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Asprosin, visfatin and subfatin as new biomarkers of obesity and metabolic syndrome

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Abstract. – OBJECTIVE: Metabolic syndrome (MetS) and obesity are important public health problems associated with adipose tissue mass. Asprosin, visfatin, and subfatin are new members of which fate in MetS and obesity has not been fully revealed yet. Thus, this study was to investigate the association between asprosin, visfatin, subfatin, and biochemical values, demographic data, and body composition measurement values in MetS patients with and without obesity.

PATIENTS AND METHODS: Blood samples were taken from a total of 90 people, including 31 MetS patients with obesity, 29 MetS patients without obesity, and 30 healthy (control). Asprosin, visfatin, and subfatin were studied by the ELISA method.

RESULTS: There was a negative correlation between asprosin and Body Mass Index (BMI) in the MetS + Obese group. The correlations between asprosin and urea and fasting insulin (FI) levels in the MetS group were positive and statistically significant ($p < 0.05$). While there was a statistically significant negative correlation ($p < 0.05$) between visfatin and BMI in the MetS + Obese group, the correlation with waist circumference in the MetS + Obese and MetS groups was statistically significant and negative ($p < 0.05$). There was a statistically significant negative relationship ($p < 0.05$) between aspartate aminotransferase value and visfatin. The results between visfatin values and asprosin and subfatin in all groups were significant ($p < 0.05$).

CONCLUSIONS: There is a direct relationship between circulating amounts of asprosin, visfatin, and subfatin hormones and age, weight, height, diastolic blood pressure, high-density lipoprotein-cholesterol, aspartate aminotransferase, alanine transaminase, and creatinine. Therefore, asprosin, visfatin, and subfatin hormones are the new biomarkers of metabolic turbulence.

Key Words:
Asprosin, Visfatin, Subfatin, Obesity, Metabolic syndrome.

Introduction

Metabolic syndrome (MetS) is a deadly endocrinopathy that starts with insulin resistance and includes risk factors, e.g., abdominal obesity, glucose intolerance or diabetes mellitus, dyslipidemia and impaired glucose metabolism, hypertension, and coronary artery disease (NCEP, 2002). MetS is very common in the world and observed in about 20-25% of the adult population. This rate is gradually increasing due to the aging population, comfortable life expectancy, poor eating habits, sedentary lifestyle, and obesity[1].

Obesity represents a disease characterized by the excessive accumulation of fat in the visceral and subcutaneous regions and excess weight that occurs with weight gain when energy intake ex-

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ceeds energy consumption. This excess weight is stored in adipose tissue consisting of adipose cells or adipocytes with an incredible capacity to store excess energy in the form of lipids[2].

The ultimate two exons of FBN1 encode asprosin. Eleven amino acids are encoded by exon 65, whereas 129 amino acids are encoded by exon 66. It represents the C-terminal cleavage product of profibrillin (encoded by FBN1). This molecule plays a role in regulating the glucose level. Asprosin occurs with excessive adiposity because WAT is capable of modulating glucose metabolism and maintaining energy homeostasis independently without increasing or disrupting the insulin effect[3]. Asprosin level was found to be high in patients with obesity and type 2 diabetes[4,5]. When asprosin function is lost, glucose and insulin levels decrease significantly. The expression of *FBN1* mRNA is also found in other tissues, such as skeletal muscles[4].

Subfatin (Meteorin-like protein; METRNL) represents a newly discovered adipokine, which is secreted by adipose tissue and skeletal muscle and has insulin-sensitizing and anti-inflammatory activity[6-8]. During exercise, it promotes WAT browning by inducing the white adipose tissue through a STAT6-mediated pathway on skeletal muscle and cold exposure, increases energy expenditure, and increases glucose tolerance[6]. Moreover, studies on subfatin transgenic mice reported that subfatin controls insulin sensitivity with PPAR-γ (Peroxisome proliferator-activated receptor-gamma), which is considered to take an essential part in the regulation of adipocyte differentiation[6]. Furthermore, in obese patients, circulating subfatin levels were revealed to be lower[9]. Nevertheless, another study demonstrated that subfatin expression in adipose tissue was higher in obese children compared to lean children[8]. In a study, serum subfatin levels correlated positively with age, gender, and body mass index (BMI), waist circumference (WC), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and correlated negatively with glycated hemoglobin A1c (HbA1c), fasting insulin (FI) and homeostasis model assessment of insulin resistance (HOMA-IR), and high-density lipoprotein cholesterol (HDL-C) in all cases examined[10]. Subfatin levels were determined to be lower in obese subjects than normal weight subjects[9].

