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• *RAPID COMMUNICATION* •

Plasma leptin and ghrelin concentrations in patients with Crohn's disease

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Abstract

AIM: To determine the concentrations of leptin and ghrelin, which have opposite effects on appetite, energy expenditure, and weight control, in the plasma of patients with Crohn's disease (CD), which is often associated with weight loss and malnutrition.

METHODS: Plasma leptin and ghrelin concentrations were determined in 28 outpatients with CD by radioimmunoassay. Age- and sex-matched controls with and without *Helicobacter pylori* (*H pylori*) infection (28 for each) were enrolled in the study. Circulating levels of these hormones were assessed with respect to CD activity, disease localization and medical treatment.

RESULTS: There were no significant differences in ghrelin levels between CD patients and *H pylori*-negative controls. However, circulating ghrelin levels were significantly lower in *H pylori*-infected subjects than in CD patients and uninfected controls. Plasma leptin levels were comparable among the groups. Localization and medication profile had no significant impact on circulating ghrelin and leptin levels.

CONCLUSION: Apart from *H pylori* infection, CD itself has no significant influence on circulating ghrelin and leptin levels in the outpatients who were mostly in inactive state.

Key words: Crohn's disease; Ghrelin; Leptin; *Helicobacter pylori*

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INTRODUCTION

Crohn's disease (CD) is characterized by a relapsing inflammatory process throughout the digestive tract[1,2]. CD is frequently accompanied with malnutrition and weight loss[1,2]. Possible explanations for these complications include malabsorption of nutrients, intestinal losses during inflammatory process, and anorexia[3]. However, the exact etiology is not completely understood.

Leptin is mainly synthesized by the adipose tissue and plays a crucial role in the homeostasis of the body weight by reducing appetite and increasing energy expenditure[4,5]. Contrary to the initial reports, leptin production is not restricted to adipocytes. It is also detected in the human placenta, muscles, and gastric chief cells[6-10].

Ghrelin is a novel endogenous ligand for growth hormone secretagogue receptor[11,12]. It was originally isolated from the stomach and has been subsequently identified in various tissues including the small and large intestine[11,12]. In addition to its potent growth hormone-releasing activity, ghrelin displays metabolic effects opposed to those of leptin[11-13]. It stimulates food intake, enhances the use of carbohydrates and reduces fat utilization. In fact, circulating ghrelin levels are decreased in obesity and increased in anorexia nervosa or cachexia[11-14].

At present, no data on the interplay of ghrelin and leptin in CD are available. This is the first report to assess the circulating ghrelin and leptin concentrations in patients with CD simultaneously.

MATERIALS AND METHODS

Patients

Twenty-eight consecutive outpatients with CD were enrolled in the study between October 2002 and

Table 1 Plasma leptin and ghrelin levels in patients with CD and controls with or without *H pylori* infection (mean±SD)

	CD	Controls	
		H pylori-infected	*H pylori*-uninfected
Body mass index (kg/m^2)	20.5±0.4 (16.2-29.9)[1]	21.3±0.8 (16.4-28.5)	20.8±0.5 (16.4-26.7)
Plasma ghrelin levels (fmol/mL)	195.7±1.5 (45.7-368.7)	143.4±7.1 (53.2-481.0)	182.4±1.4 (75.7-322.2)
Plasma leptin levels (ng/mL)	3.8±0.4 (0.0-15.0)	4.3±0.6 (0.8-8.4)	3.8±0.6 (0.0-10.8)

[1]Mean±SD (range).

Table 2 Plasma leptin and ghrelin levels in terms of various parameters (mean±SD)

	Ghrelin (fmol/mL)	Leptin (ng/mL)	Body mass index
Disease activity			
Active (n = 5)	220.6±98.8	4.7±4.1	19.9±4.5
Inactive (n = 23)	202.3±86.4	3.6±2.4	20.6±2.7
Disease location			
Small intestine (n = 3)	186.3±68.6	2.8±2.3	18.5±1.6
Large intestine (n = 10)	246.8±83.3	4.2±3.3	20.9±2.4
Small and large intestine (n = 15)	186.8±92.4	4.1±3.1	20.5±4.0
Anal lesion			
Present (n = 7)	213.4±85.1	3.4±2.5	20.1±4.8
Absent (n = 21)	206.5±92.4	4.1±3.2	20.5±2.6
Medical treatment			
5-Aminosalicylates (n = 16)	185.5±86.5	2.8±1.5	20.1±2.4
5-Aminosalicylates and elementary diet (n = 7)	229.5±98.9	4.8±3.6	20.1±5.2
No medications (n = 5)	243.4±76.8	5.7±4.8	21.8±3.1
Helicobacter pylori status			
Positive (n = 5)	265.8±109.8	3.5±2.6	20.2±3.4
Negative (n = 23)	195.7±81.5	5.5±3.9	21.8±0.1

December 2004. The study was approved by Nagasaki University Human Ethics Committee. All samples were obtained with written informed consent of the patients prior to their inclusion, in accordance with the Helsinki Declaration. A diagnosis of CD is based on the generally accepted clinical, radiographic, endoscopic and histologic criteria[1,2]. The exclusion criteria were age <18 or >80 years, pregnancy, body mass index (BMI) >30 kg/m^2, diabetes mellitus, systemic infection, thyroid and liver diseases, renal impairment, use of medications against *H pylori* during the preceding 3 mo, alcohol abuse, drug addiction, and long-term corticosteroid or nonsteroidal anti-inflammatory drug use. None underwent gastrointestinal surgery. They included 16 men and 12 women, aged between 18 and 56 years (mean, 32 years). According to the CD activity index (>150), 5 patients suffered from active CD. As for disease localization, the disease process was limited to the small bowel in 3 patients, to the colon in 10 and affected both regions in 15. Seven had anal lesions and none had upper gastrointestinal involvement. Of those, 16 were treated with 5-aminosalicylates, 7 with 5-aminosalicylates and elementary diet, and 5 received no medications.

