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ORGINAL ARTICLE

Serum levels of nesfatin-1 and irisin in obese children

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ABSTRACT. ***Background:*** **Along with the developing technology in the modern age, physical activity had decreased considerably in children and adolescents alike with a concomittant and rapid increase in the prevalence of childhood obsesity. The purpose of the present study is to measure the levels of serum nesfatin-1 and irisin in obese children.** ***Methods:*** **The present study was carried out with a total of 62 children, including 32 obese children diagnosed between June 2017 and October 2017 and 30 healthy children. Serum nesfatin-1, irisin, SOD, MDA, fasting blood glucose, total cholesterol (TC), triglyceride (TG), HDL-C, LDL-C, aspartate amino transferase (AST), alanine amino transferase (ALT)), blood urea nitrogen (BUN), C-reactive protein (CRP), calcium (Ca), sodium (Na), potassium (P), chromium (Cr), ferritin, and vitamin B_{12} data were collected for each patient.** ***Results:*** **In our study, mean nesfatin-1 and SOD values of the obesity group were lower than those of the control group ($p < 0.05$, $p < 0.001$), whereas irisin and MDA values were higher than those of the control group ($p < 0.001$).** ***Conclusion:*** **Childhood obesity is still a significant global problem, despite increased social awareness and numerous preventive healthcare interventions. We believe that all the prospective studies to be carried out to evaluate the relationship between obesity-irisin-nesfatin-1 triad, will make positive contributions to treatment of obesity.**

Key words: Irisin, nesfatin-1, obesity, oxidative stress

INTRODUCTION

Obesity, defined as abnormal or excessive fat accumulation in the body posing risk to health, is an important metabolic disorder due to genetic, environmental, metabolic, and hormonal factors and may lead to social, psychological, and medical complications [1]. Although it has an increasing prevalence worldwide, especially in developed countries, obesity is a preventable health problem [2]. Along with the developing technology, physical activity decreased considerably in children and adolescents alike and the prevalence of obesity in childhood increased rapidly [3]. World Health Organization 2010 data revealed that 43 million preschool children were overweight or obese which indicated a 4.2% increase in comparison with 1990 [4]. To define obesity clinically, body mass index is used. Accordingly, people with BMI over 25 are defined as overweight and over 30 as obese. Based on BMI percentiles for childhood and adolescence, Centers for Disease Control 2000 (CDC) defined 85-95% percentile range as overweight and over 95% percentile as obese [5, 6]. Childhood obesity and related insulin resistance pave the way for type 2 diabetes at very early ages, hypertension in children and adolescents, cardiovascular events in adult ages as a result of metabolic syndrome when accompanied by hyperlipidemia [7].

Studies have been conducted to explore the pathogenesis, in order to enable prevention, early diagnosis, and treatment of these complications [8]. The present situation regarding obesity treatment has come to the point of investigating the role of neurobiological substrates [9]. Nesfatin-1, discovered in 2006 and associated with obesity, is a satiety molecule found in the hypothalamus with a molecular weight of 9.7 kDa, consisting of 82 amino acids [10]. It can reach the brain by crossing the blood–brain barrier both endogenously and exogenously, which enables anorexigenic effect in controlling body weight, also playing a role in nutrient intake and in many metabolic processes [11, 12]. It was shown in studies that nesfatin-1 can suppress food intake via a leptin-independent but melanocortin-receptor-dependent mechanism [13, 14].

Irisin, first defined in 2012 by Bostrom et al. [15], was pointed out as a target in the treatment of diabetes and obesity due to its blood glucose level regulating and weight loss inducing effects and therefore considered a promising signal molecule. Although defined as a hormone in recent years, irisin is a myokine, the level of

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which is regulated by peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α). Irisin is released from the skeletal muscle into the blood as a result of proteolytic cleavage of protein 5 containing fibronectin type III domain (FNDC5), known as a type 1 membrane protein [16].

Oxidative stress can be defined as the shift in the balance between oxidants and antioxidants in favor of oxidants, resulting in lipid peroxidation and emergence of reactive oxygen products causing cellular damage in the organism [17]. And this is a critically important case in the pathogenesis of many diseases. One of the reasons suggested for the mechanism of the obesity-related increase in oxidative stress is the fact that obesity increases the metabolic and mechanical workload of myocard. Mitochondrial respiration increases as a result of increased oxygen consumption in the myocardium which may lead to the emergence of reactive oxygen products [18].

More effective and safer novel methods are needed today, due to the controversies regarding the effectiveness of the current treatment methods for obesity, which is an important public health problem with increasing prevalence worldwide. The most important factor in fighting obesity and related disorders is to understand the underlying causes and to develop relevant methods to struggle with the disease. To this end, oxidative stress was determined in addition to nesfatin-1 and iris serum levels in obese and healthy children in this study, and their roles in the pathogenesis of obesity were clarified.