Adipose tissue also secretes visfatin. Visfatin is also known as nicotinamide phosphoribosyltransferase (NAMPT). It is a 52 kDa mammalian protein, which is structurally synthesized in humans, chickens, and rodents by adipose tissue and numerous other tissues, such as reproductive tissues[11,12]. In humans, visfatin plasma levels increase during the development of obesity and type 2 diabetes[11]. Visfatin has been correlated with various inflammatory conditions, beta-cell functions, and cardiovascular diseases. Many scholars[12] have indicated the association between visfatin levels and lipoprotein metabolites in subjects with metabolic disorders.

In the light of all these data, this study aims to reveal in detail whether there is a relationship between asprosin, visfatin, subfatin, and biochemical values and demographic data and body composition measurement values in metabolic syndrome subjects with and without obesity.

Patients and Methods

Ninety people in total aged between 30-60 years, who applied to the polyclinic and clinic of Fırat University, Faculty of Medicine, Department of Endocrinology and Metabolism Diseases, were enrolled in our research. The required ethical permissions were obtained from the Ethics Committee of Fırat University, Faculty of Medicine for the study (Meeting No: 21, decision no: 03, 20-12-2018). The voluntary consent form was signed by the participants, and the World Medical Association Declaration of Helsinki was followed in the study. Two groups, including patients with obesity (With MetS + Obese) (n = 31) and patients without obesity (MetS) (n = 29), were formed from metabolic syndrome patients who did not receive medical treatment. The control group was formed from the control group (n = 30), not meeting any criteria of metabolic syndrome (Without MetS), not having any additional disease, and in a similar age and gender range. While forming the study groups, individuals who used alcohol, tobacco and its products, and other hormonal drugs were not enrolled in the research. While the diagnosis of MetS was made according to National Cholesterol Education Adult Treatment Panel III (ATP III) criteria, BMI values were taken as the basis in the diagnosis of obesity (obese: $\geq$30 kg/m^2)[13].

Anthropometric Measurements

BMI was calculated as weight (kg)/height (m^2) for self-reported and measured data. BMI was categorized according to the cutoffs of the World

Health Organization as follows: underweight (< 18.5 kg/m^2), healthy weight (18.5-24.99 kg/m^2), overweight (25-29.99 kg/m^2), or obese (≥ 30 kg/m^2)[13]. Furthermore, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were determined after 15 minutes of rest. The measurement of systolic and diastolic blood pressure was performed three times by sitting until the nearest double step after resting for 10 minutes by using a random zero sphygmomanometer. The demographic data (age, gender, waist and hip circumference measurement, BMI) of all study groups were recorded.

Biochemical Measurements and Test Validations

Routine biochemistry results were taken from the patient records. In blood samples without aprotinin, LDL-C (mg/dL), HDL-C (mg/dL), TG (mg/dL), TC (mg/dL), fasting blood sugar (FBS) (mg/dL), high-sensitivity C-reactive protein (hs-CRP) (ng/mL), and FI (μIU/mL) were measured. Approximately 5 ml of blood was taken into aprotinin tubes for subfatin, visfatin, and asprosin measurements, and their plasma was separated by being centrifuged at 4000 rpm for 5 minutes within 15 minutes. It was stored at -80ºC until analysis.

Asprosin levels in blood samples (of the control group, obese +With MetS and Without MetS groups) were studied with the ELISA (Enzyme Linked-Immunosorbent assay) kit [YL Biont Human Asprosin (ASPROSIN) ELISA Kit; Catalog No: YLA3950HU]. The assay range of the Human Asprosin ELISA kit was 3 pg/mL→900 pg/mL (intra-assay: CV value < 8%, inter-assay: CV value < 10%), and sensitivity was 1.56 pg/mL. Subfatin levels in blood samples (of the control group, obese + With MetS and Without MetS groups) were studied with the ELISA kit [YL Biont Human Meteorin-like protein (MTRNL) ELISA Kit; Catalog No: YLA3736HU]. The assay range of the Human Meteorin ELISA kit was 0.05 ng/mL→15 ng/mL (intra-assay: CV value < 8%, inter-assay: CV value < 10%), and sensitivity was 0.023 ng/mL. Visfatin levels in blood samples (of the control group, obese + With MetS and Without MetS groups) were studied with the ELISA kit (YL Biont Human Visfatin (Visfatin) ELISA Kit; Catalog No: YLA0808HU). The assay range of the Human Visfatin ELISA kit was 0.5 ng/mL→100 ng/mL (intra-assay: CV value < 8%, inter-assay: CV value < 10%), and sensitivity was 0.25 ng/mL.

