise training, Obestatin, Kidney function.

1. Introduction

Obesity is a chronic disease that was defined as increased body fat accumulation, and, it was associated with metabolic abnormality [1]. Park, Kim [2] and Soltani, Washco [3] declared that excessive caloric intake contributed to obesity and initiated a cascade that altered kidney functions and finally lead to obesity-related chronic kidney disease.

Previous studies declared that adipose tissue produced bioactive substances termed adipokines [4] which induced production of reactive oxygen species (ROS) causing systemic oxidative stress that increased production of proinflammatory cytokines causing renal injury [5]. On the other hand, Teixeira-Lemos, Nunes [6] proved that exercise training reduced oxidative stress.

Obestatin is, a 23-amino acid-peptide, synthesized from preproghrelin which also gives rise to ghrelin [7]. It is produced by gastric mucosa, duodenum, pancreas, kidney, brain, testis, mammary glands and adipose tissue [4, 7-9]. Grönberg, Tsolakis [10] confirmed that obestatin was expressed by both pancreatic islets and ducts. Previous studies showed controversies about effect of obestatin on body metabolism. Green and Grieve [11] stated that obestatin had positive effects on pancreatic β -cells and islets, while, Agnew, Calderwood [12] and Unniappan, Speck [13] declared that administration of obestatin to normoglycemic rats had no effect on circulating glucose or insulin.

On the other hand, Ren, Guo [14] found that obestatin inhibited glucose-induced insulin secretion. Also, Gao, Kuang [15], Lippl, Erdmann [16], Egido, Hernández [17] and Zhang, Li [18] reported an inverse relationship between circulating obestatin and insulin levels. On the contrary, Granata, Settanni [19] and Pradhan, Wu [20] confirmed the ability of obestatin to stimulate insulin secretion under hyperglycemic conditions. On the other hand, Agnew, Calderwood [12] and Nagaraj, Raghavan [21] stated that chronic obestatin treatment reduced blood triglycerides with no effect on blood cholesterol. On the other hand, Gao, Kuang [15], Gao, Kuang [22], Shen, Yu [23] and Wang, Li [24] detected a decrease in blood obestatin in obese patients with hyperglycemia, metabolic syndrome, and insulin resistance.

Also, Lee, Chen [25] confirmed that obese patients who achieved body weight reduction had increased obestatin levels. On the contrary, Arrigo, Gitto [26] and Prodam, Cadario [27] found increased obestatin levels in patients with obesity, whilst Lee, Chen [25] and Siejka, Jankiewicz-Wika [28] stated that weight loss in obese patients had no effect on obestatin levels. Also, up to our knowledge, there was no study that assessed effect of moderate exercise training and exogenous obestatin treatment on kidney function changes in case of diet-induced obesity.

So, this study was conducted to investigate the obesity-induced metabolic and kidney function changes occurred in exercised rats and to clarify the potential role of obestatin.

2. Materials and Methods

In Zagazig Faculty of Medicine Physiology Department, this study was achieved, from July 2018 to December 2018. Forty-eight adult healthy male albino rats, of local strains, weighing 180 - 220 gm were obtained from the animal house of Faculty of Medicine, Zagazig University. They were put in steel wire cages (four per cage) in the

Physiology Department research laboratory, under hygienic conditions, at room temperature (23 ±3° C) and on 12-hour light/dark cycle. For accommodation to laboratory conditions, rats were kept for one week before starting the experimental program on free access to commercial rat laboratory chow and water.

The used experimental protocol in this study was achieved according to the data guiding the use of research animals and was approved by Institutional Research Board of Faculty of Medicine, Zagazig University.

Experimental design: The rats were randomly divided into two groups I (lean) and II (obese). Group I (lean group, n=24): the animals in this group were fed on ordinary laboratory chow diet (consisted of carbohydrate 62.8%, protein 25.8% and fat 11.4% and it was obtained from Zagazig Faculty of Agriculture) for 14 weeks (duration of the study), and further subdivided into 3 subgroups; group IA (sedentary group, n=8) in which the rats remained sedentary in their cages (no exercise), group IB (exercised group, n=8) in which the rats were trained by swimming exercise training of moderate intensity (one hour/day, five days/week) [29] in the last 6 weeks of this 14-week-study, and, group IC (obestatin treated group, n=8) in which rats received a single daily i.p. dose of obestatin (64 µg/kg body weight dissolved in 0.1 ml normal saline) [30] for the last 6 weeks of the study while they remained sedentary in their cages. Rats of lean sedentary and lean exercised groups were injected i.p. daily with a single dose of 0.1 ml normal saline in the last 6 weeks of the study. Obestatin was purchased from Sigma Aldrich Co.-USA (Catalog No. O0266). Group II [obese, high fat diet (HFD)-induced obesity, n=24]: rats in this group were fed a HFD for 8 weeks; this HFD consisted of (16.4% protein, 25.6% carbohydrate and 58.0% fat in the form of cotton seed oil added to the laboratory chow diet [31] and further subdivided into 3 subgroups; group IIA (obese Sedentary, n=8) in which the rats remained sedentary in their cages (no exercise), group IIB (obese exercised, n=8) in which the rats were trained by swimming exercise training of moderate intensity in the last 6 weeks of the study as in group IB, and, group IIC (obese obestatin treated, n=8) in which the rats received a single daily i.p. dose of obestatin, as in group IC, for the last 6 weeks of the study while they remained sedentary in their cages. Rats of obese sedentary and obese exercised groups were injected i.p. daily with a single dose of 0.1 ml normal saline in the last 6 weeks of the study.