MATERIALS AND METHODS

The present study was carried out with a total of 62 children, including 32 obese children diagnosed between June 2017 and October 2017 in Ordu University Faculty of Medicine, Department of Pediatrics and 30 healthy children. The ethics committee approval was obtained from Ordu University Faculty of Medicine, Ethics Committee of Clinical Studies. The age interval of children included in the study was between 5 and 12 years. The parents completed and signed the forms covering the patients' history, along with the data of physical examination, height, weight, and age.

Height and weight measurements were taken as reference in diagnosing obesity. Body Mass Index (BMI) [weight (kg) / height^2 (m)] was calculated after the measurements and those with a BMI at or above 95. percentile were considered obese. Those with a BMI under 85. percentile were grouped as control. Serum nesfatin-1, irisin, SOD, MDA, fasting blood glucose, total cholesterol (TC), triglyceride (TG), HDL-C, LDL-C, aspartate amino transferase (AST), alanine amino transferase (ALT)), blood urea nitrogen (BUN), C-reactive protein (CRP), calcium (Ca), sodium (Na), potassium (P), chromium (Cr), ferritin, and vitamin B12 data were collected, for each patient. Serum nesfatin-1 (Chemicon® International Inc., Ph .; USA) and irisin (Chemicon® International Inc., Ph .; USA) levels were examined by enzyme-linked immunosorbent assay (ELISA) using commercial kits. Serum nesfatin-1 results were given in pg/ml while serum irisin results in ng/ml.

Serum MDA level was measured according to the method by Ohkawa et al [19]., based on the principle of measuring the absorbance of pink colored compound, formed by the reaction between MDA and thiobarbituric acid, at 532 nm. The results were given in nmol/ml. Serum SOD activity was determined by the method described by Sun et al [20]. The method is based on the spectrophotometric measurement of the color intensity of the colored compound which is formed by the nitro blue tetrazolium (NBT) and superoxide anion generated by xanthine-xanthine oxidase. The results were given in U / ml.

STATISTICAL ANALYSIS

Results are presented as sample size (n), mean, and standard deviation. The Shapiro–Wilk test was used to evaluate whether nesfatin-1, irisin, MDA, and SOD levels were normally distributed. Differences between nesfatin-1, irisin, MDA, and SOD levels of the patient and control group were calculated using the t-test for independent samples. Correlations between nesfatin-1, irisin, MDA, and SOD levels were evaluated by Pearson's correlation analysis. The chosen level of significance was $p < 0.05$. Statistical analyses were performed using IBM SPSS Statistics version 19 (SPSS Inc., Chicago, USA).

RESULTS

The biochemical findings of our study groups are presented in *table 1*. Obesity group displayed significantly higher levels of TC, TG, LDL-C, ALT, and ferritin ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.05$, respectively) while lower levels of serum calcium with respect to the control group ($p < 0.001$). However, there was no statistically significant difference in serum glucose, HDL-C, AST, BUN, CRP, Na, K, Cr, and vitamin B_{12} levels in our study groups ($p > 0.05$).

In our study, mean nesfatin-1 and SOD values of obesity group were lower than those of the control group ($p < 0.05$, $p < 0.001$), whereas irisin and MDA values were higher than the control group ($p < 0.001$) (*table 2*). The correlation between nesfatin-1, irisin, MDA, and SOD values is presented in *table 3*. Nesfatin-1 values showed a significant negative correlation with irisin and MDA values ($p < 0.05$), whereas a significant positive correlation with SOD values ($p < 0.05$). Irisin and MDA values showed a significant strong negative correlation with SOD values ($p < 0.001$), and a significant positive correlation was determined between irisin and MDA values ($p < 0.001$). It was determined that irisin and MDA values decreased while SOD values increased as nesfatin-1 values of the individuals increased. Similarly, SOD values decreased as irisin and MDA levels increase. MDA values were found to increase along with increasing values of irisin ($p < 0.001$).

DISCUSSION

Obesity was shown to be associated with many diseases such as cardiovascular diseases, type 2 diabetes, sleep apnea, hypertension and cancer and even triggering

