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Short communication

Plasma nesfatin-1 concentrations in restricting-type anorexia nervosa

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ABSTRACT

Restricting-type anorexia nervosa (AN-R) is characterized by chronic food restriction and severe emaciation due to various cognitive biases such as a distorted self-image. In spite of several treatments, AN-R continues to be a refractory disease because of its unknown pathogenesis. Although previous studies have shown that changes in feeding regulatory peptides such as ghrelin are involved in anorexia, few reports have described the relationship between AN-R and nesfatin-1, a recently identified satiety peptide. Therefore, we examined the plasma nesfatin-1 levels in AN-R patients to determine its role in AN-R. A total of 15 women participated in the study; 7 patients with AN-R and 8 age-matched healthy controls (average BMI, 13.02 ± 0.30 vs. 21.57 ± 0.48, respectively). Our results showed that plasma nesfatin-1 levels were significantly lower in AN-R group than in control group (6.23 ± 0.70 ng/ml vs. 8.91 ± 0.85 ng/ml, respectively, $P < 0.05$). Plasma acyl ghrelin and des-acyl ghrelin levels were significantly higher in AN-R group than in control group (acyl ghrelin: 62.4 ± 10.15 fmol/ml vs. 27.20 ± 5.60 fmol/ml, $P < 0.01$ and des-acyl ghrelin: 300.17 ± 55.95 fmol/ml vs. 107.34 ± 40.63 fmol/ml, $P < 0.05$). Although AN-R is associated with emaciation for a prolonged period, our result suggested that nesfatin-1 levels may be regulated by nutrition status and response to starvation.

1. Introduction

Anorexia nervosa (AN) is a serious disorder affecting adolescents and young adults, and it decreases the quality of life of affected individuals for prolonged periods [21]. Restricting-type AN (AN-R) is characterized by severe emaciation with chronic food restriction secondary to an inordinately strong desire to become thin and the fear of obesity [7]. The mechanisms underlying persistent anorexia are largely unknown, but some studies have suggested that the decrease in food intake in AN-R is because of changes in feeding regulatory peptides such as ghrelin, which is classified into acyl ghrelin and des-acyl ghrelin on the basis of their different roles in food intake [3,4]. In fact, AN-R patients have shown increased circulating total ghrelin levels [11,25], especially the plasma des-acyl ghrelin levels [9]. Des-acyl ghrelin has been shown to counteract the orexigenic effect of acyl ghrelin [10,14]. Recently, Qader et al. reported that the effects of acyl ghrelin on the secretion of insulin, glucagon, pancreatic polypeptide, and somatostatin are reduced by des-acyl ghrelin [19]. These findings indicate that des-acyl ghrelin is associated with anorexia in AN-R [13].

Nesfatin-1, a novel 82-amino acid peptide, has recently been identified as a satiety peptide derived from nucleobindin-2 (NUCB2) [18]. Nesfatin-1 is widely distributed in the central nervous system (CNS) [5] and in peripheral tissues such as adipose tissue [18] and stomach [23]. In the CNS, nesfatin-1 is distributed in the hypothalamic paraventricular nucleus, supraoptic nucleus, arcuate nucleus, lateral hypothalamic area, nucleus of the solitary tract (NTS), and dorsomedial hypothalamic nucleus [2,6,12,18], all of which play an important role in the regulation of feeding behavior and metabolism. Nesfatin-1 is thought to be involved in the physiological regulation of feeding behavior and body weight by suppressing food intake and peristalsis [18]. Peripheral administration of nesfatin-1 caused anorexia in mice, possibly due to its action on pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript neurons in the NTS via a leptin-independent mechanism [20], which indicated that similar to des-acyl ghrelin, nesfatin-1 may also be associated with persistent anorexia in AN-R. In addition, Tsuchiya et al reported that fasting nesfatin-1 concentrations negatively correlated with body mass index (BMI) in non-obese men, and these concentrations were significantly lower in high BMI group [26]. However, few reports have described the relationships between nesfatin-1 and AN-R. Therefore, in this study, we measured the plasma nesfatin-1 levels in AN-R patients to determine its role in AN-R.

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Table 1
Clinical characteristics of restricting-type anorexia nervosa patients and healthy controls.

