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Low Levels of Serum Ghrelin and Nesfatin-1 Are Associated With Anxiety Disorders in Children

Ayhan Bilgiç[1], Merve Cura[2], İbrahim Kılınç[3], and Ömer Faruk Akça[4]

[1]Department of Child and Adolescent Psychiatry, Faculty of Medicine, Izmir University of Economics, Izmir, Türkiye
[2]Department of Child and Adolescent Psychiatry, Etlik City Hospital, Ankara, Türkiye
[3]Department of Biochemistry, Necmettin Erbakan University Faculty of Medicine, Konya, Türkiye
[4]Department of Child and Adolescent Psychiatry, Necmettin Erbakan University Faculty of Medicine, Konya, Türkiye

Objectives: Because appetite-regulating hormones are implicated in neuronal survival, growth, and differentiation, they have been suggested to play a role in anxiety disorders. To date, few studies have focused on the association between these hormones and anxiety disorders in children. This study investigated the potential differences in leptin, ghrelin, and nesfatin-1 serum levels in drug-naïve children with anxiety disorders, including social anxiety disorder, separation anxiety disorder, and generalized anxiety disorder, and in healthy controls.

Methods: This study included 45 children (14 boys and 31 girls) with anxiety disorders and 35 healthy controls (13 boys and 22 girls) aged 8–18 years. The severity of anxiety disorders and additional symptoms were evaluated using the Revised Child Anxiety and Depression Scales–Child Version. Enzyme-linked immunosorbent assay (ELISA) was used to evaluate leptin, ghrelin, and nesfatin-1 serum levels.

Results: Leptin levels were significantly higher in children with anxiety disorders than in the control group, and ghrelin and nesfatin-1 levels were significantly lower in children with anxiety disorders than in the control group for girls and for the entire sample. However, only low nesfatin-1 levels were significantly associated with anxiety disorders in boys. In the entire sample, potential confounders such as age, sex, body mass index, and the severity of depressive symptoms were controlled for, and the results were the same for ghrelin and nesfatin-1 levels. However, the difference in leptin levels between groups was not significant.

Conclusion: These findings suggest that dysregulation of ghrelin and nesfatin-1 concentrations may be related to the etiopathogenesis of childhood anxiety disorders.

Keywords: Leptin; Ghrelin; Nesfatin-1; Anxiety disorder; Children.

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Address for correspondence: Ayhan Bilgiç, Department of Child and Adolescent Psychiatry, Faculty of Medicine, Izmir University of Economics, Fevzi Çakmak, Sakarya Cd. No:156, Balçova/İzmir 35330, Türkiye
Tel: +90-(232)-279-25-25, Fax: +90-(232)-279-26-26, E-mail: bilgicayhan@yahoo.com

INTRODUCTION

Anxiety disorders are psychiatric disorders that are common in childhood and cause serious impairments in social, educational, and family function [1]. Although the etiopathogenesis of anxiety disorders remains unclear, researchers have suggested several biological and environmental mechanisms [1]. Appetite-regulating hormones, including leptin, ghrelin, and nesfatin-1, have been proposed to play a role in anxiety disorders because they are implicated in neuronal survival, growth, and differentiation [2]. They also exert crucial effects on synaptic plasticity and exhibit neuroprotective and anti-apoptotic activity in different brain regions [3]. These hormones are transported to the brain through the blood-brain barrier, and an increasing number of studies have provided evidence regarding the association between peripheral levels of appetite-regulating hormones and anxiety disorders [4-9].

