Research Article

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The investigation effect of weight loss on serum vaspin, apelin-13, and obestatin levels in obese individual

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Abstract

Objectives: It was aimed to investigate if there were any significant corresponding changes on adipokine levels in obese subjects who achieved a 10% reduction in body weight.

Methods: Thirty obese and 25 healthy adults were enrolled in present study, and serum levels of vaspin, apelin-13, obestatin, and insulin were determined with the ELISA method.

Results: The serum obestatin and apelin-13 values of the obese group obtained as basal and after weight loss was significantly lower than in controls ($p < 0.05$, $p < 0.01$, $p < 0.01$, $p < 0.05$, respectively); however, weight loss did not cause significant changes on these parameters in obese groups ($p > 0.05$). The vaspin level did not differ between the groups ($p > 0.05$). The obese group had characterized increased serum insulin and insulin resistance assessment by the homeostatic assay (HOMA-IR) levels compared to controls ($p < 0.01$, $p < 0.05$, respectively); also, weight loss caused a significant decrease in these parameters compared to basal levels ($p < 0.01$). No significant correlation was detected among the vaspin, apelin-13 and obestatin levels in the obese group ($p > 0.05$).

Conclusions: Obese individuals exhibited decreased levels of apelin-13 and obestatin. Moreover, 10% weight loss caused a significant reduction of insulin resistance, but no significant change was detected on apelin-13, obestatin, and vaspin levels.

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Keywords: apelin-13; obesity; obestatin; vaspin; weight loss.

Özet

Türkçe Başlık: Kilo Kaybının Obez Bireylerde Serum Vaspin, Apelin-13 ve Obestatin Düzeyleri Üzerine Etkisinin Araştırılması

Amaç: Bu çalışmada obez bireylerin vücut ağırlıklarında % 10 azalma sağlandığında adipokin düzeylerinde anlamlı bir değişiklik olup olmadığının araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Otuz beş obez, 25 sağlıklı yetişkin çalışmaya alındı ve serum vaspin, apelin-13, obestatin ve insülin düzeyleri ELISA yöntemi ile belirlendi.

Bulgular: Obez grubun bazal ve kilo kaybı sonrası elde edilen serum obestatin ve apelin-13 değerleri, kontrollere göre anlamlı derecede düşüktü (sırasıyla $p < 0.05$, $p < 0.01$, $p < 0.01$, $p < 0.05$); ancak kilo kaybı obez grupta bu parametrelerde anlamlı değişikliğe neden olmadı ($p > 0.05$). Vaspin düzeyi gruplar arasında farklılık göstermedi ($p > 0.05$). Obez grup, kontrollere göre artmış serum insülin ve HOMA-IR düzeylerine sahipti (sırasıyla $p < 0.01$, $p < 0.05$); ayrıca bazal seviyelere göre, kilo kaybı bu parametrelerde anlamlı azalmaya neden oldu ($p < 0.01$). Obez grupta vaspin, apelin-13 ve obestatin düzeyleri arasında anlamlı ilişki saptanmadı ($p > 0.05$).

Sonuçlar: Apelin-13 ve obestatin düzeyleri obez bireylerde düşüktü. Ayrıca, %10 kilo kaybı insülin direncinde önemli azalmaya neden olurken, apelin-13, obestatin ve vaspin seviyelerinde önemli bir değişikliğe neden olmadı.

Anahtar Kelimeler: Apelin-13; obezite; obestatin; vaspin; kilo kaybı.

Introduction

Obesity, which occurs as a result of excessive accumulation of adipose tissue, is an important public health problem worldwide with social and psychological dimensions. Obesity emerges from the imbalance between energy intake and expenditures due to many reasons such as

genetic, epigenetic, physiological, behavioral, socio-cultural and environmental factors [1, 2].

Being overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. Measurement of obesity is the body mass index (BMI), the weight of person (kg) divided by the square of height (in meters). The BMI of a person is classified as overweight when it is 25 kg/m² and above and as obese when it is 30 kg/m² and above [3]. The prevalence of obesity varies from country to country and has increased worldwide [4]. According to two adult-based studies (TURDEP-I and TURDEP-II) in Turkey, conducted in 1998 in the same centers with 12 year interval, obesity prevalence was 22.3% (females 32.9% and males 13.2%); while in 2010, obesity reached 31.2% (females 44.2% and males 27.3%) [5]. Long-term epidemiological studies have shown that obesity is a significant risk factor and contributor to increased morbidity and mortality, most importantly for cardiovascular diseases (CVD), diabetes, cancer, chronic diseases, osteoarthritis, liver-kidney disease, sleep apnea, and depression [6, 7].