	Control ($n=8$)	AN-R ($n=7$)	P value
Age (years)	19.00 ± 1.18	19.29 ± 1.58	NS
Height (cm)	159.08 ± 1.51	156.16 ± 1.34	NS
Weight (kg)	54.71 ± 1.97	31.77 ± 0.89	<0.001
BMI (kg/m^2)	21.57 ± 0.48	13.02 ± 0.30	<0.001

Data are expressed as means ± standard error (SE). Statistical differences between groups were analyzed by Student's t-test. AN-R, restricting-type anorexia nervosa; BMI, body mass index; NS, not significant.

2. Materials and methods

A total of 15 women participated in the study; 7 patients with AN-R and 8 age-matched healthy controls (Table 1). All AN-R patients were diagnosed with amenorrhea according to Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV) criteria [1]. Control subjects, who were healthy volunteers recruited by placing an advertisement in the local newspaper, had no history of psychiatric illness or metabolic disease, were unrestrained eaters, and were within ±10% of their ideal body weight. The mean duration of AN-R was 23.43 ± 8.98 months before sampling; this information was collected by conducting interviews with the patients and their families. In accordance with the principles of the Declaration of Helsinki, all subjects gave informed written consent prior to participation. This study was approved by the Institutional Committee of Kagoshima University.

After overnight fasting, blood samples were obtained from each subject and were placed in chilled tubes containing ethylenedinitrilo tetraacetic acid (EDTA-2Na) (1 mg/mL) and aprotinin (500 U/mL). For the measurement of acyl ghrelin levels, blood samples were centrifuged and plasma was separated; aliquots of plasma were stored in tubes containing 1 N hydrogen chloride (HCl) at −80 °C until assay. The levels of nesfatin-1, acyl ghrelin, and des-acyl ghrelin in the samples were measured. Nesfatin-1 was determined by enzyme immunoassay (Phoenix Pharmaceuticals Inc., California, USA). Plasma acyl ghrelin and des-acyl ghrelin levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Mitsubishi Kagaku Iatron, Tokyo, Japan).

Data were presented as means ± standard error (SE). Statistical analyzes were performed using SPSS software (version 17.0). Comparisons between individual data points were made using Student's t-test. The relationships among different parameters were examined by Pearson's test. Differences were considered statistically when P value was less than 0.05.

3. Results

Clinical characteristics of the patients and healthy controls in the two groups are presented in Table 1. There were no significant differences in age and height among the groups. Patients in the AN-R group had significantly lower body weight and lower BMI than those in the control group (body weight, $P<0.001$; BMI, $P<0.001$, respectively).

Fasting plasma concentrations of nesfatin-1 are shown in Fig. 1A. Plasma nesfatin-1 levels in the AN-R group (6.23 ± 0.70 ng/ml) were significantly lower than those in the control group (8.91 ± 0.85 ng/ml, $P<0.05$). Fasting plasma concentrations of acyl ghrelin and des-acyl ghrelin are shown in Fig. 1B and C. Plasma acyl ghrelin and des-acyl ghrelin levels were significantly higher in the AN-R group than in the control group (acyl ghrelin: 62.4 ± 10.15 fmol/ml vs. 27.20 ± 5.60 fmol/ml, $P<0.01$ and des-acyl ghrelin: 300.17 ± 55.95 fmol/ml vs. 107.34 ± 40.63 fmol/ml, $P<0.05$). The ratio of acyl ghrelin to des-acyl ghrelin showed a tendency to decrease; however, this decrease was not statistically significant (0.22 ± 0.03 vs. 0.31 ± 0.03, respectively, $P<0.06$).

We also examined the correlation of plasma nesfatin-1 levels to BMI, plasma acyl ghrelin level, and des-acyl ghrelin level for the entire study population (i.e., the control and the AN-R groups combined). Plasma nesfatin-1 levels positively correlated with BMI ($r=0.520$, $P<0.05$) (Fig. 2A) but negatively correlated with plasma acyl ghrelin and des-acyl ghrelin levels (acyl ghrelin: $r=-0.534$, $P<0.05$ and des-acyl ghrelin: $r=-0.514$, $P<0.05$) (Fig. 2B and C). No significant correlation was observed between plasma nesfatin-1 levels and age ($r=-0.232$, $P=0.406$). In the AN-R group, plasma nesfatin-1 levels were not significantly correlated with the age at disease onset and the disease duration (age: $r=-0.323$, $P=0.480$ and disease duration: $r=-0.591$, $P=0.162$).