Table 1
Demographic and Laboratory Variables of the Groups

	Control group (n = 30)	Obesitiy group (n = 32)	*p value*
Age (year)	6.50 ± 1.25	5.90 ± 1.50	>0.05
Female/Male	16/14	14/18	>0.05
BMI (kg/m^2)	17.1 ± 2.4	26.3 ± 3.71	<0.05
Glucose (mg/dl)	91.40 ± 13.34	91.00 ± 7.21	>0.05
TC (mg/dl)	130.20 ± 20.44	166.43 ± 33.66	<0.001
TG (mg/dl)	78.43 ± 25.33	108.43 ± 40.63	<0.001
HDL-C (mg/dl)	52.09 ± 5.33	52.37 ± 12.27	>0.05
LDL-C (mg/dl)	62.40 ± 5.33	193.15 ± 28.02	<0.001
AST (U/L)	23.46 ± 6.17	24.15 ± 9.08	>0.05
ALT (U/L)	12.90 ± 4.00	56.72 ± 16.19	<0.001
BUN (mg/dl)	10.40 ± 2.97	9.28 ± 1.98	>0.05
CRP (mg/dl)	0.87 ± 1.26	0.56 ± 1.10	>0.05
Vitamin B_{12} (pmol/L)	284.23 ± 15.10	276.67 ± 15.75	>0.05
Ferritin (ng/ml)	26.45 ± 2.84	38.36 ± 4.79	<0.05
Ca (mg/dl)	29.18 ± 0.48	9.75 ± 0.38	<0.001
Na (mmol/L)	138.90 ± 2.52	139.38 ± 2.09	>0.05
K (mmol/L)	4.28 ± 0.41	4.42 ± 0.35	>0.05
Cr (μg/dl)	0.60 ± 0.17	0.62 ± 0.13	>0.05

BMI: body mass index; TC: total cholesterol; TG: triglyceride; HDL: high–density lipoprotein; LDL: low–density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BUN: blood urea nitrogen; CRP: c-reaktive protein; Ca: calcium; Na: sodium; K: potassium; Cr:chromium

Table 2
Serum nesfatin-1, irisin, MDA and SOD levels for control and obesity groups

	Control group (n = 30)	Obesitiy group (n = 32)	*p value*
Nesfatin-1 (pg/ml)	106.80 ± 23.32	92.60 ± 15.07	<0.05
Irisin (ng/ml)	89.75 ± 31.26	215.00 ± 76.29	<0.001
MDA (nmol/ml)	5.45 ± 0.72	9.68 ± 1.05	<0.001
SOD (U/ml)	47.40 ± 8.38	20.81 ± 5.02	<0.001

MDA: malondialdehyde; SOD: superoxide dismutase

Table 3
Relationship between nesfatin-1, irisin, MDA and SOD values

		Irisin	MDA	SOD
Nesfatin-1	**r-value**	-0.276	-0.282	0.292
	p-value	**0.033**	**0.029**	**0.024**
Irisin	**r-value**		0.648	-0.621
	p-value		**<0.001**	**<0.001**
MDA	**r-value**			-0.848
	p-value			**<0.001**

these diseases. For this reason it is essential to develop new treatment strategies to reduce obesity prevalence. Obesity is becoming widespread worldwide as a result of contemporary life standards including desk job, lack of exercise, and fast food type nutrition. Although obesity is more common in adults, it has an increasing prevalence also in childhood in recent years, and childhood obesity increases the risk of adulthood obesity twice [21, 22].

Determining obesity-related potential biomarkers that adversely affect living conditions will be very useful for the early diagnosis of the disease. Significance of the biomarkers is increasing, as the parameters that can be used in diagnosing clinical diseases, monitoring treatment response, and determining prognosis. In our study, which was conducted in light of all this information, we determined the levels of nesfatin-1 and irisin, which we considered as the new biomarkers for determining childhood obesity, along with their significant effects on metabolism. In the literature, nesfatin-1 was reported to pass through the blood–brain barrier easily, and can cause weight loss when administered as a systemic or a local drug [23]. In their study conducted in 2007, William [24] reported that regular continuous infusion of nesfatin-1 into the cerebral ventricles by osmotic pump caused significant decrease in nutrient intake, body weight, mesenteric, subcutaneous, and epididymal fat mass but no change in skeletal muscle. In another study by Li et al. [25], nesfatin-1 was noted to regulate cardiac functions, to decrease blood glucose levels, and thus, it might be a multifunctional peptide with an anorectic effect. In our study, serum nesfatin-1 levels were lower in the obesity group as compared with the control group. There are conflicting results regarding nesfatin-1 and obesity in the literature. In a study conducted with postmenopausal obese women, Celik et al. [26] reported that nesfatin-1 levels did not change statistically in the obesity group. In another study, Mirzaei et al. [27] determined nesfatin-1 levels to be lower in obese patients and noted that low levels of nesfatin-1 led to high caloric intake. Shimizu et al. [28] demonstrated a decrease in food intake after intraperitoneal nesfatin administration in mice and pointed out that a 6 months of regular nesfatin administration prevented the increase of body weight. Considering the literature results as well as our study results, we can say that further studies are needed to support them. Nevertheless, our results support the opinion that nesfatin-1 is an important anorexigenic signal in the etiology of obesity. Both fat tissue and skeletal muscle have been described as hormone secreting endocrine organs in the human body, and adipokines and myokines secreted from these organs were shown to be associated with obesity-related metabolic and vascular diseases [29, 30].