Adipose tissue is formed by the lipid-filled cells mostly named "adipocytes" which bind partly to the reticular and partly to the loose connective tissue [8]. Fat tissue, in terms of number and size of cells, shows continuous life-time variation depending on energy requirement and consumption [9]. There are two adipose tissues in body; white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is one of the most important endocrines and secretory organs that are secreting leptin, adiponectin called "adipokine" and cytokines like tumor necrosis factor- α , IL-1 β , IL-6, monocyte chemotactic protein-1, macrophage strength suppressing factor, and nerve growth factor. WAT communicates with the other cells by its secretions in endocrine, paracrine, autocrine ways. The obesity-related changes in WAT affect the production of adipokines and cytokines. Many metabolic diseases such as insulin resistance, hypertension, and impaired fibrinolysis occur due to increased adipokine levels in obesity [10, 11]. When the balance between these two groups shifts to the pro-inflammatory direction, obesity develops and obesity-related diseases occur [12]. The task of BAT is to provide thermogenesis and energy expenditure in the body. The 2–3% of the bodyweight of a newborn is BAT. However, as the individual grows older, the heat regulation mechanism becomes available and the BAT becomes WAT [9].

The apelin is produced and secreted by the brain, hypothalamus, stomach and adipose tissue in both humans and mice. There are forms such as apelin-12, 13, 36, and 40, which serve especially to provide appetite control, and the most active form in humans is apelin-13.

It affects the control of appetite, liquid, and energy balance by influencing the central nervous system [13, 14]. Obestatin is a peptide hormone consisting of 23 amino acids thought to act as autocrine and paracrine, which is synthesized mainly in the mucosa of the stomach, as well as in the spleen, pancreas, fatty tissue, skeletal muscle, liver, lung, thyroid, testis, and breast glands [15, 16]. Vaspin (Serpin A12) is synthesized from visceral adipose tissue and is defined as a member of the serine protease inhibitor family. However, other studies have shown that vaspin expression is not limited to adipose tissue, but is synthesized from the skin, stomach, pancreatic islets and rodent hypothalamus [17].

Significant associations were reported among weight loss, certain adipokines (i.e., adiponectin), leptin, and cardiovascular disease risk. Some previous studies reported evidence for adipokine profile improvement with weight loss [18, 19]. For example, 23.8% weight loss compared to baseline weight results in significant improvements in circulating leptin, PAI-1, and adiponectin levels [20]. It was also suggested that a minimum weight loss at a rate of 10% was necessary to affect some adipokine levels [21]. In the study, the purpose was to investigate the change of some parameters associated with obesity such as apelin-13, vaspin, obestatin, and insulin resistance assessment by the homeostatic assay (HOMA-IR) as a result of the diet program by targeting 10% weight loss for three months.

Materials and methods

Thirty adults (15 females and 15 males) in 18–50 age with HOMA-IR ≥ 2.5 and BMI of 25 kg/m² and above were included in the obese group; and 25 adults (10 females and 15 males) aged between 18 and 50 years with HOMA-IR < 2.5 and BMI 18.5–24.9 kg/m² were included in the control group. They were followed in the diet clinic of Ordu University between September 2016 and January 2017; and the diet program was followed for 3 months. Blood samples were taken from both the control and obese groups before the diet follow-up, and the second blood sample was taken in the obese group when the targeted 10% weight loss was reached during the follow-up period.

The exclusion criteria from the study were determined as follows; being under 18 and over 50 years of age, having any chronic disease (i.e. diabetes mellitus, heart disease, hypertension), and regular medication use. HOMA-IR calculation was made as follows: $\text{HOMA-IR} = [\text{glucose}(\text{mg/dL}) \times \text{insulin}(\mu\text{U/mL}) / 405]$, using fasting values [22]. The study was started after the approval of the Ethics Committee of Ordu University Clinical Research. The decision date of the ethics committee is 20/11/2015, and the number of ethics committee decisions is 2015/2.

The height of the obesity and control groups who applied to the dietary clinic voluntarily was measured using a wall-mounted height meter, and the body weight was measured in the morning, fasting and

with slim-light clothing by the Inbody Body Analyzer. BMI was calculated based on height (meter) and weight (kilogram) measurements. Waist circumference measurement was made at the midpoint between lower last palpable rib margin and top iliac crest. Hip circumference measurements were made around the widest part of the buttocks and waist-to-hip ratio (WHR). After taking the anthropometric measurements of the people who applied to the study, personal diet programs were prepared considering age, gender, anthropometric measurement results, general analyzes and general habits of the individuals. Attention was paid not to fall below the basal metabolic rate (BMR) when calculating the daily energy needs of individuals. The values obtained from the application of formula determined by the WHO and given below were accepted when calculating BMR [23].

18–30 age range; Male: $15.3 \times \text{body weight (kg)} + 679$, Female: $14.7 \times \text{body weight (kg)} + 496$

30–60 age range; Male: $11.6 \times \text{body weight (kg)} + 879$, Female: $8.7 \times \text{body weight (kg)} + 829$

The diet program was prepared by considering the needs of carbohydrate (g), protein (g), fat (g), vitamins and minerals after the detection of the daily energy requirements, taking into account the BMR calculations. In the daily nutrient distribution of the diet, carbohydrates were 55–60%, the proteins were 12–15% and the fats were 25–30% of the daily energy [24]. No additional food or product was used for the weight-losing process, and the food pyramid contained healthy foods.