4. Discussion

AN-R is characterized by chronic food restriction and severe emaciation due to various cognitive biases such as a distorted self-image. In spite of several treatments such as drug therapy, behavioral therapy, cognitive behavioral therapy, and family therapy, AN-R continues to be a refractory disease because of its unknown pathogenesis. Recently, many studies have reported the correlation between mechanisms regulating food intake and energy balance and thus feeding regulatory peptides is expected to be used as a novel treatment for AN-R. Acyl ghrelin, which is an orexigenic peptide, increases hunger sensation and daily energy intake and decreases gastrointestinal symptoms in AN-R [8]. On the other hand, des-acyl ghrelin reportedly counteracts the orexigenic effect of acyl ghrelin [10,14]. Similarly, an anorexic peptide nesfatin-1, which was thought to regulate food intake and energy balance, may be involved in the persistent feeding restriction in AN-R.

In our study, the plasma nesfatin-1 levels were significantly lower in the AN-R group than in the control group, and they positively correlated with BMI. We found that AN-R patients with a low BMI also had low plasma nesfatin-1 levels. This result is in disagreement with a previous study that reported negative correlation between nesfatin-1 levels and BMI [26], but is in agreement with another study reporting reduced fasting plasma nesfatin-1 level in rats [22], indicating the regulation of nesfatin-1 levels by the nutritional status and response to starvation. Although nesfatin-1 may be involved in the regulation of anxiety- and/or fear-related responses in rats [15], low nesfatin-1 levels may not be primarily involved in the anxiety effect observed in AN-R patients. However, in severe emaciation, even low nesfatin-1 levels may stimulate feeding and decrease anxiety.

Previous studies have shown that plasma total ghrelin levels [11,16,17,24,25] and plasma des-acyl ghrelin levels are higher in AN-R patients than in healthy controls, whereas plasma acyl ghrelin levels tend to be slightly higher in the former than in the latter [9]. In our study, not only the plasma des-acyl ghrelin levels but also the plasma acyl ghrelin levels were higher in the AN-R group than in the control group. Further, although previous studies have shown no significant correlation between nesfatin-1 and acyl ghrelin levels [26], we found a negative correlation between plasma nesfatin-1 levels and plasma acyl ghrelin and plasma des-acyl ghrelin levels. AN-R patients with severe starvation may show alterations in plasma nesfatin-1, acyl ghrelin, and des-acyl ghrelin levels, but these changes may not be noticeable in subjects with normal BMI who were enrolled in previous studies [26].

In conclusion, our study indicates that plasma nesfatin-1 levels in AN-R group were lower than those in the control group, which suggested that nesfatin-1 levels were regulated by nutrition status and response to starvation. Further accumulation of data from

