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Increased concentrations of serum nesfatin-1 levels in childhood with idiopathic chronic malnutrition

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Background/aim: Nesfatin-1, an anorexigenic molecule, seems to play a role in appetite regulation and energy homeostasis. The goal of this study was to evaluate the relation of nesfatin-1 with anthropometric and metabolic (ghrelin, leptin) parameters in children with idiopathic chronic malnutrition.

Materials and methods: The study included 37 underweight and 38 healthy children who were similar regarding age, sex, and pubertal status. Anthropometric and biochemical (nesfatin-1, ghrelin, and leptin levels) variables were assessed.

Results: A total of 37 underweight subjects (mean age 10.5 ± 2.6 years) and 38 heathy subjects (mean age 10.3 ± 2.3 years) were recruited. Underweight children had significantly higher nesfatin-1 (2.76 ± 0.4 vs. 1.56 ± 0.7, $P < 0.001$) and lower leptin levels (2.21 ± 2.0 vs. 5.21 ± 2.4, $P < 0.001$) than those of the control subjects. Nesfatin-1 levels were significantly associated with only leptin levels, after adjusting for age and BMI ($r = -0.371$, $P = 0.001$).

Conclusion: The present study is the first to evaluate nesfatin-1 levels in relation with anthropometric and metabolic parameters in children with chronic malnutrition, who were subsequently found to have significantly higher nesfatin-1 levels. Our study underlines that nesfatin-1 may play a role in the development of malnutrition by inhibiting food intake in children.

Key words: Nesfatin-1, chronic malnutrition, childhood, underweight, appetite regulation

1. Introduction

Malnutrition is a common clinical problem caused by insufficient food intake of at least one or more nutritional elements. Although economic development has risen, malnutrition continues to be a health problem in undeveloped and developing countries (1). To date, numerous molecules have been identified that play a role in the regulation of energy intake and expenditure, but the precise physiology of appetite regulation is not completely understood (2). Anorexigenic (appetite suppressant) and orexigenic (appetite stimulant) hormones play an important role in terminating or initiating food intake by integrating the signals among peripheral nerves, brainstem, and hypothalamus (3).

Nesfatin is a protein that was firstly identified in 2006, and is produced by posttranslational processing of Nucleobindin2 (NUCB2) precursor protein. NUCB2 is cleaved by the pro-hormone convertase yielding nesfatin-1 (amino acids 1–82), nesfatin-2 (amino acids 85–163), and nesfatin-3 (amino acids 166–396) (4). Nesfatin-1, the only molecule described to have biologic activity among three nesfatin molecules, has been shown to be present in peripheral tissues and in the central nervous system (CNS) as well. Initial reports demonstrated that nesfatin-1 injection into cerebral spinal fluid caused a reduction in food intake in rats (5,6). Several studies have shown that nesfatin-1 plays a role in the inhibition of food intake by leading to hyperpolarization in arcuate nuclei, which is responsible for neuropeptide-Y (an orexigenic protein) secretion (7–9). Moreover, experimental studies demonstrated that increased nesfatin-1 reduces appetite by reducing gastric motility and consequently leads to malnutrition (10,11). To date, there have been two studies that investigated the relation between nesfatin-1 and childhood malnutrition and the results were conflicting (12,13).

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Leptin, the protein product of the *LEP* gene, consists of 167 amino acids and is involved in regulation of food intake, energy consumption, body weight, and glucose metabolism (14,15). Studies have shown that serum leptin concentrations are positively correlated with body fat (16,17). Leptin levels are increased with food intake and decreased by fasting and malnutrition (18–20). Ghrelin, consisting of 28 amino acids, is a peripherally active orexigenic hormone that is produced mainly in the stomach and increases food intake and weight gain via its hypothalamic effects (21). Previous studies have shown that serum ghrelin concentration is negatively correlated with body fat and leptin concentration (22,23). The relationship of serum nesfatin-1 levels with anthropometric and metabolic parameters in children with idiopathic chronic malnutrition has not been investigated to date. In this study, we aimed to evaluate serum levels of nesfatin-1 and its relationship with metabolic (including ghrelin, leptin) and anthropometric parameters in chronic malnourished children.