Plates were washed by an automatic washer BioTek ELX50 (BioTek Instruments, Winooski, VT, USA), and the measurement of absorbance was performed by a ChroMate, Microplate Reader P4300 (Awareness Technology Instruments, Palm City, FL, USA). The results of the asprosin test were presented in pg/mL. Subfatin and visfatin test results were presented in ng/mL. Ninety biological specimens were analyzed for asprosin, with a number of samples being measured twice. The kits were utilized for the measurement of asprosin, subfatin, and visfatin levels in the blood and other biological specimens. Assay validation (linearity, recovery, intra-inter assay CV value) was performed, as previously specified by Aydin[14].

Statistical Analysis

The statistical analysis of the data was carried out using the Statistical Package for the Social Sciences (SPSS Inc., IBM, Armonk, NY, USA) 22 package program. In case of normally distributed variables, Student's *t*-test was conducted to reveal statistically significant differences between the groups. In case of non-normally distributed variables, the Mann-Whitney U test was conducted for the comparison of differences between independent groups. The one-way analysis of variance (ANOVA) was utilized for the comparison of continuous data between groups. The Spearman correlation test was carried out to assess the correlation between the groups. $p < 0.05$ was accepted to be statistically significant.

Results

Three groups, including subjects with MetS (n = 29), individuals both with obesity and MetS (n = 31), and control group (n = 30), were included in the study. Their means, according to the variables between the groups, are shown in Table I. In accordance with the analysis of variance data, a significant correlation was observed with age, weight, height, DBP, HDL-C, AST, ALT, and creatinine values ($p < 0.05$).

The correlation value of biochemical variables between the groups is negative (-), which indicates a negative correlation between the parameters. In other words, while one is increasing, the other one is decreasing. Positive values also indicate that there is a positive correlation. In other words, they both increase in the same direction (Tables II, III and IV).

Table I. Association of circ_001680 expression with clinicopathologic characteristics of glioma.

Variable	Control (n = 30) (15f + 15m) Means ± SD	MetS + Obese (n = 31) (16f + 15m) Means ± SD	MetS (n = 29) (16f + 13m) Means ± SD	Intergroup comparison	
				F	*p*
Age (years)	42.56 ± 8.93	43.51 ± 10.06	49.89 ± 7.74	4.888	**0.030**
Weight (kg)	72.17 ± 10.27	109.54 ± 21.20	74.12 ± 9.47	12.587	**0.001**
Height (cm)	168.1 ± 9.80	168.22 ± 8.98	164.17 ± 8.46	86.202	**0.000**
BMI (kg/m^2)	25.46± 2.34	38.46 ± 5.62	27.44 ± 1.67	0.430	0.514
WC (cm)	86 ± 9.59	121.38 ± 13.82	95.44 ± 7.28	8.327	0.244
HC (cm)	100.4 ± 4.97	124.67 ± 10.70	104.27 ± 4.78	0.008	0.930
SBP (mmHg)	109 ± 8.84	130.96 ± 15.94	121.72 ± 16.27	0.006	0.937
DBP (mmHg)	70.33 ± 8.50	105.96 ± 27.40	79.31 ± 10.66	12.446	**0.001**
TG (mg/dL)	105 ± 47.63	188.25 ± 68.24	221.48 ± 101.95	1.106	0.296
HDL-C (mg/dL)	50.61 ± 14.56	41.20 ± 7.87	42.03 ± 8.00	6.996	**0.010**
LDL-C (mg/dL)	112.61 ± 26.68	160.33 ± 39.32	145.26 ± 27.47	1.112	0.294
TC (mg/dL)	188.03 ± 32.29	210.38 ± 41.17	220.24 ± 34.72	0.713	0.401
FBS (mg/dL)	86.86 ± 6.90	123.06 ± 35.25	123.82 ± 36.25	0.365	0.547
HbA1c	5.310 ± 0.41	6.79 ± 1.71	6.59 ± 1.67	0.291	0.591
AST (U/L)	21.33 ± 4.44	22.48 ± 8.50	21.31 ± 3.98	8.786	**0.004**
ALT (U/L)	22.63 ± 8.28	32.70 ± 19.05	26.79 ± 10.63	22.725	**0.000**
Urea (mg/dL)	27.46 ± 6.00	26.96 ± 6.24	28.86 ± 7.73	1.964	0.165
Creatinine (mg/dL)	0.76 ± 0.15	0.70 ± 0.16	0.72 ± 0.15	50.633	**0.000**
FI (µIU/mL)	6.98 ± 3.94	19.21 ± 11.82	11.76 ± 8.96	0.164	0.687
hs-CRP (ng/mL)	1.83 ± 1.40	5.73 ± 3.52	2.51 ± 1.94	0.214	0.645
Asprosin (pg/mL)	56.40 ± 28.11	51.31 ± 24.09	54.31 ± 26.18	0.000	0.990
Visfatin (ng/mL)	7.26 ± 3.64	9.24 ± 3.35	8.16 ± 4.07	0.995	0.321
Subfatin (ng/mL)	1.15 ± 0.65	1.23 ± 0.41	1.31 ± 0.62	1.374	0.244