Table 1: Experimental design

Lean group (24 rats)	Lean sedentary (8 rats)	Ordinary diet	Ordinary diet + Saline
	Lean exercised (8 rats)	Ordinary diet	Ordinary diet + Exercise + Saline
	Lean obestatin treated (8 rats)	Ordinary diet	Ordinary diet + Obestatin
Obese group (24 rats)	Obese sedentary (8 rats)	HFD	HFD + Saline
	Obese exercised (8 rats)	HFD	HFD + Exercise + Saline
	Obese obestatin treated (8 rats)	HFD	HFD + Obestatin
Duration (14 weeks)		8 weeks	6 weeks

HFD= High fat diet.

Body mass index (BMI) was calculated at the end of the 8th week (as an indicator of obesity if it was more than 0.68 gm/ cm^2 [32]), and at the end of the study (to explore the changes in BMI among different groups).

This equation was used:

BMI= [Body weight (gm)] / [Length^2 (cm^2)(from nose to anus length)] [32]

Swimming exercise program: The rats in the exercised groups performed moderate intensity swimming exercise, one hour/day, five days/week for 6 weeks. Swimming was practiced in a cylindrical tank of 45 cm diameter, 80 cm high and filled with 32- 35 ° C water 45 cm deep [33]. Swimming rats were initially trained for 15 minutes/day and duration was gradually increased such that the rats were able to perform exercise for one hour/day, which was achieved in one week [34]. Exercise was performed between 9- 10 am. At the end of each exercise session, the animals were kept

to dry in a warm environment [35]. The animals that practiced exercise were sacrificed 48 h after the end of the last training session to minimize the acute effects of the exercise [6].

At the end of experimental period, all rats were placed individually in metabolic cages for 24 hr. Urine was collected, centrifuged and stored at -20° C to avoid urea degradation [36] until analysis.

After 12 hours fasting, the blood samples were obtained from all rats after their scarification by decapitation. Blood samples were allowed to clot at room temperature (23 ± 3° C) before centrifuging for 15 minutes at 3000 revolutions per minute (rpm). The separated serum was stored at -20° C in dark containers for biochemical assay.

Serum levels of obestatin, insulin, glucose, triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), tumor necrosis factor alpha (TNFα), C-reactive protein (CRP), creatinine and urea were measured using commercial kits from Sigma-Aldrich, USA (Cat. No. RAB0208, RAB0904, GAGO20, TR0100, MAK043, MAK045, RAB0479, RAB0097, MAK080 and MAK006, respectively). Also, commercial kits were used for estimating serum levels of C-Peptide (ALPCO, Cat. No. 80-CPTRT-E01), total protein (Biocompare.com, Cat. No. ABIN996403) and albumin (abcam.com, Cat. No. ab235642). Moreover, commercial kits were used for estimating serum and urine levels of Na^+ (abcam.com, Cat. No. ab211096) and K^+ (Sigma-Aldrich, USA, Cat. No. 37202). Furthermore, commercial kits were used for measuring the urinary levels of creatinine (Sigma-Aldrich, USA, Cat. No. MAK080), total protein (Chondrex.com, Cat. No. 9040) and albumin (abcam.com, Cat. No. ab235642).

Homeostasis model assessment of insulin resistance (HOMA-IR) index was measured by the formula:

HOMA-IR= [(Insulin in μIU/L) X (Glucose in mg/dl)] / 405 [37]

Homeostatic Model Assessment of beta cell function (HOMA-B) index was measured by the formula:

HOMA-B = [(20 X Fasting Insulin in μIU/L) / (Fasting Glucose in mmol/L - 3.5)] [38]

Low-density lipoprotein (LDL) serum level was calculated using Friedewald formula:

LDL (mg/dl) = [TC] - [(HDL) + (TG / 5)] [39]

Atherogenic index was calculated using the formula:

Atherogenic index= [TC - HDL] / HDL [40]

Adiposity index (%) was determined by the sum of epididymal, visceral and retroperitoneal fat weights divided by body weight x100 [41].

Calculation of creatinine clearance:

Creatinine clearance (ml/min) = [(Urine Creatinine in mg/dl) X (Urine Volume in ml/day)] / [(Serum Creatinine in mg/dl) X (Time in min)] [42]

Statistical Analysis: The obtained data was expressed as mean values ± standard deviation (Mean ± SD). Means were compared by one-way analysis of variance (ANOVA) and Tukey HSD for Post hoc Multiple Comparisons using (IBM SPSS Statistics Version 25 Software for Windows) for statistical significance. Pearson correlation analysis using (GraphPad Prism Version 7 Software for Windows) was performed to study the associations between serum obestatin and different studied parameters within obese sedentary and obese exercised groups. P value ≤ 0.05 indicated significance.