2. Materials and methods

2.1. Study design

Thirty-seven idiopathic chronic malnourished children with both weight and height below –2 SDS who presented to our department with poor appetite and 38 control subjects of similar age, sex, and pubertal stage distribution were consecutively enrolled. For calculation of weight and height SDS, data from the CDC were used (24). The patients with chronic malnutrition were divided into two groups according to ideal body weight. The patients who had chronic malnutrition with <90% of ideal body weight were classified as the acute on chronic malnutrition group (Group IA) and the remaining as the chronic malnutrition group (Group IB) (25,26).

At the outset of the study, all of the patients and control subjects underwent a thorough physical examination. The malnourished patients underwent a laboratory evaluation including thyroid function tests, complete blood count, renal and liver function test, serum electrolyte levels, and celiac antibodies profile measurement for a probable endocrine pathology. Those with chronic diseases (cardiovascular, gastrointestinal, nephrologic, and respiratory), history of acute infection with or without fever, history of drug use (antibiotics, steroids, and antipsychotics), an endocrine pathology (isolated growth hormone deficiency and hypothyroidism), or suspected syndromes were excluded from the study.

The study participants were recruited from Dokuz Eylül University Pediatric Clinic between September 2015 and February 2016. This study was initiated upon approval of the local ethics committee (approval date/protocol number; 10.06.2016/2286-GOA) of Dokuz Eylül University, Faculty of Medicine in light of the Helsinki Declaration. A written informed consent of the parent(s) of each subject was also obtained before the study.

2.2. Clinical measurements

Height was measured using a Harpenden stadiometer with a sensitivity of 0.1 cm and weight was measured using a SECA scale with a sensitivity of 0.1 kg. The weight of each subject was measured with all clothing removed except undergarments. BMI was calculated by dividing weight (kg) by height squared (m^2). Waist circumference (WC) and mid-upper arm circumferences (MAC) were measured using standard techniques. Triceps skin fold thickness (TSF) (millimeters) was measured with a Harpenden skin-fold caliper. The percentage of body fat (PBF) (%) was measured using bioelectric impedance analysis (Tanita BC-418, Tokyo, Japan).

Findings for pubertal development were evaluated according to Tanner staging (27). A testicular volume of ≥4 mL in males and breast development of stage 2 and over in females were considered to be findings of puberty.

2.3. Laboratory measurements

The venous blood samples were collected in plain tubes after 10–12 h of night fasting. The tubes were centrifuged at 1200 × *g* for 10 min and serum samples were removed from clots into Eppendorf tubes using plastic Pasteur pipettes. Serum samples for nesfatin-1, ghrelin, and leptin were stored at –80 °C until analysis.

Serum leptin (catalogue no: EK0437, Boster Biological Technology Co Ltd, Wuhan, China), nesfatin-1 (EK1138, Boster Biological Technology Co Ltd), and ghrelin (catalogue no: CSB-E13398h, CUSABIO Co Ltd, Wuhan, China) levels were measured by enzyme linked immunosorbent assay kit (ELISA) based on the principle of sandwich enzyme immunoassay. The microplate in the kit is precoated with antibody specific to analyte. Standard is reconstituted and prepared by serial dilution with sample diluent. Serum samples are diluted with sample diluent 1:10 for leptin and nesfatin-1 assays and 1:200 for ghrelin assays. Standards and samples are loaded into the appropriate microtiter plate wells and any analyte present is bound by immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific to analyte is added to the wells. After washing, avidin-conjugated peroxidase is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added and color develops in proportion to the amount of analyte. The color development is stopped and the intensity of the color is measured spectrophotometrically at a wavelength of 450 nm. A standard curve of known concentration of analyte is established and the concentration of analyte in the samples is calculated accordingly. The ELISA assays of leptin, nesfatin-1, and ghrelin had a sensitivity of <10

pg/mL, <10 pg/mL, and <0.156 pg/mL; a detection range of 62.5–4000 pg/mL, 31.2–2000 pg/mL, and 0.625–40 pg/mL; and intraassay CV of <10% and interassay CV of <15%, respectively.