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: Body mass index; DBP: diastolic blood pressure; f: female; FBS: Fast blood sugar; FI: Fasting Insulin; HbA1c: glycated hemoglobin A1c; HC: Hip Circumference; HDL-C: High-density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein; LDL-C: Low-density lipoprotein cholesterol; m: male; MetS: group with metabolic syndrome; pg/mL: picogram per mililiter; SBP: systolic blood pressure; SD: standard deviation; TC: Total cholesterol; TG: Triglyceride; WC: Waist circumference; Values are means ± SD. cm: centimeter; kg: kilogram; kg/m^2: kilograms per square meter; mg/dL: miligram per deciliter; mmHg: Millimetres of Mercury at 0 deg C Pressure Unit; U/L: units per litre; ng/mL: nanogram per mililiter; pg/mL: picogram per mililiter; µIU/mL: Micro International units per millilitre.

In terms of asprosin concentration, there was a statistically significant positive relationship with visfatin (r = 0.578, p = 0.001) and subfatin (r = 0.725, p = 0.000) in the control group, a statistically significant positive relationship with visfatin (r = 0.482, p = 0.013) and subfatin (r = 0.707, p = 0.000) in the MetS group, a statistically significant positive relationship with visfatin (r = 0.580, p = 0.001) in the MetS+Obese group (p < 0.05). Its positive relationship with subfatin (r = 0.246, p = 0.182) was not statistically significant (p > 0.05). A negative correlation was found between asprosin and BMI in the MetS + Obese group (r = -0.187, p = 0.313). A statistically significant positive relationship was determined between asprosin and urea (r = 0.439, p = 0.025) and between asprosin and FI (r = 0.469, p = 0.024) levels in the MetS group (p <0.05). Although there was a positive relationship with other parameters [weight, height, BMI, WC, Hip Circumference (HC), TG, HbA1c, urea, FI, and hs-CRP], this correlation was not statistically significant (p > 0.05) (Table II).

Concerning visfatin concentration, there was a statistically significant positive relationship with asprosin (r = 0.578, p = 0.001) and subfatin (r = 0.605, p = 0.001) in the control group, a statistically significant positive relationship with asprosin (r = 0.482, p = 0.013) and subfatin (r = 0.638, p = 0.000) in the MetS group, and a statistically significant positive correlation with asprosin (r = 0.580, p = 0.001) and subfatin (r = 0.446, p = 0.012) in the MetS + Obese group (p < 0.05). A statistically significant negative relationship was revealed between visfatin and BMI in the MetS+Obese group (r = -0.469, p = 0.008). There was a statistically significant negative relationship (p <0.05) between visfatin

Table II. Asprosin statistical comparison values between the groups.