3. Results

In this study, we investigated the effects of both exercise training and obestatin treatment on both metabolic and kidney function changes in diet-induced obesity. Obesity was induced by giving rats HFD for 8 weeks in the obese groups, then, rats of obese exercised group were exposed to a moderate exercise and those of obese obestatin treated group were administered obestatin for extra 6 weeks.

In Table 2, there was a significant ($P<0.001$) increase in both BMI and adiposity % in obese groups (sedentary, exercised and obestatin treated), in comparison to those of the lean groups. On the other hand, with either, performing exercise training (in obese exercised group) or treatment with obestatin (in obese obestatin treated group), for next 6 weeks, both BMI and adiposity % were significantly ($P<0.05$) reduced in comparison to obese sedentary group. Changes in serum obestatin showed a significant ($P<0.01$) increase in lean obestatin treated group in comparison to lean sedentary group.

On the other hand, a significant (P<0.001) reduction was detected in serum obestatin in obese groups (sedentary, exercised and obestatin treated), in comparison to those of the lean groups. On the contrary, a significant (P<0.01) increase in serum obestatin occurred in both obese exercised and obese obestatin treated groups in comparison to the obese sedentary group. Also, a significant (P<0.001) increase was found in serum level of glucose, insulin and C-peptide, and, HOMA-IR in obese groups (sedentary, exercised and obestatin treated), in comparison to those of the lean groups. On the other hand, a significant (P<0.001) decrease in serum level of both glucose and C-peptide occurred in both obese exercised and obese obestatin treated groups in comparison to those in the obese sedentary group.

Also, a significant (P<0.01) decrease in serum insulin was detected in both obese exercised and obese obestatin treated groups in comparison to that in the obese sedentary group. In comparison to obese exercised group, a significant (P<0.05) increase in serum level of insulin, C-peptide and HOMA-IR was found in obese obestatin treated group. On the other hand, a significant (P<0.05) decrease was found in HOMA-B in obese groups (sedentary, exercised and obestatin treated), in comparison to that of the lean groups.

In comparison to lean groups (sedentary, exercised and obestatin treated), a significant (P<0.001) increase was found in serum level of TC, TG and LDL, but a significant (P<0.001) decrease was detected in serum HDL in obese groups. On the other hand, a significant (P<0.001) decrease was declared in serum level of TC, TG and LDL, but, a significant (P<0.01) increase was detected in serum HDL, in both obese exercised and obese obestatin treated groups in comparison to obese sedentary group.

Moreover, a significant (P<0.01) increase was found in atherogenic index in obese groups (sedentary, exercised and obestatin treated), in comparison to that in the lean groups. On the other hand, a significant (P<0.001) decrease was declared in atherogenic index in both obese exercised and obese obestatin treated groups in comparison to obese sedentary group. In comparison to lean groups (sedentary, exercised and obestatin treated), a significant (P<0.001) increase was found in serum CRP and TNFα in the obese groups. On the other hand, a significant (P<0.001) decrease was detected in serum CRP and TNFα in both obese exercised and obese obestatin treated groups in comparison to the obese sedentary group. But, a significant (P<0.05) increase was confirmed in serum CRP in obese obestatin treated group in comparison to obese exercised group.

In Table 3, kidney function changes among different groups were assessed. In comparison to lean groups (sedentary, exercised and obestatin treated), a significant (P<0.001) increase was detected in serum levels of creatinine, urea and K^+, and in urinary Na^+, total protein, albumin and urine volume, but, a significant (P<0.001) decrease was confirmed in serum levels of Na^+, total protein and albumin, and, in urinary creatinine, K^+, and, creatinine clearance, in obese groups. In comparison to obese sedentary group, a significant (P<0.001) decrease was found in serum levels of creatinine, urea, K^+, and, in urinary Na^+, total protein, albumin, and, urine volume, but, a significant (P<0.05) increase was detected in serum levels of Na^+, total protein, albumin, and, in urinary creatinine, K^+, and, creatinine clearance, in both obese exercised and obese obestatin treated groups.

In comparison to obese exercised group, a significant (P<0.001) increase was estimated in serum creatinine, urea, K^+, and, in urinary Na^+, total protein, albumin, and urine volume, but, a significant (P<0.001) decrease was found in serum Na^+, total protein, albumin, and, in urinary creatinine and K^+, in obese obestatin treated group.

In Table 4, correlations within the obese sedentary group between serum obestatin and different studied parameters showed negative associations with final BMI (r=-0.959, P<0.001), serum glucose (r=-0.981, P<0.001), serum insulin (r=-0.964, P<0.001), HOMA-IR (r=-0.978, P<0.001), serum TC (r=-0.836, P<0.01), atherogenic index (r=-0.926, P<0.01), serum TNFα(r=-0.769, P<0.05), serum creatinine (r=-0.935, P<0.001), serum urea (r=-0.809, P<0.05) and urinary Na^+ (r=-0.804, P<0.05), but, positive associations with HOMA-B (r=0.986, P<0.001), urinary K^+ (r=0.729, P<0.05) and creatinine clearance (r=0.783, P<0.05).