Since ghrelin is primarily produced by X/A-like cells in the gastric fundus[11,12], its production and release may be affected by inflammatory and atrophic events associated with *H pylori* infection[15,16]. Thus, *H pylori* status was assessed, as described below.

Age- and sex-matched 23 *H pylori*-negative healthy subjects served as controls. Moreover, age- and sex-matched 23 *H pylori*-infected patients with non-ulcer dyspepsia were enrolled in the study.

Plasma leptin and ghrelin concentrations

Blood samples were taken between 9 and 11 a.m. after an overnight fast, transferred into chilled tubes containing eth ylenediaminetetraacetic acid-2Na and aprotinin, stored on ice during collection and centrifuged. Then, the plasma was separated, and stored at -80 ℃ until assay. Plasma ghrelin concentrations were measured in-house in duplicate by radioimmunoassay (RIA), as described previously[17]. This assay system employs a rabbit polyclonal antibody against the C-terminal fragment of human ghrelin. Plasma leptin concentrations were measured in duplicate by commercial RIA kit (Linco Research Co., St. Charles, USA), based on the protocol provided by the manufacturer.

Detection of H pylori infection

H pylori status was assessed by anti-*H pylori* immunoglobulin G antibody (HELp TEST, an enzyme linked immunosorbent assay kit, AMRAD Co., Melbourne, Australia) using the stored plasma and ^{13}C-urea breath test (UBiT, Otsuka Pharmaceutical Co., Tokyo, Japan).

Statistical analysis

Statistical analyses were performed using Fisher's exact, χ^2, Student's t, Mann-Whitney U, Kruskal-Wallis, Spearman's rank, and Wilcoxon signed ranks tests, as appropriate. P<0.05 was considered statistically significant. Data were expressed as mean±SD.

RESULTS

There were no significant differences in ghrelin levels between CD patients and *H pylori*-negative controls (Table 1). However, circulating ghrelin levels were significantly lower in *H pylori*-infected subjects than in CD patients ($P<0.01$, Table 1) and controls negative for the infection ($P<0.05$, Table 1). On the other hand, circulating leptin levels were comparable between the groups (Table 1).

Other parameters such as disease activity, localization and medication profile and *H pylori* status had no significant impact on circulating ghrelin and leptin levels (Table 2).

There was a significant positive correlation between plasma leptin levels and BMI ($r = 0.61$, $P<0.005$). Plasma ghrelin concentrations tended to decrease with increase in BMI, *albeit* insignificantly. There was no significant correlation between circulating ghrelin and leptin levels.

DISCUSSION

Our results suggest that circulating ghrelin levels are not altered in CD patients who mainly consisted of those with the inactive disease. In our study, plasma concentrations of leptin, the opposing metabolic counterpart of ghrelin[10-13], were not affected by the disease. In addition, there were no significant association of such factors as localization and medication profile with circulating ghrelin and leptin. These findings suggest that alterations in these hormones involved in appetite and energy metabolism are unlikely to mediate nutrition state in CD. However, these results must be interpreted within the context of the limitations in our study. First, the sample size was relatively small. Second, severe patients with wasting symptoms or malnutrition were not enrolled in this study, as it was in the outpatient-based setting. Although our series were not associated with upper gastrointestinal lesions, where ghrelin is primarily produced[10,17], such involvement might have an impact on the circulating ghrelin levels.

Murch *et al*[18] demonstrated that suppression of growth velocity in children with CD correlates with circulating tumor necrosis factor (TNF) alpha concentrations. In cachectic state, a positive correlation has been found between ghrelin and TNF alpha circulating levels[19]. On the other hand, there is a significant association between serum levels of leptin and TNF receptor 1[20]. We did not measure TNF alpha and TNF receptor 1 levels in the present series of CD, but accumulating evidence indicates that the TNF system is activated in CD[1,2].

Recently, Suzuki *et al*[15] demonstrated that *H pylori* infection modifies gastric and plasma ghrelin dynamics in Mongolian gerbils. There are contradictory reports on the relationship between *H pylori* and ghrelin. A Turkish study reported that *H pylori* has no effect on plasma ghrelin levels[21], whereas a British study demonstrated that circulating ghrelin increases following the cure of *H pylori* infection[16]. In our series, the principal determinant of circulating ghrelin might be the *H pylori* status. The exact reason for such a discrepancy is not clear, but the following factors should be considered: differences in the study populations of diverse races, nutrient status and dietary habits, small sample size and inadequate assessment of *H pylori* status, i.e., only by histology, leading to underestimation of infection in their series[21]. In turn, circulating leptin concentrations are not associated with *H pylori* status, consistent with previous reports[8,22]. On the other hand, plasma leptin concentrations significantly correlate with BMI, as the primary contributor of circulating leptin is exclusively the adipose tissue[4,5].

In conclusion, CD itself has no significant influence on the circulating levels of leptin and ghrelin. Further evaluation of a larger population with the active disease or response to medical treatment including parenteral and enteric nutrition and infliximab is warranted. Plasma ghrelin dynamics may be affected by *H pylori* status in human beings.

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