2.4. Statistical analyses

Statistical analyses of the data were conducted with SPSS 16.0.1 (SPSS Inc., Chicago, IL, USA). The distribution of data was evaluated with the Kolmogorov–Smirnov test. For numerical comparisons, Student's t-test or Mann–Whitney U-tests were used according to normal distribution of the measured parameters. Categorical variables were compared using the chi-square test. A correlation analysis was performed using Spearman's correlation analyses. A partial correlation was also performed with serum nesfatin-1 as a dependent variable controlling for potential confounders such as age and BMI. In all statistical tests, P values <0.05 were considered significant.

3. Results

Overall, 37 idiopathic chronic malnourished subjects (mean age 10.5 ± 2.6 years) and 38 control subjects (mean age 10.3 ± 2.3 years) we recruited. Age, sex, and pubertal status were not significantly different between the two groups. There were significant differences between the idiopathic chronic malnourished and control subjects in terms of weight SDS, height SDS, BMI SDS, ideal body weight (IBW %), WC, MAC, TSF, fat mass, and PBF ($P < 0.05$) (Table 1). Idiopathic chronic malnourished subjects had significantly higher nesfatin-1 and lower leptin levels than the control subjects did ($P < 0.05$), while no differences was observed regarding the ghrelin levels ($P > 0.05$) (Table 1).

According to the ideal body weight, the idiopathic chronic malnourished subjects were divided into two groups. Of the 37 malnourished subjects, 18 (46.2%) were classified as acute on chronic malnutrition (Group IA) and 19 (53.8%) had chronic malnutrition (Group IB). Those two subgroups were similar in terms of leptin, nesfatin-1, and ghrelin levels ($P > 0.05$), while PBF and TSF were significantly lower in patients in Group IA than those of Group IB ($P < 0.05$) (Table 2).

Among all subjects (n = 74), nesfatin-1 levels showed a significant negative correlation with weight SDS, height SDS, BMI SDS, WC, MAC, TSF, fat mass, PBF, and leptin, but no correlation was found between nesfatin-1 and ghrelin ($P > 0.05$) (Table 3). In addition, ghrelin levels had no correlation with anthropometric and metabolic parameters ($P > 0.05$) (Table 3). After adjusting for age and BMI, nesfatin-1 levels were significantly associated with only leptin levels ($r = -0.371$, $P = 0.001$). The correlation between serum nesfatin-1 and leptin levels is shown in the Figure.

Table 1. The clinical and laboratory characteristics of underweight children and those of control subjects.

	Underweight subjects (n = 37)		Control subjects (n = 38)		P
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	
Age (years)	10.5 ± 2.6	10.3 (4.5)	10.3 ± 2.3	9.75 (3.4)	0.481[b]
Sex (M/F)	16/21		17/21		0.896[c]
Pubertal/prepubertal	12/25		16/22		0.387[c]
Weight SDS	−2.73 ± 1.1	−2.6 (0.98)	−0.01 ± 0.7	−0.01 ± 1.2	<0.001[b]
Height SDS	−2.57 ± 0.5	−2.50(0.5)	0.08 ± 0.9	0.04 ± 1.4	<0.001[b]
BMI (kg/m^2)	14.5 ± 1.1	14.3 (1.6)	17.3 ± 2.3	16.9 (2.9)	<0.001[b]
BMI SDS	−1.68 ± 0.8	−1.48 (1.0)	−0.05 ± 0.7	−0.02 (1.1)	<0.001[b]
IBW %	89.7 ± 6.0	90.0 (7.3)	100.1 ± 11.0	98.6 (16.0)	<0.001[a]
WC (cm)	20.3 ± 3.7	20.0 (3.6)	54.2 ± 8.8	54.2 (2.8)	<0.001[b]
MAC (cm)	18.4 ± 6.3	17.5 (6.0)	20.9 ± 3.8	21 (6.2)	<0.001[b]
TSF (mm)	8.9 ± 2.8	8.1 (3.3)	13.5 ± 5.1	12.6 (5.2)	<0.001[b]
Fat mass (kg)	3.66 ± 1.3	3.50 (1.8)	6.76 ± 2.8	5.90 (2.9)	<0.001[b]
PBF (%)	16.1 ± 3.7	17.1 (5.9)	19.5 ± 3.9	19.3 (5.0)	<0.001[a]
Ghrelin (ng/mL)	1.64 ± 0.7	1.69 (1.3)	1.60 ± 0.7	1.51 (1.13)	0.803[b]
Leptin (ng/mL)	2.21 ± 2.0	1.23 (2.4)	5.21 ± 2.4	5.6 (3.5)	<0.001[b]
Nesfatin-1 (ng/mL)	2.76 ± 0.4	2.71 (0.5)	1.56 ± 0.7	1.47 (1.3)	<0.001[b]