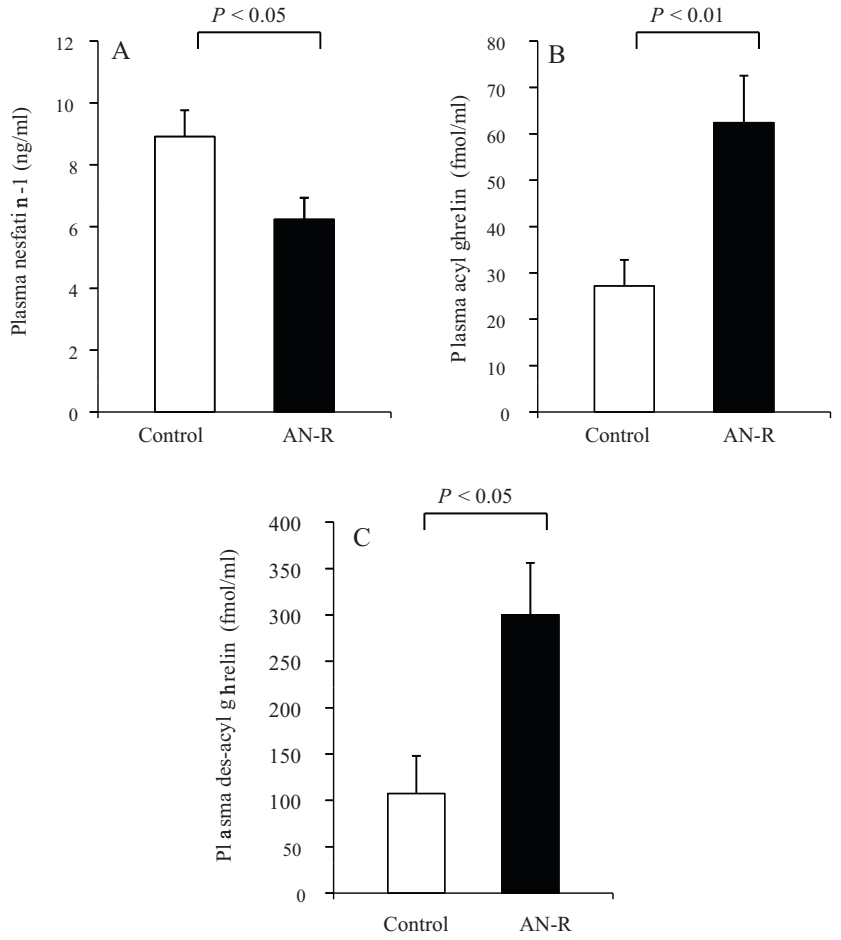

Fig. 1. (A) Fasting plasma nesfatin-1 concentrations (means ± SE) in healthy controls ($n = 8$) and in patients with restricting-type anorexia nervosa (AN-R; $n = 7$). (B) Fasting plasma acyl ghrelin concentrations (means ± SE) in healthy controls ($n = 8$) and in patients with restricting-type anorexia nervosa (AN-R; $n = 7$). (C) Fasting plasma des-acyl ghrelin concentrations (means ± SE) in healthy controls ($n = 8$) and in patients with restricting-type anorexia nervosa (AN-R; $n = 7$). Statistical differences between groups were analyzed by Student's *t*-test.

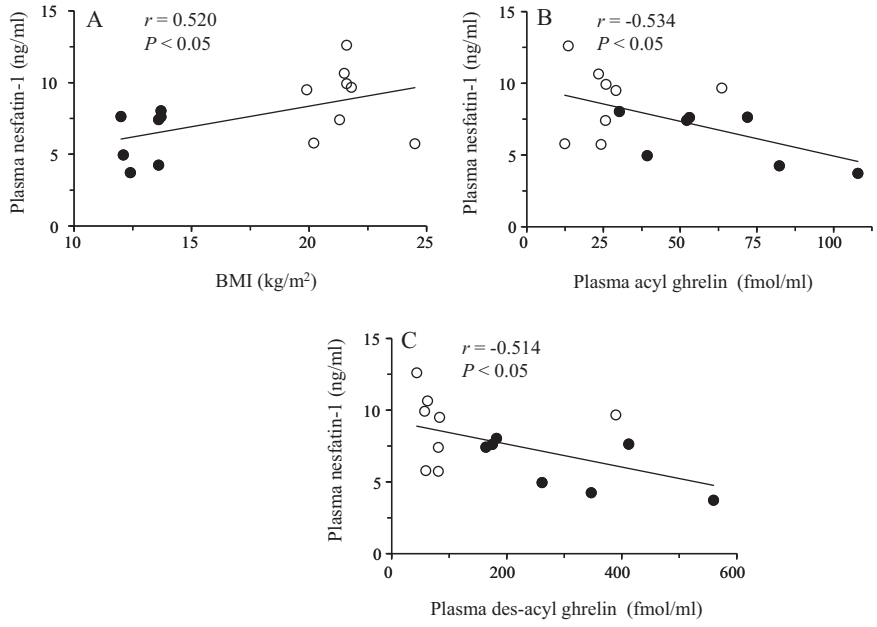

Fig. 2. Relationships between plasma nesfatin-1 concentrations and body mass index (BMI) (A) and plasma acyl ghrelin concentrations (B) and plasma des-acyl ghrelin concentrations (C) in healthy controls (○, $n = 8$) and in patients with restricting-type anorexia nervosa (●, AN-R, $n = 7$). Correlation analyzes was also performed for the entire population ($n = 15$).

clinical observations of subjects with other types of eating disorder, including binge eating-/purging-type AN and bulimia nervosa is required to clarify the role of nesfatin-1 in feeding behavior.

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