The blood samples taken after 8–12 h fasting from obesity and control groups were transferred to gel tubes (BD-Belliver, Industrial Estate, UK) which did not contain anticoagulants, and were kept at room temperature for 30 min. Then, serum samples obtained by centrifugation at $1800 \times g$ for 12 min were stored at -80°C until the analysis time.

Serum vaspin was determined by enzyme immune absorbent assay (ELISA) (Human Vaspin, BioVendor R&D Products, Czech Republic), with an intraassay coefficient of variation (CV) $< 7.60\%$, interassay CV $< 7.70\%$, and sensitivity 0.01 ng/mL . Serum apelin-13 was determined by ELISA (Elabscience Biotechnology Co., Ltd. EL-H0458, USA), with an intraassay CV $< 6.61\%$, interassay CV $< 6.79\%$, and sensitivity 75.00 pg/mL . Serum obestatin was determined by ELISA (Elabscience Biotechnology Co., Ltd. E-EL-H0458, USA. Elabscience Biotechnology Co., Ltd. E-EL-H1989, USA), with an intraassay CV $< 8.33\%$, interassay CV $< 7.69\%$, and sensitivity 0.47 ng/mL . Serum insulin was also determined by ELISA (Human insulin, R&D Products, Czech Republic), with an intraassay CV $< 6.0\%$, interassay CV $< 9.0\%$, and sensitivity $0.17 \text{ } \mu\text{IU/mL}$. Results were read at a 450 nm wavelength on an ELISA reader (BioTek ELX800 reader, BioTek ELX50 washer, Winooski, Vermont, United States). Serum glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) measurements were performed colorimetrically on an auto analyzer (Architect C8000, Abbott Diagnostics, USA) by using commercial kits (Architect, Abbott Diagnostics, USA). Low-density lipoprotein cholesterol (LDL-C) level was determined with Friedewald formula [25].

Statistical evaluation of data

Descriptive statistics of the variables are given as mean $\pm$ standard deviation (SD) and minimum and maximum values. The normality assumption of the variables was tested with Kolmogorov–Simirnov

test. To compare independent and dependent groups for the non-normal distributed variables, Mann–Whitney U and Wilcoxon tests were used, respectively. Student t and Paired-t tests were performed for the normally distributed variables. To determine linear relations among the variables, the Pearson Correlation Analysis was made. The statistical significance level was taken to be 5%; and SPSS (ver: 21) statistical program was used for analyses.

Results

There were no age differences between obese and control groups ($p > 0.05$). BMI, waist, and hip circumference values of the obese group, both basal and after the weight loss, were found to be higher at significant levels than the control group ($p < 0.01$); however, weight loss caused a significant decrease on all these parameters compared to basal levels in obese individuals ($p < 0.01$). The basal WHR of the obese group was higher at significant levels than in controls, but there was no significant difference after the weight loss (Table 1).

Serum insulin and HOMA-IR levels of the obese group obtained both basal ($18.56 \pm 11.41 \text{ } \mu\text{IU/mL}$, 4.44 ± 2.61 , respectively) and after weight loss ($11.10 \pm 7.88 \text{ } \mu\text{IU/mL}$, 2.55 ± 1.81 , respectively) were found to be significantly higher than the control group ($7.26 \pm 2.05 \text{ } \mu\text{IU/mL}$, 1.63 ± 0.46 , $p < 0.01$, $p < 0.05$, respectively), also, weight loss caused a significant decrease on these parameters as compared to basal levels in obese individuals ($p < 0.01$).

The serum obestatin and apelin-13 values of the obese group obtained at both basal ($18.15 \pm 7.60 \text{ ng/mL}$, $3.44 \pm 1.38 \text{ ng/mL}$, respectively) and after weight loss ($17.32 \pm 6.36 \text{ ng/mL}$, $4.01 \pm 1.20 \text{ ng/mL}$, respectively) were found to be significantly lower than the control group ($22.93 \pm 5.90 \text{ ng/mL}$, $4.78 \pm 1.08 \text{ ng/mL}$, $p < 0.05$, $p < 0.01$, $p < 0.01$, $p < 0.05$, respectively), but weight loss did not cause significant changes on these parameters in obese individuals ($p > 0.05$). The comparison of apelin, obestatin, and insulin resistance index (HOMA-IR) between the control and obese groups are presented in Figure 1.

The vaspin level in controls was $0.09 \pm 0.07 \text{ ng/mL}$, basal levels of the obese group were $0.12 \pm 0.16 \text{ ng/mL}$, and also after the weight loss was $0.10 \pm 0.09 \text{ ng/mL}$, these differences were not statistically significant ($p > 0.05$).