Leptin was first identified as an anorexigenic adipose-tissue-derived hormone that regulates food intake and energy consumption [10]. Research has revealed that leptin contributes to various neuropsychiatric mechanisms, including learning, memory, stress regulation, and emotional processes [11,12]. Several studies in adults have investigated its potential role in anxiety disorders and yielded conflicting findings [11,13,14]. Naufel et al. [14] found a positive association between blood leptin levels and anxiety symptoms in overweight middle-aged women. Another study found that elevated cerebrospinal fluid leptin levels were related to greater anxiety symp-

tom severity and a hyperactive hypothalamic–pituitary–adrenal (HPA) axis in female suicide attempters [11]. However, Masdrakis et al. [13] showed a link between low serum leptin levels and greater severity of panic disorder symptoms in women. To date, only one study has examined circulating leptin levels in childhood anxiety disorders and reported no alterations [8]. In summary, there is no conclusive evidence regarding whether this hormone plays a role in anxiety disorders.

Ghrelin is an orexigenic hormone produced in the stomach that stimulates feeding and opposes the effects of leptin on appetite [15]. In addition to its function in regulating feeding behaviors, ghrelin activates the HPA axis, and its circulating levels have been shown to be related to psychosocial stressors [16]. Therefore, ghrelin may be involved in the development or triggering of anxiety disorders via its effect on the HPA axis or other unknown mechanisms [17]. Little research has thus far evaluated the association between ghrelin and anxiety disorders [6,8,18]. Gul et al. [6] found higher circulating ghrelin levels in subjects with panic disorder with agoraphobia than in subjects with panic disorder without agoraphobia and controls. Atescelik et al. [18] demonstrated a positive relationship between serum ghrelin levels and increased anxiety among adult suicide attempters. Consistent with these studies, Ozmen et al. [8] found a positive link between circulating ghrelin levels and anxiety disorders in children. Several animal studies have also demonstrated that ghrelin administration to the brain leads to anxious behaviors [19,20]. However, these data are not unique, and others have reported anxiolytic and antidepressant effects in mice [21,22].

Nesfatin-1, similar to leptin, was first identified as an anorexigenic peptide that suppresses food intake [23]. Other important effects include glucose homeostasis, anti-inflammatory activity, increased blood pressure, puberty initiation, and regulation of emotional stress responses [24]. A variety of animal studies demonstrated the anxiogenic and anhedonic effects of nesfatin-1 [24,25]. Several studies in humans have specifically scrutinized the potential role of nesfatin-1 in anxiety disorders but have yielded divergent results. Gunay et al. [7] observed decreased peripheral nesfatin-1 levels in adult males diagnosed with generalized anxiety disorder. However, another study found increased peripheral nesfatin-1 levels in mixed adult male and female subjects with panic disorder [5]. Additionally, some studies have provided evidence for a sex-specific association between nesfatin-1 levels and anxiety severity in obese individuals [26,27]. Although a recent study found low nesfatin-1 levels in adolescents with major depression [28], to our knowledge, no study has investigated the potential effect of nesfatin-1 on anxiety disorders in childhood and adolescence.

The exact mechanism by which appetite-regulating hormones influence stress responses and anxiety levels remains unclear. Animal studies have suggested that hippocampal neurogenesis can be promoted by leptin and ghrelin via neuroplasticity [29]. Wang et al. [30] detected a key role of leptin in reducing fear conditioning-induced synaptic potentiation in the thalamic-lateral amygdala through NMDA receptors. Although the molecular mechanisms are complex and unclear, Chang et al. [31] found that ghrelin may exhibit anxiolytic effects by binding to the growth hormone secretagogue receptors in the nucleus accumbens. Merali et al. [32] suggested that nesfatin-1 exerts its anxiety-producing effects by collaborating with corticotropin-releasing hormones or by affecting the melanocortin system. It has been suggested that in triggering the HPA axis, nesfatin-1 exerts serotonin activity in the raphe nuclei and norepinephrine activity in the locus coeruleus [33]. Studies have also proposed that the effects of these hormones on anxiety levels are determined by a variety of factors, such as the duration of stress, existence of stress, and feeding status [31,32].