Table 2: Biochemical changes among different groups

Parameter \ Group	Group I (Lean)			Group II (Obese)		
	Sedentary (IA)	Exercised (IB)	Obestatin treated (IC)	Sedentary (IIA)	Exercised (IIB)	Obestatin treated (IIC)
BMI (gm/cm^2)	0.51±0.04	0.45±0.05	0.51±0.04	0.94±0.07$^{a,b\&c}$	0.84±0.07$^{a,b,c\&d}$	0.81±0.06$^{a,b,c\&e}$
Serum obestatin (ng/ml)	157.9±6.7	161±9.1	172.6±8.6^{f}	105.9±6.6$^{a,b\&c}$	123.4±6.4$^{a,b,c\&e}$	132.9±9.7$^{a,b,c\&e}$
Serum glucose (mg/dl)	86.6±9.3	73.9±6.8	81.4±7.6	296.3±19.4$^{a,b\&c}$	228.4±17.3$^{a,b,c\&g}$	240.3±18.9$^{a,b,c\&g}$
Serum insulin (μIU/L)	12.2±1.8	9.5±1.2	11.1±1.8	35.8±2.9$^{a,b\&c}$	25.9±2.4$^{a,b,c\&e}$	29.7±3.6$^{a,b,c,e\&h}$
HOMA-IR	2.6±0.5	1.7±0.3	2.2±0.4	26.1±2.5$^{a,b\&c}$	14.6±1.7$^{a,b,c\&e}$	17.6±2.4$^{a,b,c,e\&i}$
HOMA-B	225.1±22.1	291.1±34.2	261.1±29.8	55.7±7.3$^{j,b\&l}$	57±7.7$^{j,b\&l}$	61±10.1$^{j,k\&l}$
Serum C-Peptide (ng/L)	104.1±11.3	86±8.2^{f}	94.4±10.7	193±12.7$^{a,b\&c}$	148.5±7.7$^{a,b,c\&g}$	168.1±6.7$^{a,b,c,g\&i}$
Serum TC (mg/dl)	180.9±10.9	169.3±10.7	174±10.2	315±31.4$^{a,b\&c}$	243.8±18.3$^{a,b,c\&g}$	266.4±10.7$^{a,b,c\&g}$
Serum TG (mg/dl)	130.5±5.7	115.8±4.6	122.5±4.5	249.4±30.5$^{a,b\&c}$	193.1±13.3$^{a,b,c\&g}$	202±13.6$^{a,b,c\&g}$
Serum HDL (mg/dl)	47.12±2.7	60.6±3.1^{a}	53.6±4.2$^{i\&k}$	25.1±3.6$^{a,b\&c}$	38.5±4.3$^{a,b,c\&g}$	33.5±4.1$^{a,b,c\&e}$
Serum LDL (mg/dl)	107.7±10.7	85.5±11.8	95.9±11.2	240±30.1$^{a,b\&c}$	166.6±19.9$^{a,b,c\&g}$	192.5±12.4$^{a,b,c\&g}$
Atherogenic index	2.8±0.3	1.8±0.2	2.3±0.4	11.9±2.9$^{a,b\&c}$	5.4±1.1$^{f,b,l\&g}$	7.1±1.2$^{a,b,c\&g}$
Serum CRP (μg/ml)	0.29±0.08	0.23±0.08	0.25±0.04	5.2±0.56$^{a,b\&c}$	2.59±0.29$^{a,b,c\&g}$	3.04±0.27$^{a,b,c,g\&h}$
Serum TNFα(pg/ml)	21.1±1.5	17.3±1.7	18.8±1.4	82.2±7.7$^{a,b\&c}$	48.4±5.4$^{a,b,c\&g}$	52.5±5.1$^{a,b,c\&g}$
Adiposity %	5.4±0.4	4.8±0.3^{f}	5.1±0.2	10.1±0.3$^{a,b\&c}$	7.6±0.4$^{a,b,c\&g}$	8.3±0.4$^{a,b,c,g\&i}$

Data was expressed as Mean±SD. ^{a}P<0.001 in comparison to lean sedentary group. ^{b}P<0.001 in comparison to lean exercised group. ^{c}P<0.001 in comparison to lean obestatin treated group. ^{d}P<0.05 in comparison to obese sedentary group. ^{e}P<0.01 in comparison to obese sedentary group. ^{f}P<0.01 in comparison to lean sedentary group. ^{g}P<0.001 in comparison to obese sedentary group. ^{h}P<0.05 in comparison to obese exercised group. ^{i}P<0.01 in comparison to obese exercised group. ^{j}P<0.05 in comparison to lean sedentary group. ^{k}P<0.01 in comparison to lean exercised group. ^{l}P<0.01 in comparison to lean obestatin treated group. BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-B, homeostasis model assessment of beta cell function; TC, total cholesterol; TG, triglycerides; HDL, high density lipoproteins; LDL, low density lipoproteins; CRP, C-reactive protein; TNFα, tumor necrosis factor alpha.