The basal serum glucose levels in the obese group were significantly higher than controls ($98.23 \pm 9.09 \text{ mg/dL}$, $91.41 \pm 6.27 \text{ mg/dL}$, $p < 0.01$, respectively), and also weight loss caused a significant decrease on glucose levels ($93.47 \pm 8.34 \text{ mg/dL}$) compared to basal levels in the obese group ($p < 0.01$).

The basal serum TG, TC, and LDL-C levels in obese group ($140.90 \pm 67.94 \text{ mg/dL}$, $201.66 \pm 35.33 \text{ mg/dL}$,

Table 1: The descriptive characteristics of the obese, obtained both as basal and after weight loss, and control groups.

Parameters	Control Group (n=25) X ± SD	Obese Group (basal level. n=30) X ± SD	Obese Group (after weight loss. n=30) X ± SD
Male	15 (50%)	15 (50%)	15 (50%)
Female	10 (40%)	15 (60%)	15 (60%)
Age, year	30.51 ± 7.26	33.20 ± 8.98	–
BMI, kg/m ²	23.47 ± 1.55 (20.2–25.07)	34.05 ± 6.40 ^{a**c} (24.76–52.04)	29.30 ± 5.63 ^{a**} , ^{b**c} (22.43–47.4)
WC, cm	84.89 ± 7.61 (70.0–96.0)	101.93 ± 13.89 ^{a**} (83.0–144.0)	94.80 ± 12.98 ^{a**} , ^{b**} (74.0–135.0)
HC, cm	94.91 ± 4.27 (90.0–105.0)	107.46 ± 10.37 ^{a**} (81.0–132.0)	103.95 ± 8.79 ^{a**} , ^{b**} (92.5–128.0)
WHR	0.89 ± 0.07	0.95 ± 0.09 ^{a*}	0.91 ± 0.08

*p<0.05, **p<0.01,

Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist hip ratio.

^a compared with control group.

^b compared with basal level in obese group.

^c Mann–Whitney U test.

126.58 ± 37.00 mg/dL, respectively) were significantly higher than controls (74.65 ± 27.29 mg/dL, 180.30 ± 31.22 mg/dL, 105.04 ± 28.25 mg/dL, p<0.001, p<0.05, respectively), and also weight loss caused significant decrease on TG, and TC levels compared to basal levels in obese group (110.12 ± 61.94 mg/dL, 185.00 ± 38.90 mg/dL, p<0.01, respectively). HDL-C levels in obese groups, obtained both as basal and after weight loss, were significantly lower than in controls (45.67 ± 8.03 mg/dL, 43.42 ± 11.4 mg/dL, 7, 60.33 ± 13.31 mg/dL, p<0.001, p<0.01, respectively) (Table 2).

The basal serum insulin concentrations in obese individuals correlated at significant levels with HOMA-IR (r=0.99, p<0.01), BMI (r=0.606, p<0.01), waist circumference (r=0.638, p<0.01), age (r=–0.516, p<0.01), AST (r=0.374, p<0.05), and ALT (r=0.435, p<0.05); also, obestatin correlated at significant levels with WHR (r=–0.410, p<0.05). Besides, serum insulin concentrations in obese individuals after weight loss correlated at significant levels with HOMA-IR (r=0.995, p<0.01), BMI (r=0.736, p<0.01),

waist circumference (r=0.588, p<0.01), and TG (r=0.587, p<0.01), serum vaspin was also correlated at significant levels with HDL-C (r=–0.668, p<0.01), also serum apelin correlated at significant levels with WHR (r=–0.405, p<0.05). There were also found significant correlations between serum apelin and obestatin (r=0.834, p<0.01), TC (r=–0.495, p<0.05), LDL-C (r=–0.509, p<0.05), vaspin and BMI (r=–0.497, p<0.01), waist circumference (r=–0.497, p<0.01), insulin and HOMA-IR (r=0.970, p<0.01), and age (r=–0.484, p<0.05) in the control group. The relations among the other parameters are also presented in Table 3.

Discussion

Obesity is defined as an increase in body weight which exceeds the physical requirement limits as a result of excessive fat accumulation. Due to its hyperplasia and hypertrophic properties, adipose tissue is a type of tissue

Figure 1: The comparison of apelin, obestatin, and insulin resistance index (HOMA-IR) between the control and obese groups.

a; compared with control group, b; compared with basal level in obese group, *, p<0,05, **, p<0,01.

Table 2: The comparison of metabolic parameters of the obese, obtained both as basal and after weight loss, and control groups.