To gain further data on the relationship between appetite-regulating hormones and childhood anxiety disorders, this study aimed to evaluate serum leptin, ghrelin, and nesfatin-1 levels in children with anxiety disorders (social anxiety disorder, separation anxiety disorder, and generalized anxiety disorder) who were not taking medication. As previous studies in adults have reported different findings between the two sexes [25,26], this study also aimed to evaluate whether there is a sex-specific association between these hormones and childhood anxiety disorders. We hypothesized that serum levels of appetite-regulating hormones may be linked to anxiety disorders in children.

METHODS

Participants

Patients with anxiety disorders, including social, separation, and generalized anxiety disorders, were recruited from the Child and Adolescent Psychiatry Outpatient Clinic of the Necmettin Erbakan University Faculty of Medicine. The exclusion criteria were as follows: having a psychiatric disorder, including intellectual disability, autism spectrum disorder, mood disorders, a tic disorder, obsessive-compulsive disorder, or psychotic disorders; having a major physical illness (e.g., epilepsy and autoimmune diseases) or an active infection; use of psychotropic, corticosteroid, or antihistaminic medication or medication that may have affected one's appetite in the previous 3 months; and not being fluent in Turkish. Children who had used psychiatric medication in the last 6 months were also excluded from the study. The control

group consisted of healthy volunteers with no psychiatric disorders or significant physical illnesses. The same exclusion criteria were applied to patients with anxiety disorders in the control group.

Necmettin Erbakan University Ethics Committee reviewed and approved the research protocol (ethics approval number: 2018/1478), and the children's parents provided written informed consent. Verbal assent was also obtained from all children and their parents. For the study group, 67 children with anxiety disorders were recruited for inclusion; however, 19 were excluded according to the exclusion criteria and three refused to participate. For the control group, 42 children were recruited and seven were excluded based on the exclusion criteria.

Assessment procedures

An experienced child and adolescent psychiatrist (M.C.) conducted psychiatric interviews with all participants using the Schedule for Affective Disorders and Schizophrenia for School-Age Children, Present and Lifetime Version (K-SADS-PL) [34]. The severity of the anxiety disorders and depression symptoms was determined using the Revised Child Anxiety and Depression Scales−Child Version (RCADS-CV) [35]. This Likert-type scale comprises 47 items that assess DSM-IV-based anxiety disorders and depression symptoms in childhood. Its subscales correspond to separation anxiety disorder, social anxiety disorder, panic disorder, generalized anxiety disorder, obsessive-compulsive disorder, and major depressive disorder. The reliability and validity of the RCADS-CV have been demonstrated in a Turkish population [35]. Cronbach's α coefficients of all Turkish version of RCADS-CV subscales were above 0.70. The participants' body mass index (BMI) was calculated by measuring their height and weight on the day their blood samples were collected.

Blood sample collection

Participants' blood samples were collected from the antecubital vein into biochemistry tubes between 8:30 and 9:30 in the morning after an overnight fast. Blood samples were collected within one week of K-SADS-PL and RCADS-CV administration. They were centrifuged at 4000 rpm for 5 min, and until analyzed, the obtained serum was stored at -80°C. Serum concentrations of leptin, ghrelin, and nesfatin-1 were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the procedures provided by USCN Life Science. Inc. (SEA084Hu, CEA991Hu, and CEA-242Hu).

Statistical analysis

The distribution of variables was evaluated using the Shapiro−Wilk test. Differences in sex distribution between the two groups were evaluated using a chi-squared test. Age, BMI, and appetite-regulating hormone levels of the two groups were compared using the Mann−Whitney U test or Student's t-test based on the distribution of variables. Correlations between serum appetite hormone concentrations and RCADS-CV scores were evaluated using Spearman or Pearson correlation tests. Pearson's correlation analysis was used when variables were normally distributed and Spearman's correlation analysis was used when variables were not normally distributed.