Variable	Control (n = 30) (15f + 15m)		MetS + Obese (n = 31) (16f + 15m)		MetS (n = 29) (16f + 13m)	
	Correlation	p	Correlation	p	Correlation	p
Visfatin (ng/mL)	**0.578****	**0.001**	**0.482***	**0.013**	**0.580****	**0.001**
Subfatin (ng/mL)	**0.725****	**0.000**	**0.707****	**0.000**	0.246	0.182
Age (years)	-0.007	0.972	-0.067	0.745	0.190	0.305
Weight (kg)	0.059	0.755	0.078	0.706	-0.227	0.220
Height (cm)	0.080	0.674	-0.079	0.701	-0.129	0.491
BMI (kg/m^2)	0.071	0.711	0.294	0.145	-0.187	0.313
WC (cm)	0.109	0.568	-0.074	0.718	-0.293	0.110
HC (cm)	0.091	0.634	-0.189	0.355	-0.233	0.207
TG (mg/dL)	0.128	0.502	0.168	0.412	-0.118	0.527
HDL-C (mg/dL)	-0.180	0.341	-0.004	0.984	0.091	0.627
LDL-C (mg/dL)	-0.002	0.993	0.174	0.395	0.201	0.279
TC (mg/dL)	-0.062	0.745	0.132	0.520	0.197	0.288
FBS (mg/dL)	-0.058	0.760	-0.016	0.939	-0.008	0.965
HbA1c	0.047	0.809	-0.123	0.550	-0.019	0.919
AST (U/L)	-0.118	0.533	-0.104	0.614	0.054	0.772
ALT (U/L)	-0.058	0.761	0.249	0.220	0.050	0.789
Urea (mg/dL)	0.127	0.503	0.439*	0.025	0.349	0.054
Creatinine (mg/dL)	-0.020	0.915	0.308	0.126	-0.128	0.493
FI (µIU/mL)	0.355	0.064	0.469*	0.024	0.013	0.944
hs-CRP (ng/mL)	0.153	0.420	-0.287	0.164	0.124	0.513

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: Body mass index; f: female; FBS: Fast blood sugar; FI: Fasting Insulin; HbA1c: glycated hemoglobin A1c; HC: Hip Circumference; HDL-C: High-density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein; LDL-C: Low-density lipoprotein cholesterol; m: male; MetS: group with metabolic syndrome; SD: standard deviation; TC: Total cholesterol; TG: Triglyceride; WC: Waist circumference; Values are means ± SD. cm: centimeter; kg: kilogram; kg/m^2: kilograms per square meter; mg/dL: milligram per deciliter; U/L: units per liter; ng/mL: nanogram per milliliter; µIU/mL: Micro International units per milliliter. **Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level.

and waist circumference (r = -0,395; p = 0.046) in the MetS group, and between visfatin and waist circumference (r= -0,418; p = 0,019) (p < 0.05) in the MetS + Obese group. There was a statistically significant negative relationship (p < 0.05) between AST value and visfatin (r = -0.534, p = 0.005).

Concerning subfatin concentration, there was a statistically significant positive relationship with asprosin (r = 0.725, p = 0.000), and visfatin (r = 0.605, p = 0.001) in the control group, and a statistically significant positive relationship with asprosin (r = 0.707, p = 0.000) and visfatin (r = 0.638, p = 0.000) in the MetS group (p <0.05). The relationship with asprosin (r = 0.246, p = 0.182) in the MetS+Obese group was positive but not statistically significant (p > 0.05). A statistically significant positive relationship was found between subfatin and visfatin (r = 0.446, p = 0.012) in the Mets + Obese group. Although there was a positive correlation between subfatin and some other parameters in the control, MetS, and MetS + Obese groups, the results were not significant (p > 0.05).

According to gender, serum asprosin, visfatin, and subfatin levels in the control, MetS+Obese, and MetS groups are presented in Figure 1. According to gender, asprosin, visfatin, and subfatin levels in the control, MetS+Obese, and MetS groups were observed to be higher in females. No significant difference was revealed in terms of BMI, HC, asprosin, visfatin, and subfatin levels in the analyses of variance by gender (Table V) (p > 0.05). The difference, according to WC, was statistically significant (p <0.05).

Discussion

MetS represents an essential public health problem worldwide. In the current research, according to gender, asprosin, visfatin, and subfatin levels were determined to be higher in

Table III. Visfatin statistical comparison values between the groups.