Parameters	Control X ± SD	Obese group (basal level) X ± SD	Obese group (after weight loss) X ± SD
Glucose, mg/dL	91.41 ± 6.27 (80.0–102.0)	98.23 ± 9.09 ^{***} (82.0–120.0)	93.47 ± 8.34 ^{b**} (76.0–111.0)
AST, U/L	19.14 ± 8.97 (13.0–55.0)	22.45 ± 10.39 ^c (13.0–57.0)	18.80 ± 5.65 ^{b**c} (11.0–36.0)
ALT, U/L	18.32 ± 10.17 (6.0–47.0)	32.31 ± 24.13 ^{a**c} (6.0–99.0)	23.00 ± 9.68 ^{a**c} (10.0–45.0)
TC, mg/dL	180.30 ± 31.22 (120.0–235.0)	201.66 ± 35.33 ^{a*} (138.0–272.0)	185.00 ± 38.90 ^{b**} (128.0–306.0)
HDL-C, mg/dL	60.33 ± 13.31 (35.6–88.2)	45.67 ± 8.03 ^{***} (31.1–61.7)	43.42 ± 11.47 ^{a**} (8.3–67.2)
LDL-C, mg/dL	105.04 ± 28.25 (57.1–156.7)	126.58 ± 37.00 ^{a**c} (39.1–212.8)	117.96 ± 34.48 ^c (58.9–237.6)
TG, mg/dL	74.65 ± 27.29 (33.0–135.0)	140.90 ± 67.94 ^{a***} (48.0–295.0)	110.12 ± 61.94 ^{a*,b*} (58.0–339.0)
HOMA-IR	1.63 ± 0.46 (0.64–2.40)	4.44 ± 2.61 ^{a**} (2.32–11.81)	2.55 ± 1.81 ^{a*,b**} (1.13–8.08)
Vaspin, ng/mL	0.09 ± 0.07 (0.03–0.34)	0.12 ± 0.16 ^c (0.04–0.88)	0.10 ± 0.09 ^c (0.04–0.53)
Apelin-13, ng/mL	4.78 ± 1.08 (2.51–6.09)	3.44 ± 1.378 ^{a**} (1.36–6.48)	4.01 ± 1.20 ^{a*} (2.25–7.24)
Obestatin, ng/mL	22.93 ± 5.90 (10.84–37.08)	18.15 ± 7.6 ^{a*} (3.96–41.34)	17.32 ± 6.36 ^{a**} (8.93–30.93)
Insulin, µU/mL	7.26 ± 2.05 (3.0–10.77)	18.56 ± 11.41 ^{a***} (8.8–50.0)	11.10 ± 7.88 ^{b**c} (4.8–34.8)

*p<0.05, **p<0.01, ***p=0.000.

Abbreviations: AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; TC, total cholesterol; HDL-C, High density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TG, triglycerides; TC, total cholesterol; HOMA-IR, insulin resistance assessment by the homeostatic assay.

^a compared with control group.

^b compared with basal level in obese group.

^c Mann–Whitney U test.

that can change more than other tissues [1]. Adipose tissue is no longer just a neutral tissue that stores fat; it is expressed as an endocrine organ that can synthesize some active compounds that regulate metabolic dynamics and homeostasis [8]. Each tissue in the human body has a different sensitivity to insulin. In the development of obesity-induced insulin resistance, the first case is indicated as triglyceride accumulation in fat cells. With increasing cellular triglyceride accumulation, many changes in the production and release of some adipose tissue-derived peptide complement factors and cytokines occur. This situation triggers the development of insulin resistance in other tissues [7–14]. In the present study, the mean basal BMI of the obese group was 34.05 kg/m², the average of these values decreased to 29.30 kg/m² in terms of statistical significance. Similarly, a statistically significant decrease was observed (p<0.001) in the mean fasting glucose, insulin, HOMA-IR, TG and TC levels after the weight loss in the obese group. Also, when we look at the relationship between HOMA-IR and BMI measured both as basal and after weight loss in the obese group, a statistically significant relationship was found between these two parameters. These results showed that weight loss at about a 10% rate might contribute to a decrease in the insulin resistance, and also lipid levels in obese individuals. A significant and negative relation was detected only between vaspin and HDL-C levels; there were no significant relationships among the other lipid parameters and adipokines that were measured in the present study. The contributions of adipokines in pathophysiological aspect

of obesity have not been identified fully; however, recent data show significant roles of adipokines in lipid metabolism. Changes in lipid metabolism in liver and peripheral tissues are dealt with particular reference to adipose and muscle tissues, and the mechanisms by which some adipokines mediate the regulation of fatty acid oxidation in those tissues [26].

Apelin, an endogenous regulatory peptide hormone, and its receptor are expressed at high levels in the body, and play cytoprotective roles in multiple physiological processes. After the identification of apelin as an adipokine, its relationship with obesity has been extensively investigated. Apelin is generally involved in neuroendocrine, energy homeostasis and food intake regulation mechanisms [13]. Different studies emphasized apelin's role regarding energy metabolism. It was shown that the central administration of apelin decreased food intake in rats; however, it was also reported that there were also contrasting effects. Apelin promotes endothelial vasodilation because it stimulates nitric oxide and acts as anti-inflammatory secretagogue and angiotensin II antagonist, and decreases vascular tone and angiogenesis [27]. In this study, obese individuals had characterized decreased apelin-13 levels. Although weight loss caused an increase in apelin-13 levels, this difference was statistically insignificant. Besides, a statistically negative and significant relationship was found between apelin-13 and WHR in the obese group after weight loss. Apelin, as a member of the adipose tissue-derived peptides, might contribute to the obesity-related disorder. However, the findings of studies that examined apelin's role in obesity are

Table 3: The correlation matrix between variables for control, basal level and after weight loss.