To control for confounding factors, multivariate analysis of covariance (MANCOVA) was also performed. This assessed the main effect of a group using all serum appetite-regulating hormone outcome measures and accounted for potential confounders, such as age, sex, BMI, and depressive symptom severity. If a significant difference between groups was found using the MANCOVA test, a separate one-way analysis of covariance (ANCOVA) was conducted. Before these tests were conducted, variables that did not show a normal distribution (leptin and nesfatin-1) were logarithmically transformed. Statistical significance was set at $p<0.05$ (two-sided).

RESULTS

Sociodemographic and clinical characteristics of the subjects

A total of 45 children (31 girls and 14 boys) with anxiety disorders and 35 healthy controls (22 girls and 13 boys) constituted the final study population, and no significant difference was found between the groups in terms of sex distribution ($\chi^2=0.320$, $p=0.571$). No significant difference was detected in mean age between the patient (12.8±2.7 years) and control (11.7±3.1 years) groups (range, 8−18 years; $z=1.854$; $p=0.064$). The BMI did not show a significant difference between the anxiety disorder patients (20.6±5.4 kg/m^2) and the controls (18.7±3.2 kg/m^2) ($t=1.869$, $p=0.066$). In the anxiety disorder group, 57% of the patients (n=26) had social anxiety disorder, 33% (n=15) had generalized anxiety disorder, and 29% (n=13) had separation anxiety disorder. All RCADS-CV scores were significantly higher in the anxiety disorder group than in the control group (Table 1).

Comparison of appetite-regulating hormone concentrations between study groups

Serum leptin concentrations were significantly higher, whereas serum ghrelin concentrations were significantly lower, in the anxiety disorder group than in the healthy control group for girls and the entire sample (Table 1 and Fig. 1). However, the differences in serum leptin and ghrelin levels

Table 1. Demographic and clinical characteristics of children with anxiety disorders and controls

Characteristics	Boys				Girls				Total sample			
	Patients (n=14)	Controls (n=13)	t or z or χ^2	p	Patients (n=31)	Controls (n=22)	t or z or χ^2	p	Patients (n=45)	Controls (n=35)	t or z or χ^2	p
Sex, male/female	NA	NA	NA	NA	NA	NA	NA	NA	14/31	13/22	0.320*	0.571
Age (yr)	13.1±3.0	10.4±2.6	2.299†	0.022	12.8±2.6	12.4±3.1	0.667‡	0.154	12.8±2.7	11.7±3.1	1.854†	0.064
BMI (kg/m^2)	22.6±5.8	18.1±3.0	1.893†	0.061	19.6±5.0	19.1±3.3	0.390‡	0.698	20.6±5.4	18.7±3.2	1.869‡	0.066
RCADS-CV-SAD	9.1±5.6	2.8±2.2	3.959‡	0.001	7.2±5.3	3.8±3.9	2.416†	0.016	7.8±5.4	3.4±3.4	3.823†	<0.001
RCADS-CV-SP	14.9±12.8	5.7±4.5	3.162†	0.001	16.7±6.5	5.8±3.8	7.619‡	<0.001	16.1±8.8	5.8±4.0	6.995‡	<0.001
RCADS-CV-GAD	10.0±3.7	2.4±2.1	6.551‡	<0.001	9.5±4.4	4.3±2.6	4.257†	<0.001	9.7±4.2	3.6±2.6	6.108†	<0.001
RCADS-CV-PD	11.9±6.3	1.6±2.2	4.131†	<0.001	10.8±8.0	2.2±1.9	4.315†	<0.001	11.1±7.4	2.0±2.0	6.017†	<0.001
RCADS-CV-OCD	8.8±4.5	1.5±1.8	3.705†	<0.001	8.3±5.0	3.8±2.5	4.294‡	<0.001	8.4±4.8	2.9±2.5	5.004†	<0.001
RCADS-CV-MDD	13.3±7.3	2.7±2.9	4.987‡	<0.001	12.5±7.9	5.0±4.3	4.459‡	<0.001	12.8±7.6	4.1±4.0	5.082†	<0.001
Leptin (ng/mL)	1.99±1.24	1.36±0.76	1.820†	0.068	2.04±1.41	1.37±0.71	2.437†	0.015	2.03±1.35	1.36±0.71	3.080‡	0.002
Ghrelin (ng/mL)	1.51±0.43	1.79±0.54	-1.452‡	0.159	1.48±0.39	1.72±0.45	-2.079‡	0.043	1.49±0.40	1.75±0.48	-2.594†	0.011
Nesfatin-1 (ng/mL)	21.8±8.3	45.8±28.1	-2.962‡	0.010	20.0±8.4	34.4±17.2	-3.087†	0.002	20.6±8.3	38.7±22.2	-4.035‡	<0.001