Irisin is a novel myokine, which is suggested to be involved in the pathophysiology of obesity and the regulation of glucose metabolism. Irisin is mainly secreted from the skeletal muscle, and influences thermogenesis, energy consumption, and glucose metabolism via stimulating brown adipocytes [31, 32]. In obesity, excessive amount of fat accumulates in the body as a result of the energy intake exceeding the energy consumption. Irisin has been

thought to play a role in the development of obesity and relevant studies on this topic have been conducted on humans and rats. In our study, irisin levels were found to be significantly higher in the obese group, compared to the control group. There are conflicting results in the literature regarding the correlation of obesity and irisin levels. Low levels of irisin were determined in obese people in some of these studies but high levels in some others. Stengel et al. [33] found higher levels of irisin in obese individuals in comparison with controls, similar to our study. In addition, serum irisin levels were noted to increase with increasing fat mass. In another study by Reinehr et al. [34], serum irisin levels were higher in obese patients with respect to healthy controls. There are also some results in the literature contrary to our study results. In a study on obese children with metabolic syndrome, Shim et al. [29] found lower irisin levels in obese children compared to healthy children.

Various mechanisms were suggested explaining the increase in oxidative stress in obese individuals. Changes in lipid and glucose metabolism, chronic inflammation [35], tissue dysfunction [36], and abnormal ROS formation [37] are some of these mechanisms. Increased free radicals in obesity influence hypothalamic neurons and act in controlling hunger and satiety and accordingly in controlling body weight. Lipid peroxidation is the degradation of polyunsaturated fatty acids by free oxygen radicals in mammalian cell membranes, into various products such as peroxides, alcohols, aldehydes, hydroxy fatty acids, ethane, pentane, malondialdehyde (MDA) [38]. MDA, one of the most important degradation products of lipid peroxidation, reacts with functional groups of various compounds in the cell and causes cell damage [39, 40] pointed out in a relationship between the extent of abdominal fat accumulation and increased lipid peroxidation. In their study on obese children, Yilmaz et al. [41] found significantly higher MDA levels in comparison with the control group. In our study with compatible results with the literature, serum MDA levels were found to be significantly higher in obesity group, compared with the control group.

There are enzymatic and nonenzymatic antioxidant molecules to prevent the damage induced by free radicals. The oxidant-antioxidant status of the body can be evaluated by measuring the activation and concentration of antioxidant enzymes separately. In our study, serum SOD enzyme activities were higher in the obesity group, compared to healthy controls. In a study with obese adolescents, Khaled et al. [42] reported significantly lower SOD enzyme activity with respect to the control group. In a study with nondiabetic obese children and adolescents, Habib et al. [43] also noted significantly decreased SOD activity. Ozata et al. [44] reported lower SOD levels in obese individuals when compared with those with normal weight, and lower levels with other antioxidant enzymes, namely, catalase and glutathione peroxidase activities as well. In our study, a significant positive correlation was found between serum nesfatin-1 and SOD levels while a negative correlation was found between serum irisin and MDA levels. There was a positive correlation between serum irisin levels and serum MDA levels. The correlations we observed in our study suggest that nesfatin-1 decrease and irisin levels increase as oxidative stress increases. Further studies are needed on inflammatory cytokines that take an important part/are involved in the pathogenesis of obesity, in order to investigate the difference in the levels of nesfatin-1 and irisin.

In our study, we also evaluated the lipid profiles of the groups. The TG, TC, and LDL-C levels of the obesity group were significantly higher than those of the control group. However, there was no significant difference between the groups in terms of HDL-C values. In a study by Lima et al., [45] TC and LDL-C levels were higher in obese children than normal-weight children, but HDL-C levels displayed no significant difference. Juonala et al. [46] stated that TG and LDL-C levels were higher and HDL-C levels were lower in obese children and adults. In conclusion, considering our results of lipid profile, we can interpret decreased antioxidant enzyme activity and increased lipid peroxidation in obesity as one of the results of hyperlipidemia.

There is a risk of fatty liver associated with obesity, which triggers a process that can progress to cirrhosis. Mild or moderately increased levels of ALT and AST are the most common laboratory findings in patients with fatty liver disease [47]. We determined statistically significantly higher levels of serum ALT in the obesity group when compared to the control group whereas serum AST levels showed no statistically significant difference between the groups.

Childhood obesity is still a significant global problem, despite increased social awareness and numerous preventive healthcare interventions. We believe that all the prospective studies to be carried out to evaluate the relationship between obesity-irisin-nesfatin-1 triad, will make positive contributions to treatment of obesity.

CONFLICT OF INTEREST

The authors declared no conflicts of interest.

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