Groups	Vaspin	Apelin	Obestatin	Insulin	Glucose	AST	ALT	T.C	HDL-C	LDL-C	TG	Age	BKI	WC	HC	HOMA-IR	WHR
Vaspin	Basal level After weight loss Control	1															
Apelin	Basal level After weight loss Control	-0.236 -0.80 -0.086	1														
Obestatin	Basal level After weight loss Control	-0.117 -0.250 0.266	-0.039 -0.057 0.834**	1													
Insulin	Basal level After weight loss Control	-0.010 -0.046 -0.112	-0.182 -0.148 0.166	0.154 0.293 0.012	1												
Glucose	Basal level After weight loss Control	-0.288 -0.156 0.071	0.134 -0.112 0.059	0.038 -0.121 0.224	-0.254 -0.099 -0.156	1											
AST	Basal level After weight loss Control	-0.220 -0.078 -0.125	0.006 0.070 0.027	0.181 0.183 0.026	0.374* 0.067 0.417	1											
ALT	Basal level After weight loss Control	-0.297 -0.321 -0.164	0.081 0.055 0.180	0.018 0.140 0.124	0.435* 0.267 0.397	0.844** 0.708** 0.812**	1										
TC	Basal level After weight loss Control	-0.257 -0.105 0.036	0.113 0.107 -0.495*	0.043 -0.193 -0.383	-0.032 0.061 -0.048	0.168 0.172 0.201	0.259 0.332 0.203	1									
HDL-C	Basal level After weight loss Control	-0.005 -0.668** 0.329	-0.105 0.286 -0.062	0.042 0.216 -0.034	-0.300 -0.217 0.157	-0.120 -0.031 0.312	-0.151 0.065 -0.012	0.259 0.089 0.326	1								
LDL-C	Basal level After weight loss Control	-0.089 -0.088 -0.054	0.114 0.063 -0.509*	-0.032 -0.173 -0.398	-0.284 -0.044 -0.135	0.333 0.467* -0.217	-0.030 0.242 0.187	0.613** 0.959** 0.927**	0.021 0.000 -0.019	1							
TG	Basal level After weight loss Control	-0.352 -0.053 -0.314	0.076 -0.077 -0.042	0.106 -0.214 -0.045	0.340 0.587** 0.075	0.108 0.122 -0.351	0.284 0.490* 0.171	0.490** 0.483* 0.127	-0.135 -0.234 -0.476*	-0.021 0.304 0.172	1						
Age	Basal level After weight loss Control	-0.095 0.066 0.025	0.058 0.069 -0.231	-0.063 -0.476 -0.134	-0.516** -512 -0.484*	0.454* 0.281 0.085	-0.116 -0.074 -0.338	0.204 0.263 0.528**	0.274 -0.042 0.151	0.038 0.277 0.516*	0.020 0.081 -0.015	1					
BMI	Basal level After weight loss Control	-0.336 -0.222 -0.497**	0.095 -0.137 -0.270	0.189 0.163 -0.321	0.606** 0.736** 0.119	0.076 0.127 -0.082	0.181 -0.012 0.157	0.114 0.259 0.265	-0.202 0.048 -0.132	-0.079 0.164 0.299	0.313 0.466* 0.291	-0.310 -0.245 0.056	1				

Table 3: (continued)