Table 3: Kidney function changes among different groups

Parameter \ Group	Group I (Lean)			Group II (Obese)		
	Sedentary (IA)	Exercised (IB)	Obestatin treated (IC)	Sedentary (IIA)	Exercised (IIB)	Obestatin treated (IIC)
Serum creatinine (mg/dl)	0.62±0.03	0.56±0.02	0.59±0.03	2.11±0.06$^{a,b\&c}$	1.53±0.11$^{a,b,c\&d}$	1.86±0.09$^{a,b,c,d\&e}$
Serum urea (mg/dl)	45±0.2	44.3±0.3	44.6±0.3	56.1±0.5$^{a,b\&c}$	48.7±0.8$^{a,b,c\&d}$	51±0.9$^{a,b,c,d\&e}$
Serum Na^+ (mg/dl)	141.9±0.4	141.5±0.5	141.7±0.3	122.7±0.7$^{a,b\&c}$	134.7±0.6$^{a,b,c\&d}$	129.5±0.9$^{a,b,c,d\&e}$

Serum K^+ (mg/dl)	2.73±0.08	2.66±0.05	2.72±0.05	5.58±0.15[a,b&c]	3.36±0.16[a,b,c&d]	4.2±0.2[a,b,c,d&e]
Serum total protein (gm/dl)	6.37±0.14	6.35±0.17	6.34±0.18	4.57±0.23[a,b&c]	5.61±0.17[a,b,c&d]	5.09±0.16[a,b,c,d&e]
Serum albumin (gm/dl)	2.91±0.12	2.91±0.12	2.9±0.09	1.55±0.08[a,b&c]	2.44±0.05[a,b,c&d]	2.01±0.09[a,b,c,d&e]
Urinary creatinine (mg/dl)	41.4±0.5	41.1±0.3	40.9±0.2	28.5±0.7[a,b&c]	38.4±0.7[a,b,c&d]	35.6±0.8[a,b,c,d&e]
Urinary Na^+ (mg/dl)	132.6±1	133.1±0.4	132.8±0.8	148.5±0.4[a,b&c]	137±0.6[a,b,c&d]	139.7±0.5[a,b,c,d&e]
Urinary K^+ (mg/dl)	126.4±0.6	126.4±0.6	126.1±0.6	74.4±4.2[a,b&c]	102.6±1[a,b,c&d]	88.7±0.5[a,b,c,d&e]
Urinary total protein (gm/dl)	0.44±0.01	0.4±0.02	0.42±0.02	3.09±0.07[a,b&c]	2.17±0.07[a,b,c&d]	2.75±0.06[a,b,c,d&e]
Urinary albumin (gm/dl)	0.36±0.03	0.34±0.02	0.35±0.02	1.73±0.05[a,b&c]	0.94±0.05[a,b,c&d]	1.24±0.06[a,b,c,d&e]
Urine volume (ml/24hr)	7.62±0.21	7.63±0.21	7.55±0.21	13.64±0.62[a,b&c]	9.98±0.75[a,b,c&d]	11.61±0.96[a,b,c,d&e]
Creatinine clearance (ml/min)	0.36±0.02	0.39±0.02f	0.36±0.02	0.13±0.01[a,b&c]	0.17±0.01[a,b,c&d]	0.16±0.02[a,b,c&g]

Data was expressed as Mean±SD. [a]P<0.001 in comparison to lean sedentary group. [b]P<0.001 in comparison to lean exercised group. [c]P<0.001 in comparison to lean obestatin treated group. [d]P<0.001 in comparison to obese sedentary group. [e]P<0.001 in comparison to obese exercised group. [f]P<0.01 in comparison to lean sedentary group. [g]P<0.05 in comparison to obese sedentary group.

Table 4: Pearson's correlation coefficient (r) between serum level of obestatin and some biochemical parameters in both obese sedentary and obese exercised groups

	Serum obestatin			
	Obese sedentary group		Obese exercised group	
	r	P	r	P
Final BMI	-0.959	<0.001	-0.944	<0.001
Serum glucose	-0.981	<0.001	-0.92	<0.01
Serum insulin	-0.964	<0.001	-0.765	<0.05
HOMA-IR	-0.978	<0.001	-0.97	<0.001
HOMA-B	0.986	<0.001	0.712	<0.05
Serum TC	-0.836	<0.01	-0.736	<0.05
Atherogenic index	-0.926	<0.01	-0.955	<0.001
Serum TNFα	-0.769	<0.05	-0.816	<0.05
Serum creatinine	-0.935	<0.001	-0.72	<0.05
Serum urea	-0.809	<0.05	-0.747	<0.05
Urinary Na^+	-0.804	<0.05	-0.813	<0.05
Urinary K^+	0.729	<0.05	0.79	<0.05
Creatinine clearance	0.783	<0.05	0.897	<0.01

P<0.05 indicated statistical significance. BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-B, homeostasis model assessment of beta cell function; TC, total cholesterol; TNFα tumor necrosis factor alpha.