[a]Student's t-test, [b]Mann–Whitney U-test, [c]Chi-square test, BMI: body mass index, BMI-SDS: standard deviation score of body mass index, IBW: ideal body weight, WC: waist circumferences, MAC: mid arm circumference, TSF: triceps skin fold, PBF: percent body fat

Table 2. Comparison of clinical and laboratory characteristics in chronic malnutrition (Group IA) and those of acute on chronic malnutrition (Group IB) subjects.

	Group IA (n = 18)	Group IB (n = 19)	P*
Age (years)	12.0 (6.0)	10.0 (2.7)	0.169
Sex (M/F)	11/7	5/14	0.05[b]
Weight SDS	–3.23 (1.4)	–2.48 (0.4)	0.019
Height SDS	–2.37 (0.5)	–2.50 (0.7)	0.178
BMI (kg/m^2)	13.8 (2.1)	14.6 (1.3)	0.008
BMI SDS	–2.30 (1.5)	–1.15 (0.7)	<0.001
IBW %	86.8 (6.8)	94.1 (4.9)	<0.001
MAC (cm)	16.8 (2.5)	18 (2.9)	0.199
TSF (mm)	7.2 (1.4)	10.0 (3.3)	0.04
WC (cm)	54.8 (11.1)	54.2 (6.6)	0.988
Fat mass (kg)	3.6 (2.2)	3.5 (18.0)	0.599
PBF (%)	15.6 (6.8)	17.8 (3.0)	0.012
Ghrelin (ng/mL)	1.69 (1.6)	1.69 (1.2)	0.845
Leptin (ng/mL)	1.53 (2.2)	1.11 (2.6)	0.443
Nesfatin -1 (ng/mL)	2.65 (0.43)	2.76 (0.62)	0.159

*Mann–Whitney U-test, [b]Chi-square test, Data are given median (IQR)
BMI: body mass index, BMI-SDS: standard deviation score of body mass index, IBW: ideal body weight, WC: waist circumferences, MAC: mid arm circumference, TSF: triceps skin fold, PBF: percent body fat

Table 3. Correlation coefficients of nesfatin-1 and ghrelin levels with anthropometrics and laboratory parameters.

Parameters	Nesfatin-1		Ghrelin	
	r	*P	r	*P
Age (years)	0.051	0.662	–0.003	0.976
Weight SDS	–0.647	<0.001	–0.109	0.350
Height SDS	–0.667	<0.001	–0.073	0.531
BMI SDS	–0.583	<0.001	–0.020	0.866
IBW %	–0.456	<0.001	0.119	0.310
WC (cm)	–0.417	<0.001	–0.088	0.452
MAC (cm)	–0.456	<0.001	0.142	0.223
TSF (mm)	–0.359	0.002	–0.006	0.960
Fat mass (kg)	–0.527	<0.001	0.028	0.813
PBF (%)	–0.246	<0.001	–0.054	0.644
Leptin (ng/mL)	–0.456	<0.001	0.074	0.531
Ghrelin (ng/mL)	–0.109	0.353	-	-