Groups	Vaspin	Apelin	Obestatin	Insulin	Glucose	AST	ALT	T-C	HDL-C	LDL-C	TG	Age	BKI	WC	HC	HOMA-IR	WHR
WC																	
Basal level	-0.272	-0.186	-0.130	0.638**	0.083	0.503**	0.628**	0.202	-0.316	-0.060	0.428*	-0.080	0.556**	1			
After weight loss	-0.316	-0.238	0.014	0.588**	0.075	0.219	0.377	0.246	-0.101	0.157	0.637**	-0.068	0.711**				
Control	-0.497**	0.097	-0.037	-0.222	-0.249	-0.152	0.157	-0.233	-0.675*	0.005	0.287	-0.365	0.297				
HC															1		
Basal level	-0.264	-0.036	0.275	0.672**	-0.100	0.260	0.445*	-0.007	-0.301	-0.257	0.331	-0.277	0.707**	0.653**			
After weight loss	-0.245	0.111	-0.002	0.676**	0.017	0.096	0.305	0.227	0.034	0.114	0.544**	-0.274	0.838**	0.693**			
Control	-0.169	0.377	0.345	-0.319	0.281	-0.187	0.118	-0.041	-0.253	0.041	0.172	0.111	0.137	0.427*			
HOMA-IR																1	
Basal level	-0.053	-0.162	0.166	0.991**	-0.131	0.421*	0.459*	0.023	-0.307	-0.271	0.364	-0.462*	0.637**	0.670**	0.681**		
After weight loss	-0.057	-0.159	0.277	0.995**	-0.003	0.094	0.283	0.090	-0.216	-0.015	0.604**	-0.484**	0.749**	0.591**	0.679**		
Control	-0.109	0.177	0.070	0.970**	0.085	0.464*	0.471*	-0.070	0.236	-0.180	0.003	-0.475*	0.110	-0.268	-0.255		
WHR																	1
Basal level	-0.127	-0.199	-0.410*	0.184	0.221	0.460*	0.461*	0.265	-0.190	0.160	0.272	0.115	0.087	0.676**	-0.109	0.212	
After weight loss	-0.282	-0.405*	0.018	0.218	0.088	0.232	0.276	0.143	-0.135	0.122	0.404*	0.078	0.263	0.788**	0.107	0.222	
Control	-0.461*	-0.112	-0.241	-0.080	-0.284	-0.079	0.112	-0.222	-0.599**	-0.003	0.210	-0.089	0.258	0.866**	-0.081	-0.162	

*p<0.05, **p<0.01,

not consistent, and there are gaps in this field [28]. Apelin's role is still not clear, and experimental findings are inconsistent. Recently, it was shown that infusion of apelin to diabetic rats not only improved insulin sensitivity and glucose disposal at significant levels, but also promoted endogenous pancreatic β cell proliferation, as well as raising plasma contents of insulin and C-peptide [29]. Also, apelin's chronic administration reduced hepatic steatosis because it reduced *de novo* lipogenesis in mice that were resistant to insulin; however, this effect seems mainly based on overall amelioration of insulin sensitivity instead of apelin's direct effect on liver since apelin had no effects on isolated hepatocytes [30]. Plasma apelin-12 concentration was not altered in obese and high-fat diet female mice that were resistant to insulin, but gene expression apelin level was high in WAT and decreased in brown-adipose tissue, liver, and kidneys, which suggest that apelinergic system, could be implicated in several dysfunctions in these tissues under obesity [31]. It may be possible to explain opposite findings by differential apelin expression across tissues. Similar to our study results, it was reported that the apelin-12 plasma level was decreased in obese young patients compared to healthy ones, and this might be attributed to severity of insulin resistance and adiposity [32, 33]. Various studies concluded that apelin secretion could be regulated by insulin [34, 35]. Some clinical studies show that plasma apelin levels are increased in hyperinsulinemia associated with obesity. In one of these studies, Cavallo et al. [36] investigated serum apelin levels in Type 2 Diabetes Mellitus (T2DM) patients, T1DM and non-diabetic healthy individuals. These investigators reported that T2DM group had the highest serum apelin level; and also, serum apelin levels decreased significantly related to changing insulin secretion in these patients after bariatric surgery operation. We think that statistically insufficient change in the apelin-13 levels after weight loss in the obese group may be related to the small number of cases in the present study.

Obestatin is a peptide encoded by the ghrelin gene, which slows nutrient uptake and bowel movements and suppresses the sensation of thirst. In contrast to ghrelin, obestatin has a very small circulating half-life as about 2 min [37]. Obestatin is degraded at a fast pace and may reflect faster variations in food intake, glucose levels, or insulin action compared to ghrelin, which increased during weight reduction in obese patients. It is interesting that it was paralleled by increases in circulating obestatin concentrations [38]. In the present study, obese individuals had characterized lower serum obestatin values, and also weight loss did not cause significant change on obestatin levels. Besides, there were no significant relations between obestatin and other parameters

in the obese group. In various studies, it has been reported that overweight and obese individuals had low obestatin levels compared to people who had normal weight, similar to the results of our study [39–43]. Reinehr et al. [44] reported that overweight children and adolescents showed high plasma obestatin levels at significant levels in overweight group compared to the study group with normal weight, and also weight loss caused an increase in obestatin levels in the overweight group. Ghanbari-Niaki et al. [45] reported similar findings, only with this difference that the level of obestatin increased at significant levels after weight loss in their study; however, before weight loss, the overweight children had low obestatin levels compared to the normal-weight children. It was also demonstrated that weight loss after gastric banding in morbidly obese patients caused an increase in obestatin levels [38]. However, Roth et al. [40] reported that postoperative obestatin levels of patients who underwent Roux-en-Y gastric surgery did not significantly change by two years after surgery. Martins et al. [41] reported that serum obestatin levels were higher at significant levels in the third year after Roux-en-Y gastric surgery than in the control group. As experimentally, similar to effects of apelin, the chronic obestatin administration for 30 days of animals with T2DM causing low body and liver weights, improves liver functions, carbohydrate and lipid metabolism and insulin-resistance. The effect mechanism of obestatin on improving the insulin sensitivity related to increased mRNA and protein expression, which is associated with insulin signaling pathways [46]. Looking at our study and different results of other studies, further studies are needed to demonstrate correctly the effect of obestatin in the energy homeostasis and pathophysiology of obesity.