On the other hand, correlations within the obese exercised group between serum obestatin and different studied parameters showed negative associations with final BMI (r=-0.944, P<0.001), serum glucose (r=-0.92, P<0.01), serum insulin (r=-0.765, P<0.05), HOMA-IR (r=-0.97, P<0.001), serum TC (r=-0.736, P<0.05), atherogenic index (r=-0.955, P<0.001), serum TNFα (r=-0.816, P<0.05), serum creatinine (r=-0.72, P<0.05), serum urea (r=-0.747, P<0.05) and urinary Na^+ (r=-0.813, P<0.05), but, positive associations with HOMA-B (r=0.712, P<0.05), urinary K^+ (r=0.79, P<0.05) and creatinine clearance (r=0.897, P<0.01).

4. Discussion

In this study, rats in the obese groups (sedentary, exercised and obestatin treated) were fed HFD to induce obesity that was confirmed by the use of BMI and adiposity % as a significant increase was found in their values in comparison to those of the lean groups. This was supported by EL-Gohary and Hussien [36] who declared that HFD-induced obesity was associated with increased BMI.

On performing exercise training (in obese exercised group) or treatment with obestatin (in obese obestatin treated group), for the last 6 weeks of the study, both BMI and adiposity % were significantly reduced in comparison to obese sedentary group. These results confirmed that exercise training and obestatin treatment deteriorated HFD-induced obesity which was supported by EL-Gohary and Hussien [36]. In contrary, Chow, Greenlund [43] declared that exercise alone is not effective for weight loss. This controversy may be referred to species difference, duration and type of exercise performed.

On the other hand, a significant reduction was detected in serum obestatin in obese groups (sedentary, exercised and obestatin treated), in comparison to that of the lean groups. Also, a negative correlation was estimated within the obese sedentary and obese exercised groups between serum obestatin and BMI. This was in agreement with Lacquaniti, Donato [44], Granata, Gallo [7] and Wang, Li [24] who confirmed that serum obestatin was inversely correlated with BMI. This may confirm that obesity had an inhibitory effect on obestatin production causing lack of its anorectic effect leading to more weight gain. On the contrary, significant increases in serum obestatin levels occurred in both obese exercised and obese obestatin treated groups in comparison to the obese sedentary group.

These results declared that obestatin may have a role in exercise-induced modifications on metabolic and renal changes with obese rats. This was supported by Shen, Yu [23] who declared that obestatin treatment decreased body weight by reduction of food intake, delay of gastric emptying, suppression of jejunal contraction and inhibition of ghrelin biological actions as it acted as an endogenous antagonist for ghrelin. Also, [18] stated that obestatin crossed blood-brain barrier and acted on brain centers to inhibit hunger. On the contrary, Wang, Chen [45] stated that obestatin had no effect in exercise-induced change body weight. This controversy may be explained by the difference in type and duration of exercise training. Also, significant increases were found in serum levels of glucose, insulin and C-peptide, and in HOMA-IR in obese groups (sedentary, exercised and obestatin treated), in comparison to those of the lean groups.

Moreover, a negative correlation was found within the obese sedentary and obese exercised groups, between serum obestatin and serum glucose, serum insulin and HOMA-IR. These results confirmed increased insulin secretion and development of insulin resistance. This was supported by EL-Gohary and Hussien [36] and El Sawy, El-Sherbiny [46] who declared that HFD-induced obesity was associated with increased serum glucose, serum insulin and HOMA-IR. El Sawy, El-Sherbiny [46] explained the increased insulin level in HFD-induced obesity by fat accumulation in adipocytes, muscles and liver causing insulin resistance.

On the other hand, significant decreases in serum level of both glucose and C-peptide occurred in both obese exercised and obese obestatin treated groups in comparison to the obese sedentary group. This was in agreement with Saengsirisuwan, Perez [47] who confirmed the decrease in blood glucose with exercise and they owed this to increased glucose transport by increased GLUT-4 concentration in skeletal muscle and elevated activity of both hexokinase II and glycogen synthase enzymes. Also, Lacquaniti, Donato [44]

confirmed that obestatin treatment improved glucose homeostasis. Also, a significant decrease in serum insulin was detected in both obese exercised and obese obestatin treated groups in comparison to the obese sedentary group. But, in comparison to obese exercised group, a significant increase in serum insulin was found in obese obestatin treated group.

Also, a significant increase was detected in both serum C-peptide and HOMA-IR in obese obestatin treated group in comparison to obese exercised group. These results confirmed that muscular exercise improved metabolic parameters more than obestatin treatment did. But, with exercise, obestatin serum levels were significantly increased which indicated that mechanism of actions of exercise was partly mediated through increasing serum obestatin levels. These results were supported by Savini, Catani [48], EL-Gohary and Hussien [36], Gibb, Epstein [29] and EL-Gohary [49] who confirmed that exercise training deteriorated insulin resistance as evidenced by the decrease in plasma glucose, plasma insulin, and HOMA-IR. Gibb, Epstein [29] stated that exercise training increased both skeletal muscle glucose transporter (GLUT4) activity, and hepatic insulin clearance.