*Spearman's correlation analysis; Serum nesfatin-1 and ghrelin levels as dependent variable
BMI: body mass index, BMI-SDS: standard deviation score of body mass index, IBW: ideal body weight, WC: waist circumferences, MAC: mid arm circumference, TSF: triceps skin fold, PBF: percent body fat

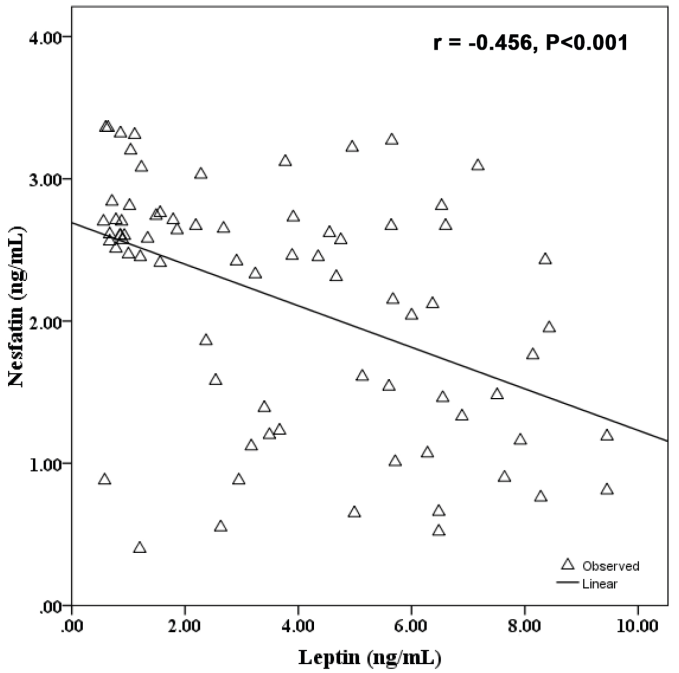

Figure. The negative correlation between serum nesfatin-1 and leptin levels in all participants.

4. Discussion

Nesfatin-1, a recently described anorexigenic and satiety peptide, is secreted by different parts of the central nervous system (predominantly in the hypophysis and hypothalamus) and peripheral tissue such as the gastric mucosa, adipose tissue, and pancreas beta cells (4,8,28–30). Experimental studies have revealed that cerebroventricular and peripheral nesfatin-1 injection in rats inhibited food intake (9,31). On the other hand, in light of the results of previous studies, it was shown that the injection of antiserum to nesfatin-1 leads to increased food intake (7). Kahraman et al. (13) found lower serum nesfatin-1 levels in acute malnourished children with <90% of ideal body weight. In another study conducted by Kaba et al., (12) significantly higher serum nesfatin-1 levels were demonstrated in children with acute malnutrition, a finding similar to our results. In these two studies, conflicting results were reported regarding the relation between BMI and nesfatin. Kaba et al. (12) found no significant association between BMI SDS and nesfatin-1 (neither in underweight children nor in control groups), while Kahraman et al. (13) demonstrated a positive correlation between BMI SDS and nesfatin-1. Similarly, conflicting results regarding the nesfatin-1 level were observed in studies performed in obese children (32–34). Abaci et al. (32) previously reported lower serum nesfatin-1 levels in obese subjects compared to those of healthy controls and a negative correlation between nesfatin-1 and BMI in nonobese males. In addition, it has been speculated that low levels of this satiety peptide may be one of the reasons for inadequately controlled food intake. Moreover, it has recently been described that nesfatin-1 leads to a feeling of satiety and delayed gastric emptying due to the effect on inhibiting gastric activity (10,11). In the present study, higher serum nesfatin-1 levels were detected in children with chronic malnutrition when compared to those of healthy subjects and a negative correlation was found between nesfatin-1 levels and BMI. The findings suggested that high nesfatin-1 may be one of the reasons for chronic malnutrition in otherwise healthy patients by causing poor appetite due to leading to the feel of satiety. Nesfatin synthesis and serum nesfatin-1 levels may differ based on nesfatin gene polymorphism (35,36). According to this finding, we thought that this circumstance may also be associated with polymorphisms causing increased nesfatin synthesis.