Values are presented as mean±standard deviation. *chi-square test; †Mann–Whitney U test; ‡Student's t-test. BMI, body mass index; GAD, generalized anxiety disorder; MDD, major depressive disorder; OCD, obsessive compulsive disorder; PD, panic disorder; RCADS-CV, Revised Child Anxiety and Depression Scales–Child Version; SAD, separation anxiety disorder; SP, social phobia (social anxiety disorder)

Fig. 1. Serum leptin (A), ghrelin (B), and nesfatin-1 (C) concentrations in medication-free children with anxiety disorders and healthy controls. Horizontal lines represent the mean value for each group.

between the groups were not statistically significant in boys. Serum nesfatin-1 concentrations were significantly lower in the anxiety disorder group than in the control group for both sexes and for the entire sample (Table 1 and Fig. 1). The mean appetite-regulating hormone concentrations in the two groups are presented in Table 1.

A MANCOVA test was also conducted on the entire sample to control for confounding factors. While age, sex, and BMI were used as covariates, this test was conducted with the patient and control groups as independent variables. The re-

Table 2. Comparison of serum appetite-related hormone levels in children with anxiety disorders and controls according to ANCOVA

	Patients (n=45)	Controls (n=35)	ANCOVA*			ANCOVA†		
			F	p	np^2	F	p	np^2
log-Leptin (ng/mL)	0.45±0.15	0.36±0.12	5.458	0.022	0.068	2.716	0.104	0.035
Ghrelin (ng/mL)	1.49±0.40	1.75±0.48	5.458	0.022	0.068	5.433	0.022	0.068
log-Nesfatin-1 (ng/mL)	1.28±0.18	1.52±0.25	22.397	<0.001	0.230	19.612	<0.001	0.210

Values are presented as mean±standard deviation. *covariates: age, sex, BMI; †covariates: age, sex, BMI, RCADS-CV-MDD. ANCOVA, analysis of covariance; BMI, body mass index; MDD, major depressive disorder; RCADS-CV, Revised Child Anxiety and Depression Scales–Child Version

sult demonstrated significant overall group differences between the two groups on serum appetite-regulating hormone concentrations [V (Pillai's trace)=0.252, $F_{(3,73)}$=8.216, p<0.001, np^2=0.252]. Separate univariate ANCOVAs on the outcome variables demonstrated significant differences between the two groups concerning the all appetite-regulating hormone levels. Serum levels of log-leptin [$F_{(1,75)}$=5.458, p=0.022, np^2=0.068] were high, whereas serum levels of ghrelin [$F_{(1,75)}$=5.458, p=0.022, np^2=0.068] and log-nesfatin-1 [$F_{(1,75)}$=22.397, p<0.001, np^2=0.230] were lower in the anxiety disorder group than the healthy control group (Table 2).