It was shown that vaspin plays a regulatory role in lipid and glucose metabolism and is a therapeutic adipokine against impaired glucose tolerance encountered in obese individuals. It is also indicated that serum vaspin expression is decreased with progressive diabetes, but this level has reached normal levels with insulin therapy [47]. In our study, we did not find a statistically significant difference in serum vaspin levels between obese and control groups ($p>0.05$). Also, 10% of weight loss did not significantly change serum vaspin levels in the obese group. It was reported in previous studies that vaspin is increased at significant levels in serum of obese people, and were significantly associated with BMI and WHR [48, 49]. Contrary to the above, similar to the results of our study, in a study investigating 108 participants who had normal glucose tolerance, no association was detected between serum vaspin and glucose tolerance and peripheral insulin

sensitivity measured with hyperinsulinemic-euglycemic clamp technique and HOMA-IR, respectively; and no change was observed in vaspin levels even in the presence of fat-induced insulin resistance [50]. Sperling et al. [51] also stated that no significant differences were detected between vaspin levels in the groups of 37 obese and 27 healthy individuals. In a study of Vink et al. [52], 26 males and 30 female obese individuals were applied low-caloric diet for 12 weeks (1250 kcal/day) and a very low-calorie diet (500 kcal/day) for five weeks. A weight loss of approximately 10% was found at the end. Also, the value of serum vaspin did not change according to the initial value. A study in Seoul-Korea was performed on 63 obese subjects. Participants applied a diet program for 12 weeks and those who lost 2% of the initial weight were evaluated at the end. A statistically significant decrease was found in the vaspin levels of the participants who responded to the program [53]. As a result, the studies investigating the relationship between obesity and vaspin have conflicting results. Some studies show that serum vaspin levels can vary with weight loss, but some studies indicate that weight loss does not cause a significant change in serum vaspin concentration as in our study. Whether vaspin influences the insulin signaling pathway to improve insulin resistance in peripheral tissues is still unknown. Recently, Liu et al. [54] demonstrated that vaspin might enhance glucose tolerance and insulin sensitivity after promoting IRS/PI3K/Akt/Glut signal transduction pathway in peripheral tissue of high-fat diet-fed rats. However, Heiker et al. [55] speculated that vaspin enhanced glucose tolerance with serine protease inhibitory features. Hida et al. [47] reported that vaspin could improve insulin sensitivity of WAT following the regulation of gene expression related with metabolic disorders like glucose transport protein, leptin, resistin, and adiponectin. However, a significantly negative relation was found between vaspin and HDL-C levels after the weight loss in obese individuals in the present study. Various studies have reported that vaspin concentration correlated with lipid parameters, others, however, did not correlate it with lipid parameters in obesity-related diseases. For example, Ye et al. [56] reported that serum vaspin and lipid levels were associated with TG and HDL-C at significant levels in T2DM. Jian et al. [57] reported that vaspin levels were not related to TG or TC levels in control individuals or individuals with T2DM. Sperling et al. [51] also showed that vaspin levels did not correlate with TG or TC levels in obese individuals. The underlying reasons for variability in serum vaspin concentrations are still not clear. Serum vaspin concentrations showed a specific daily profile according to food intake with peak levels in the early morning fasting period and a significant postprandial

decrease 2 h after breakfast, as this trend has remained the same at the other meals during the day, as well, which shows its role in metabolic regulation [58]. Breitfeld et al. [59] conducted a genome-wide association study and identified several single nucleotide polymorphisms (SNPs) in the vaspin locus of 14th chromosome attributed to serum vaspin levels, and inferred that genetic variations were the most probable reason for serum vaspin variations. Therefore, we think that it is necessary to further studies that investigate vaspin and obesity relations.

Conclusion

In conclusion, it would appear that obese individuals had characterized lower obestatin and apelin-13 levels and also similar vaspin levels compared to healthy controls. However, the effects of 10% weight loss in obese individuals on levels of obestatin, vaspin and apelin-13 are insignificant. The decreased insulin resistance after the weight loss on obese individuals possibly resulted in improvements in fasting levels of glucose, triglyceride and total cholesterol. It was suggested that there is an insignificant relationship among apelin 13, vaspin, obestatin and insulin in obese people both in baseline values and after weight loss; and therefore, the secretion of these parameters might be performing independently with each other. The mean time to reach 10% weight loss in our study was approximately 6 weeks. The reason for no significant change in obestatin, apelin-13 and vaspin levels may be due to the low rate of weight loss as the targeted and shorter duration in our study. Therefore, we think that there is a need for further studies with a large number of participants and long-term diet and nutrition programs to clarify the relationship between obesity and these parameters.

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