Ghrelin is an orexigenic hormone that regulates food intake and energy homeostasis. Circulating ghrelin concentration increases during fasting and decreases immediately after food intake (37,38). Higher serum ghrelin concentration in anorexia nervosa and lower serum ghrelin concentration in obese subjects compared to healthy controls were reported in several studies (39–41). These findings suggested that circulating ghrelin concentration increases in malnutrition. In contrast to reported studies to date, we demonstrated that fasting

serum ghrelin levels did not differ between chronic malnutrition and healthy controls. Additionally, in the present study, serum ghrelin concentrations were similar even among subgroups (acute on chronic malnutrition and chronic malnutrition). In the present study, serum ghrelin levels were not correlated with nesfatin-1 level, leptin level, and anthropometric parameters such as BMI, MAC, TSF, and WC. These findings suggested that ghrelin is not a valuable predictor in chronic malnutrition. Although high ghrelin concentrations did not lead to an increase in feeding behavior in patients with anorexia nervosa and, on the other hand, obese subjects even with low levels of ghrelin continue to feel hunger, which leads to increased food intake and weight gain, a variety of peptides are thought to be involved in appetite regulation.

Leptin, a newly discovered obese (*LEP*) gene product, is an anorexigenic peptide mainly synthesized by adipose tissue (18). Leptin concentration changes in nutritional status such as being decreased by fasting and subsequently increased by food intake (18,42). Most studies revealed significant lower leptin levels and a negative correlation with BMI in patients with acute and chronic malnutrition (20,42,43). Buyukgebiz et al. (44) investigated serum leptin levels in malnourished children and showed significantly lower leptin levels in patients with malnutrition. In addition, positive correlations between leptin levels and anthropometric parameters such as weight, IBW (%), and BMI were found. Consistent with the literature, we observed significantly lower leptin levels and a positive correlation between leptin and BMI, emphasizing that serum leptin concentration may be an important marker for evaluating nutritional status. To the best of our knowledge, the present study was the first to investigate the relationship between nesfatin-1 and leptin levels. We found a negative correlation and speculate that an increase in serum nesfatin-1 levels results in suppression of food intake and weight loss. A subsequent decline in body fat leads to a decrease in leptin release into the circulatory system. However, additional studies are necessary to endorse this hypothesis.

To date, the mechanism of food intake inhibition via nesfatin-1 has not been clarified. Possible mechanisms have been claimed to explain that nesfatin-1 can inhibit food intake via melanin system activation, independent of the leptin pathway or by suppressing neuropeptide Y, a potent anorexigenic peptide (7,9,30). Unexpectedly, we found a negative correlation between two anorexigenic peptides such as nesfatin-1 and leptin levels. While low levels of leptin secreted by adipocyte tissue were elucidated by a low percentage of BMI and PBF, we could not explain the mechanism of how and why nesfatin-1 is secreted in higher concentration in patients with malnutrition than in healthy subjects. Nesfatin-1 genetic polymorphism may play a potential role in variation of nesfatin-1 concentration in individuals (35,36). Our study suggested that a high level of an anorexigenic peptide such as nesfatin-1 was involved in the etiology of otherwise healthy patients with chronic malnutrition.

In conclusion, this is the first study to evaluate the relationship of nesfatin-1 levels with anthropometric and metabolic parameters in underweight children. We observed higher serum nesfatin-1 and lower leptin levels in children with chronic malnutrition. Serum nesfatin-1 levels were correlated negatively with serum leptin levels and BMI SDS. Our results underline that undefined higher serum nesfatin-1 levels may play a role in inhibiting food intake and subsequent development of malnutrition. Further studies including genetic analyses assessing individual variation of nesfatin-1 gene expression would be beneficial.

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