In addition to age, sex, and BMI, depression severity was regarded as a covariate, and a one-way MANCOVA test was performed. Similar to the first MANCOVA analysis, significant overall group differences were detected between the two groups for serum appetite-regulating hormone levels [V (Pillai's trace)=0.229, $F_{(3,72)}$=7.116, p<0.001, np^2=0.229]. Then, separate univariate ANCOVAs on the outcome variables were conducted, and significant differences for serum concentrations of ghrelin [$F_{(1,74)}$=5.433, p=0.022, np^2=0.068] and log-nesfatin-1 [$F_{(1,74)}$=19.612, p<0.001, np^2=0.210] were found between the two groups. However, serum log-leptin concentrations were not significant between the groups [$F_{(1,74)}$=2.716, p=0.104, np^2=0.035] (Table 2).

Correlations between appetite-regulating hormone concentrations and RCADS-CV scores

Correlations between serum appetite-regulating hormone concentrations and RCADS-CV scores were also assessed in the anxiety disorder group. No association was found between appetite-regulating hormone concentrations and RCADS-CV scores for either sex or for all groups (Table 3).

DISCUSSION

The present study showed that serum levels of leptin were high, whereas those of ghrelin and nesfatin-1 were lower in children with anxiety disorders than in healthy controls. While controlling for potential confounders, including age, sex, and BMI, the link between serum appetite-regulating hormone levels and the presence of anxiety disorders did not change. However, after controlling for the severity of depression, age, sex, and BMI, the link between high serum leptin levels and the presence of anxiety disorders was not significant. This study also investigated the probable sex-specific relationships of appetite-regulating hormones with anxiety disorders and provided some data for the possible sex-specific relationship between leptin and ghrelin and anxiety disorders in childhood.

We detected a link between elevated serum leptin levels and anxiety disorders in children, independent of age, sex, and BMI. However, after controlling for depression severity, the difference in serum leptin levels between the groups was not statistically significant. This finding suggests that leptin cannot be implicated in the origin of childhood anxiety disorders separate from depressive symptoms. Only one previous study investigated the role of leptin in childhood anxiety disorders and found no relationship between serum leptin levels and the presence of anxiety disorders [8]. Data on leptin levels in anxiety disorders or psychological distress in adults are discordant so far, as some studies showed decreased leptin levels, while others showed increased circulating leptin levels, probably due to differences in sex, anxiety disorder subtype, and severity of coexisting psychiatric problems [36-38]. For instance, Yoshida-Komiya et al. [38] analyzed plasma leptin levels in 29 women with anxiety disorders and mild depression and 26 healthy controls. Lower leptin concentrations were observed in the patient group than in the control group. However, another study detected elevated serum leptin levels in men who perceived psychological stress compared to controls [37]. A depression study also pointed out the potential importance of the psychiatric disorder subtype, showing an association between elevated leptin levels and atypical symptoms of depression such as leaden paralysis, increased weight, and hyperphagia, although no relationship was found between serum leptin levels and overall depression [36]. Therefore, the discrepant findings in anxiety studies might be attributable to differences in anxiety disorder subtypes, the clinical heterogeneity of anxiety disorders, or the presence of coexisting depressive symptoms among the study samples. Additionally, similar to the relationship between the HPA axis and anxiety disorders, which was found to be dif-

Table 3. Correlations between serum appetite-related hormone levels and the RCADS-CV scores

	SAD			SP			OCD			PD			GAD			MDD		
	Boys	Girls	Total	Boys	Girls	Total	Boys	Girls	Total	Boys	Girls	Total	Boys	Girls	Total	Boys	Girls	Total
Leptin	0.27	-0.14	-0.05	0.11	0.19	0.14	-0.40	0.05	-0.07	0.02	-0.12	-0.10	0.27	-0.09	-0.03	0.15	-0.04	-0.01
Ghrelin	-0.20	-0.01	-0.09	-0.01	0.00	-0.02	0.15	0.12	0.04	0.14	0.19	0.16	-0.41	0.19	0.02	-0.12	0.15	0.03
Nesfatin-1	-0.12	0.09	0.06	0.11	0.13	0.03	-0.02	0.17	0.45	0.28	0.09	0.16	-0.15	0.23	0.14	-0.03	0.19	0.12

There was no statistical significance for any correlation ($p>0.05$). GAD, generalized anxiety disorder; MDD, major depressive disorder; OCD, obsessive compulsive disorder; PD, panic disorder; RCADS-CV, Revised Child Anxiety and Depression Scales–Child Version; SAD, separation anxiety disorder; SP, social phobia

ferent in children and adults [39], leptin may play different roles in child and adult anxiety disorders.