Also, Dewal, Stanford [50] declared that moderate exercise training increased glucose uptake by adipocytes. Moreover, Mafra, Guebre-Egziabher [51], Seim, Walpole [52] and Zhang, Li [18] declared that plasma obestatin was negatively associated with plasma insulin, plasma glucose and HOMA-IR. Furthermore, Granata, Gallo [7] and Green and Grieve [11] confirmed that obestatin improved glucose tolerance and insulin sensitivity in HFD-induced obesity. On the other hand, a significant decrease was found in HOMA-B in obese groups (sedentary, exercised and obestatin treated), in comparison to those of the lean groups. Also, a positive correlation was detected within the obese sedentary and obese exercised groups between serum obestatin and HOMA-B. These results confirmed that obesity impaired beta cell function which was partially improved by exercise and obestatin treatment, although, this improvement was insignificant.

This was supported by Granata, Settanni [19], Favaro, Granata [53] and Green and Grieve [11] who confirmed that obestatin improved β-cell function through enhancing its viability and inhibiting its apoptosis. In comparison to the lean groups (sedentary, exercised and obestatin treated), a significant increase was found in serum TC, TG and LDL, but a significant decrease was detected in serum HDL in obese groups. Also, a negative correlation was detected within the obese sedentary and obese exercised groups between serum obestatin serum TC. These results confirmed occurrence of impaired metabolic function with obesity. On the other hand, a significant decrease was declared in serum TC, TG and LDL in both obese exercised and obese obestatin treated groups in comparison to obese sedentary group.

On the contrary, a significant increase was detected in serum HDL in both obese exercised and obese obestatin treated groups in comparison to obese sedentary group. These results confirmed that exercise improved metabolic functions partially through increasing serum obestatin. These results were partially supported by El Sawy, El-Sherbiny [46] who found that obestatin treatment of obese rats decreased serum TC, serum TG and BMI, but they reported insignificant changes in both serum glucose and insulin levels. The partial difference in the results of that study may be related to the duration of treatment with obestatin. Mechanisms involved in improvement of lipid metabolism by obestatin include its ability to decrease food intake and body weight via reduction of perirenal and epididymal fat with reduction of stored fat [54]. Also, obestatin reduced intestinal absorption of TG [12]. Moreover, obestatin increased fatty acid uptake, but, it inhibited lipolysis [44].

Furthermore, obestatin reduced serum lipids through stimulation of leptin secretion [46]. On the other hand, a significant increase was found in atherogenic index in obese groups (sedentary, exercised and obestatin treated), in comparison to those of the lean groups. This was in agreement with Ivanova, Myasoedova [55] who declared that HFD-induced obesity was manifested by increased blood LDL which passed through the endothelial fenestrations entering subendothelial spaces where it can be transformed into plaques that caused atherosclerosis and kidney disease progression. But, a significant decrease was reported in atherogenic index in both obese exercised and obese obestatin

treated groups in comparison to obese sedentary group. Also, a negative association was found within the obese sedentary and obese exercised groups between serum obestatin and atherogenic index.

These changes in atherogenic index with obese exercised and obese obestatin treated groups can be explained by the improvement in the lipid profile that occurred in these groups in comparison to the obese sedentary group. In comparison to lean groups (sedentary, exercised and obestatin treated), a significant increase was found in serum levels of both CRP and TNFα in the obese groups. Also, a negative correlation was declared within the obese sedentary and obese exercised groups between serum levels of both obestatin and TNFα. These results confirmed presence of inflammatory reaction with obesity. This was supported by Speretta, Rosante [56] who explained the obesity-induced insulin resistance by the increase in serum TNFα which increased insulin resistance through increasing the release of free fatty acids in adipocytes and by blocking the synthesis of adiponectin.

On the other hand, a significant decrease occurred in serum CRP and TNFα in both obese exercised and obese obestatin treated groups in comparison to obese sedentary group. These results confirmed that both exercise and obestatin had anti-inflammatory activities. This was supported by Speretta, Rosante [56] and EL-Gohary and Hussien [36] who declared that TNFα was reduced among obese exercised rats. Also, Savini, Catani [48] stated that exercise had antioxidant and anti-inflammatory effects that prevented obesity-associated complications through improving glucose homeostasis and antioxidant body defenses.

In comparison to lean groups (sedentary, exercised and obestatin treated), a significant increase was detected in serum levels of creatinine, urea and K^+, and in urinary Na^+, total protein, albumin and urine volume in obese groups. Also, a negative correlation was detected within the obese sedentary and obese exercised groups between serum obestatin and each of serum creatinine, serum urea and urinary Na^+. On the other hand, a significant decrease was detected in serum levels of Na^+, total protein and albumin, and, urinary creatinine, K^+, and, creatinine clearance in obese groups (sedentary, exercised and obestatin treated) in comparison to lean groups.