Variable	Control (n = 30) (15f + 15m)		MetS + Obese (n = 31) (16f + 15m)		MetS (n = 29) (16f + 13m)	
	Correlation	p	Correlation	p	Correlation	p
Asprosin (pg/mL)	**0.578****	**0.001**	**0.482***	**0.013**	**0.580****	**0.001**
Subfatin (ng/mL)	**0.605****	**0.001**	**0.638****	**0.000**	**0.446***	**0.012**
Age (years)	-0.081	0.676	-0.112	0.587	0.122	0.513
Weight (kg)	0.146	0.449	-0.289	0.153	**-0.479****	**0.006**
Height (cm)	0.161	0.405	-0.342	0.087	-0.108	0.563
BMI (kg/m^2)	-0.093	0.631	0.127	0.537	**-0.469****	**0.008**
WC (cm)	0.098	0.613	**-0.395***	**0.046**	**-0.418***	**0.019**
HC (cm)	0.191	0.322	-0.245	0.227	-0.160	0.390
TG (mg/dL)	-0.141	0.465	-0.044	0.829	-0.242	0.190
HDL-C (mg/dL)	-0.024	0.903	0.131	0.524	0.313	0.086
LDL-C (mg/dL)	-0.016	0.934	0.027	0.894	0.140	0.454
TC (mg/dL)	0.035	0.855	0.086	0.675	0.159	0.393
FBS (mg/dL)	-0.101	0.601	-0.139	0.499	-0.116	0.541
HbA1c	0.025	0.899	0.021	0.919	0.043	0.817
AST (U/L)	0.091	0.638	**-0.534****	**0.005**	-0.025	0.894
ALT (U/L)	0.055	0.775	-0.232	0.254	-0.105	0.576
Urea (mg/dL)	0.083	0.667	0.065	0.753	0.222	0.231
Creatinine (mg/dL)	0.029	0.883	-0.260	0.200	-0.175	0.347
FI (µIU/mL)	-0.085	0.672	0.051	0.818	-0.230	0.212
hs-CRP (ng/mL)	-0.057	0.767	-0.075	0.722	-0.051	0.788

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: Body mass index; f: female; FBS: Fast blood sugar; FI: Fasting Insulin; HbA1c: glycated hemoglobin A1c; HC: Hip Circumference; HDL-C: High-density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein; LDL-C: Low-density lipoprotein cholesterol; m: male; MetS: group with metabolic syndrome; SD: standard deviation; TC: Total cholesterol; TG: Triglyceride; WC: Waist circumference; Values are means ± SD. cm: centimeter; kg: kilogram; kg/m^2: kilograms per square meter; mg/dL: milligram per deciliter; U/L: units per liter; ng/mL: nanogram per milliliter; µIU/mL: Micro International units per milliliter. **Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level.

females in the control, MetS+Obese, and MetS groups. Baykus et al[15] found a positive correlation between birth weights of neonates and arterial and venous asprosin levels in pathological and normal pregnancies. According to the gender of neonates, mean asprosin levels in the cord blood of female neonates were revealed to be significantly higher than those in males. Wiecek et al[16] reported that asprosin is not increased in the obesity. Furthermore, the concentration of asprosin in the blood of the elderly was similar in obese and non-obese subjects as underlined by Wiecek et al[16].

A correlation was also found between some biochemical parameters and asprosin. There were positive (statistically significant) relationships between asprosin concentration and fasting glucose, HOMA-IR, TG, BMI and waist-to-hip ratio (WHR) in individuals with T2DM[5]. Li et al[17] found a correlation between asprosin concentration and BMI. However, Wiecek et al[18] did not find a correlation between plasma asprosin concentration and BMI in older women with metabolic disorders. Greenhill[19] found that asprosin administration to mice increased blood glucose levels. Ugur et al[20] demonstrated that as HDL-C levels decreased, BMI values decreased, and accordingly, LDL-C and asprosin values increased. In the study, the researchers suggested that asprosin was directly related to obesity. Again, in the same study, they reported that asprosin was first identified in saliva and that increased asprosin level in saliva was associated with an increase in BMI[20]. Asprosin was suggested to be a centrally effective oxygenic hormone that causes adiposity and body weight as well as appetite stimulation[21]. Then, it was also found to be associated with insulin resistance in females with polycystic ovary syndrome[22].

Also, it was found that there was a relationship between MetS, obesity, and visfatin. In a case-control study conducted on 120 non-obese subjects, Chen et al[23] indicated that individuals with type 2 diabetes mellitus had higher plasma levels than

Table IV. Subfatin statistical comparison values between the groups.