Our findings showed that serum ghrelin concentrations were lower in children with anxiety disorders than in healthy controls and did not change after accounting for all potential confounders. Previously, only Ozmen et al. [8] had scrutinized the link between circulating ghrelin levels and anxiety disorders in children, and they reported higher serum ghrelin levels in the patient group than in the control. In addition, these researchers detected a relationship between trait anxiety scores in girls and ghrelin levels and proposed that increased ghrelin levels could stem from chronic stress induced by anxiety. Several studies in adults have suggested a positive relationship between circulating ghrelin and anxiety [6,18]. Additionally, some animal studies have supported the anxiogenic action of ghrelin, showing anxiety-like behavior induced by ghrelin administration in different regions of the brain [19,20]. However, these findings are not universal; Mahbod et al. [22] reported that life-long exposure to high levels of endogenous ghrelin reduces anxiety-like behavior in mice. Another study also demonstrated that ghrelin decreases anxiety levels after acute stress by stimulating the HPA axis in mice and suggested that ghrelin may regulate acute stress and have therapeutic effects on stress and mood disorders in humans [17,27]. Örüm et al. [40] recently reported low serum ghrelin levels in drug-naïve adult patients with panic disorders. Therefore, we believe that the low ghrelin levels in our patients may have impaired the regulation of anxiety, leading to anxiety disorders. Human and animal studies have suggested that ghrelin may have both anxiolytic and anxiogenic effects, depending on the source and duration of stress, which may be the reason for the conflicting results. Future studies are required to investigate the role of ghrelin in anxiety disorders.

Similar to ghrelin, we found significantly lower serum nesfatin-1 concentrations in children with anxiety disorders than in healthy controls, and this finding remained significant after accounting for all potential confounders. Although the present study was the first to assess circulating nesfatin-1 levels in children with anxiety disorders, a recent study evaluated the potential implications of nesfatin-1 in adolescents with depression. Consistent with these findings, the present study reported lower nesfatin-1 levels in the patient group than in the control group. It has been proposed that an increased concentration of stress-induced cortisol may have an inhibitory effect on the release of nesfatin-1 [28]. Studies in adults have yielded conflicting results regarding the potential role of nesfatin-1 in anxiety disorders. Similar to our findings, Gunay et al. [7] found lower plasma nesfatin-1 levels in normal-weight patients diagnosed with generalized anxiety

disorder than in controls. Örüm et al. [40] also found low serum nesfatin-1 levels in drug-naïve adult patients with panic disorders. However, Bez et al. [5] found an association between higher plasma nesfatin-1 concentrations and panic disorder in normal-weight individuals. Similarly, a study in obese women reported elevated plasma concentrations of nesfatin-1 in subjects with high anxiety levels compared to those in the low anxiety group [41]. Furthermore, a sex-specific association between nesfatin-1 levels and anxiety disorders has been evaluated by a research group that reported a positive relationship between anxiety and circulating nesfatin-1 levels in obese female patients and a negative relationship in obese males [26]. Our results do not support a sex-specific role of nesfatin-1 in children with anxiety disorders. Örüm et al. [40] proposed that the partly different fasting-satiety status and duration of the participants in the patient and control groups may be responsible for the conflicting results among studies. However, the exact reason for conflicting results regarding the link between circulating nesfatin-1 levels and anxiety disorders remains unclear.