Also, a positive correlation was found within the obese sedentary and obese exercised groups between serum obestatin and each of urinary K^+ and creatinine clearance. These results confirmed that obesity deteriorated kidney function and this was accompanied by low obestatin level. This was supported by Lacquaniti, Donato [44] who found low obestatin levels in chronic kidney disease. Also, Amin, Kamel [57] and EL-Gohary and Hussien [36] declared that obese rats had a significant elevation in serum urea, serum creatinine, and urinary albumin. Moreover, Soltani, Washco [3] explained the disturbances occurred in electrolytes and nitrogenous waste products with kidney disease by the essential role exerted by the kidney in regulation of these substances.

Furthermore, Wang, Chen [58] supported the deterioration in kidney function that occurred with obesity and they owed this to the binding of the elevated glucose irreversibly to proteins in the kidney forming advanced glycosylation end products that finally stimulated fibrotic growth factors causing renal damage. In addition, Kume, Uzu [59] stated that HFD caused an imbalance between lipolysis and lipogenesis in the kidney in addition to the systemic metabolic changes that finally lead to renal lipid accumulation with subsequent kidney injury. This was supported by Choudhary, Naheed [60] who confirmed that hyperlipidemia was associated with renal function deterioration.

Also, Tokuyama, Wakino [61] declared that obesity increased glomerular injury and caused glomerular hyperfiltration. Moreover, Serra, Romero [62] confirmed that prolonged obesity was manifested by proteinuria. Furthermore, El-Wakf, Serag [63] stated that HFD-induced obesity caused kidney dysfunction that was characterized by changes in both serum and urine of Na^+ and K^+. Soltani, Washco [3] explained the presence of proteinuria and changed serum and urinary Na^+ and K^+ with obesity-induced kidney disease by occurrence of glomerular membrane degeneration, and renal tubular injury.

On the other hand, Darouich, Goucha [64] detected occurrence of renal injury with obesity and they owed this to oxidative stress that has been

detected in obesity-related renal diseases. EL-Gohary and Hussien [36] explained occurrence of obesity-induced oxidative stress by the increased adipose tissue which enhanced proinflammatory cytokines as TNFα that increased production of reactive oxygen species. This was agreed with Fernández-Sánchez, Madrigal-Santillán [5] who stated that obesity was considered as a state of chronic inflammation as adipocytes produced proinflammatory cytokines including TNFα.

Also, Noeman, Hamooda [41] declared that renal lipids are rich in long-chain polyunsaturated fatty acids which made kidney to be more liable to damage by ROS which oxidized lipids and proteins causing cellular injury and increasing glomerular and renal tubule damage and proteinuria [65]. In comparison to obese sedentary group, a significant decrease was found in serum levels of creatinine, urea, K^+, and, in urinary Na^+, total protein, albumin, and, urine volume in both obese exercised and obese obestatin treated groups. But, a significant increase was found in serum levels of Na^+, total protein, albumin, and, in urinary creatinine, K^+, and, creatinine clearance in both obese exercised and obese obestatin treated groups, in comparison to obese sedentary group.

Also, in comparison to obese exercised group, a significant increase was estimated in serum creatinine, urea, K^+, and, urinary Na^+, total protein, albumin, and urine volume, in obese obestatin treated group. On the other hand, a significant decrease was found in serum Na^+, total protein, albumin, and, urinary creatinine and K^+ in obese obestatin treated group in comparison to obese exercised group. These results confirmed that moderate exercise improved kidney function through increasing serum obestatin and decreasing proinflammatory cytokines and improving body metabolism. This was supported by Lin, Lin [66] and EL-Gohary and Hussien [36] who declared that serum creatinine, urea and albuminuria were declined in obese exercised rats. Also, Park, Kim [2] confirmed that moderate exercise protected against renal injury in HFD-induced obesity in rats.

Moreover, Toyama, Sugiyama [67] stated that regular exercise improved renal function in obesity-associated renal injury in rats and they referred this to elevation of HDL and reduction of advanced glycosylation end products formation. Furthermore, Ishikawa, Gohda [68] reported that exercise training decreased albuminuria in obese rats and they owed this to reduction of proinflammatory cytokines. In addition, Gleeson, Bishop [69] confirmed the anti-inflammatory action of exercise training and they owed it to visceral fat mass reduction. Therefore, exercise training acted as a natural anti-inflammatory strategy that prevented obesity-induced kidney dysfunction.

On the other hand, Park, Kim [2] and EL-Gohary and Hussien [36] declared that exercise training improved the antioxidant state and hence improved kidney function. Also, Aragno, Mastrocola [70] found that obestatin had antioxidant activity through increasing activity of prosurvival kinases. Thus, the antioxidant activity of obestatin can be considered as another mechanism by which obestatin improved kidney function changes with obesity. This was confirmed by Koç, Kumral [71] who reported that obestatin exerted anti-inflammatory and antioxidant activities in chronic kidney diseases.

5. Conclusion

This study found that moderate exercise training improved both metabolic and kidney function changes that occurred with HFD-induced obesity through its anti-inflammatory effect and increasing obestatin secretion.

Conflict of Interest

None declared.

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