Variable	Control (n = 30) (15f + 15m)		MetS + Obese (n = 31) (16f + 15m)		MetS (n = 29) (16f + 13m)	
	Correlation	p	Correlation	p	Correlation	p
Asprosin (pg/mL)	**0.725****	**0.000**	**0.707****	**0.000**	0.246	0.182
Visfatin (ng/mL)	**0.605****	**0.001**	**0.638****	**0.000**	**0.446***	**0.012**
Age (years)	0.025	0.898	-0.225	0.269	0.175	0.347
Weight (kg)	-0.028	0.882	-0.114	0.578	-0.210	0.257
Height (cm)	-0.018	0.925	-0.169	0.410	-0.066	0.723
BMI (kg/m^2)	0.015	0.938	0.154	0.453	-0.177	0.342
WC (cm)	-0.006	0.976	-0.271	0.180	-0.104	0.576
HC (cm)	0.074	0.698	-0.212	0.299	-0.039	0.834
TG (mg/dL)	0.086	0.652	0.081	0.694	-0.170	0.361
HDL-C (mg/dL)	-0.217	0.250	0.175	0.394	0.042	0.821
LDL-C (mg/dL)	-0.004	0.982	0.180	0.378	0.154	0.409
TC (mg/dL)	-0.048	0.801	0.030	0.886	0.172	0.353
FBS (mg/dL)	-0.072	0.704	-0.231	0.256	0.041	0.832
HbA1c	0.219	0.254	-0.195	0.339	0.187	0.313
AST (U/L)	-0.195	0.301	-0.312	0.121	0.030	0.875
ALT (U/L)	-0.051	0.788	-0.105	0.609	-0.023	0.904
Urea (mg/dL)	0.076	0.688	0.120	0.558	0.024	0.898
Creatinine (mg/dL)	-0.134	0.479	0.013	0.952	-0.159	0.392
FI (µIU/mL)	0.242	0.215	0.170	0.439	-0.243	0.188
hs-CRP (ng/mL)	0.033	0.862	-0.086	0.682	0.074	0.699

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: Body mass index; f: female; FBS: Fast blood sugar; FI: Fasting Insulin; HbA1c: glycated hemoglobin A1c; HC: Hip Circumference; HDL-C: High-density lipoprotein cholesterol; hs-CRP: high-sensitivity C-reactive protein; LDL-C: Low-density lipoprotein cholesterol; m: male; MetS: group with metabolic syndrome; TC: Total cholesterol; TG: Triglyceride; WC: Waist circumference; Values are means ± SD. cm: centimeter; kg: kilogram; kg/m^2: kilograms per square meter; mg/dL: miligram per deciliter; U/L: units per litre; ng/mL: nanogram per mililiter; pg/mL: picogram per mililiter; µIU/mL: Micro International units per millilitre. **Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level.

non-diabetic individuals after the gender, age, and BMI adjustment of circulating visfatin. In a case-control study (120 patients and 80 controls), serum visfatin levels were found to be significantly positively correlated with body composition parameters (BMI, WHR, etc.)[24]. In the present research, although visfatin values were negatively associated with WC and AST values in the MetS +

Table V. Intergroup comparison of BMI, WC, HC, asprosin, visfatin, and subfatin data according to gender.

Variable	Intergroup ANOVA test data according to gender				
	Gender	n	Means ± SD	F	p
BMI (kg/m^2)	Female	45	30.12 ± 6.24	0.430	0.514
	Male	42	31.07 ± 7.50		
WC (cm)	Female	45	96.06 ± 17.48	8.327	0.005
	Male	42	106.88 ±18.02		
HC (cm)	Female	45	110.12 ± 13.18	0.008	0.930
	Male	42	109.88 ± 13.08		
Asprosin (pg/mL)	Female	45	53.93 ± 25.61	0.000	0.990
	Male	42	54.00 ± 26.60		
Visfatin (ng/mL)	Female	45	8.63 ± 3.62	0.995	0.321
	Male	42	7.83 ±3.83		
Subfatin (ng/mL)	Female	45	1.29 ± 0.53	1.374	0.244

BMI: Body mass index; HC: Hip Circumference; WC: Waist circumference; SD: standard deviation; Values are means ± SD. cm: centimeter; kg: kilogram; kg/m^2: kilograms per square meter; ng/mL: nanogram per milliliter; pg/mL: picogram per milliliter.