In the present study, no relationship was found between leptin, ghrelin, or nesfatin-1 levels and the severity of anxiety disorders and depressive symptoms. Similar to our findings, Ozmen et al. [8] found no correlation between serum ghrelin or leptin levels and state anxiety scores in children in the whole group. However, they also found a positive correlation between serum ghrelin levels and trait anxiety scores in girls. In a study conducted in adults, Hofmann et al. [41] found a positive correlation between perceived stress and depression severity and nesfatin-1 levels in women, whereas no correlation was found in men. These authors also reported a stronger positive association between nesfatin-1 levels and anxiety severity in women, whereas there was an inverse correlation in men. Masdrakis et al. [13] investigated the link between plasma leptin levels and anxiety symptoms in women with panic disorder and detected significant negative correlations between plasma leptin levels and the severity of anxiety symptoms. The difference between the results of our study and those of previous studies may be due to a variety of different factors, such as sex-related differences becoming more pronounced in adulthood, the sample not being sufficiently homogeneous in terms of anxiety disorder subtypes, and cultural eating habits.

Although previous studies have suggested a sex-specific relationship between nesfatin-1 levels and anxiety disorders [26,27], our results are not consistent with this assumption. However, this study revealed findings suggesting a sex-specific relationship between ghrelin and leptin and anxiety disorders in children. We propose that the small male sample size in our study may have prevented the same relationship from being detected. Despite the lack of statistical significance, the fact that the direction of the difference between the two groups for ghrelin and leptin levels in the male group was similar to that in the female group supports the idea that statistical significance was not observed because of the small sample size. Therefore, although our study provides some data on the possible sex-specific relationship between leptin, ghrelin, and anxiety disorders in childhood, studies with larger sample sizes are needed to obtain definitive information on this subject.

Including only patients who were not taking medication, controlling for BMI and depression symptoms, and investigating sex-specific associations were the strengths of the present study. However, this study has several limitations. Due to the small sample size, the lack of separate evaluation of anxiety disorder subtypes is the main limitation of the current study. The lack of assessment of dietary patterns and weight changes in the participants was also a limitation. Another limitation is the lack of consideration of menstrual cycle phases in girls. In addition, only the serum levels of appetite-regulating hormones were measured, and the samples were analyzed only once. The fact that the effects of other hormones that play a role in the etiopathogenesis of anxiety disorders, such as cortisol and corticotropin-relaxing hormones, could not be controlled can also be considered a limitation. Additionally, it should be noted that the eating culture and dietary habits of different countries may have affected the results.

CONCLUSION

In summary, this study suggests that low serum ghrelin and nesfatin-1 levels may be involved in the development of childhood anxiety disorders. However, these findings do not support a potentially independent role of leptin in childhood anxiety disorders. Ghrelin and nesfatin-1 may play different roles in the regulation of anxiety. For instance, ghrelin may exert anxiolytic effects by binding to the growth hormone secretagogue receptor in the nucleus accumbens. In contrast, nesfatin-1 can be effective in anxiety regulation by cooperating with corticotropin-releasing hormones or through its effects on the melanocortin system. The specific role of appetite-related hormones in anxiety regulation may play a role in the etiopathogenesis of anxiety disorders. Future studies should include larger sample sizes and must target to clarify the potential mechanisms that play a role in the link between appetite-regulating hormones and childhood anxiety disorders.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

Author Contributions

Conceptualization: all authors. Data curation: Merve Cura, İbrahim Kılınç. Formal analysis: İbrahim Kılınç. Funding acquisition: Ayhan Bilgiç. Project administration: Ayhan Bilgiç. Writing—original draft: Ayhan Bilgiç, İbrahim Kılınç, Ömer Faruk Akça. Writing—review & editing: Ayhan Bilgiç.

ORCID iDs

Ayhan Bilgiç	https://orcid.org/0000-0003-0703-2630
Merve Cura	https://orcid.org/0000-0002-3156-8657
İbrahim Kılınç	https://orcid.org/0000-0002-7729-7557
Ömer Faruk Akça	https://orcid.org/0000-0002-1165-2974

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