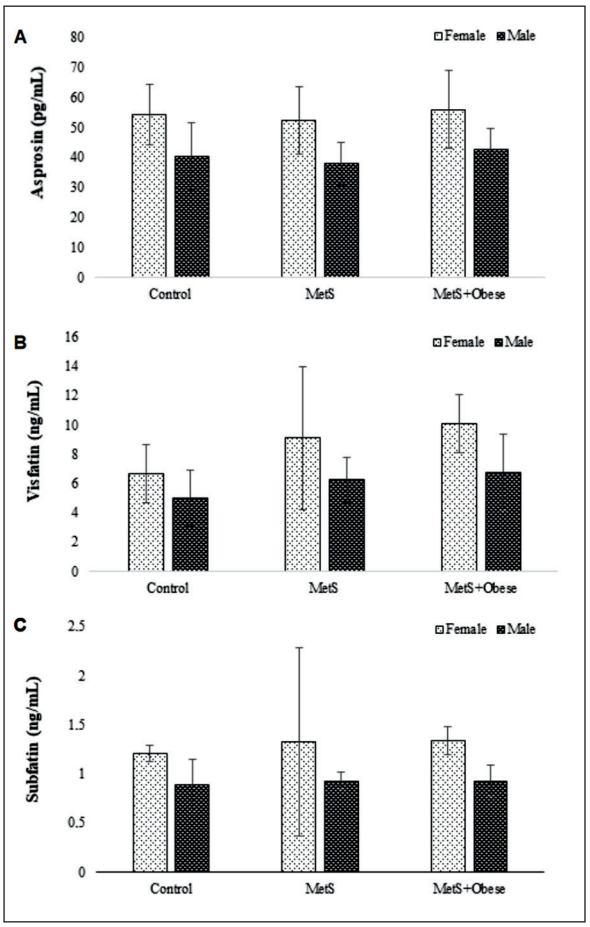

Figure 1. A, Serum asprosin levels. MetS: group with metabolic syndrome. **B,** Serum visfatin levels. MetS: group with metabolic syndrome. **C,** Serum subfatin levels. MetS: group with metabolic syndrome.

Obese group, the result was found to be significant. In the MetS group, it showed a negative correlation with BMI, WC, and weight, but the result was found to be significant. The correlations with other variables were not found to be significant. Wafa et al[25] reached the conclusion that serum visfatin was correlated with impaired glucose metabolism and obesity. Abd Rabo et al[26] determined that visfatin levels were related to hyperglycemia in obese diabetic subjects compared to obese controls. Controversial findings were reported concerning visfatin levels in many metabolic diseases. Although some scholars[27] reported that circulating visfatin levels increased in obesity, type 2 diabetes, and metabolic syndrome, a number of researchers argued that circulating visfatin levels did not change or were lower in comparison with healthy controls[28]. In our study, no significant difference was also revealed between the groups ($p > 0.05$).

Again, in this research, subfatin levels were determined to increase in the case of MetS and obesity. The results of previously published studies demonstrated that serum subfatin levels increased in T2DM subjects, but the results were controversial. Lee et al[29] reported low serum subfatin levels in subjects with newly diagnosed T2DM, whereas Chung et al[30] determined increased serum subfatin levels. Furthermore, after the adjustment of age, gender, and BMI, the serum MTERNL level was significantly associated with lipid profile, glucose profile, and insulin resistance. The previous research has shown that serum subfatin levels significantly increased in subjects with T2DM and increased the risk of T2DM independently of insulin resistance[10]. Subfatin increases energy expenditure, and it increases insulin sensitivity by inducing the expression of genes related to brown fat thermogenesis in mice[6]. Besides, in our current study, there was a correlation ($r = 0.015$) between subfatin and BMI, but there was no significant difference ($p = 0.938$). It has previously demonstrated that serum subfatin levels increased in overweight and obese individuals and correlated significantly with BMI. Löffler et al[8] also stated that subfatin expression in adipocytes in obese children increased in comparison with lean children. On the other hand, Chung et al[30] reported that serum subfatin levels were not correlated with body weight, BMI, WC, and adipose tissue mass.

Conclusions

This study is the first to investigate the interaction of MetS, asprosin, visfatin, and subfatin hormones together. Each hormone of asprosin, visfatin, and subfatin has been previously investigated independently in case of obesity, and contradictory results have been obtained. The current study demonstrates that these molecules have a direct relation with MetS and obesity. Since MetS and obesity are an important public health problem with metabolic turbulence, we can say based on the findings of the present research that measuring asprosin, visfatin, and subfatin together will provide information about the condition and course of the disease.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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