의학석사 학위논문

기능성 소화불량증 환자에서의 Ghrelin 과 Leptin 의 발현

Ghrelin and Leptin in Patients with Functional Dyspepsia

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최윤진

Abstract

Ghrelin and Leptin in Patients with Functional Dyspepsia

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Background and aims: Ghrelin and leptin have been indicated one of the etiological factors in functional dyspepsia (FD). In spite of several studies, the association between plasma ghrelin level and FD still remains controversial. Furthermore, only a few studies about leptin in FD have been introduced. We compared the major two forms of plasma ghrelin, serum leptin and gastric mRNA expression of both hormones between FD and control patients to understand underlying pathophysiology of FD.

Methods: In total, two hundred and thirty two subjects (FD, n = 129 and healthy control, n = 103), who had undertaken endoscopy in Seoul National University Bundang Hospital from January 2011 to March 2013

were enrolled. FD patients were classified into two groups according to Rome III classification; postprandial distress syndrome (PDS, n = 88) and epigastric pain syndrome (EPS, n = 41). Self-reported questionnaire regarding FD, GERD and IBS symptoms was used. Acyl, des-acyl ghrelin and leptin levels in the fasting blood samples before endoscopy and mRNA expression of preproghrelin and leptin in the body mucosal tissue were measured by ELISA method and real time PCR, respectively.

Results: Plasma acyl ghrelin was lower in PDS than in control and EPS (control: 13.0, PDS: 8.6, EPS: 13.9 fmol/ml, $P < 0.001$), while plasma des-acyl ghrelin, leptin and gastric mRNA expression of preproghrelin did not show any significant difference. Gastric leptin mRNA was significantly higher in patients with postprandial fullness (without fullness: 1.28, fullness: 1.80 $P = 0.040$). mRNA expression of preproghrelin of *H. pylori* (HP) positive patients (HP-positive: 0.70, HP-negative: 3.05, $P < 0.001$) was lower than the others but this does not lead to decrease in circulating ghrelin level. Women with HP infection had relatively higher expression of leptin mRNA than the others (HP-negative: 0.62, HP-positive: 1.45, $P = 0.044$).

Conclusions: Plasma acyl ghrelin can play a certain role for development of PDS. However, it might be determined by not reducing mRNA expression but using posttranslational mechanism. On the other hand, elevated gastric leptin mRNA may cause gastric dysmotility and postprandial fullness. While HP infection with active or atrophic gastritis

may reduce expression of preproghrelin mRNA, it can cause increase in expression of leptin mRNA by active gastritis.

Keywords: Functional dyspepsia, Ghrelin, Acyl ghrelin, Des-acyl ghrelin, Leptin

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Introduction

Functional dyspepsia (FD), one of the most common gastrointestinal disorders is diagnosed by symptom-based diagnostic criteria in absence of structural, infectious, or metabolic causes. Under the Rome III classification, FD has been subclassified into two different disease categories; postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS) (1). Even though FD does not lead to mortality, due to considerably high prevalence it has imposed huge amount of social costs. Many researchers have tried to find the pathophysiology of FD but it is still unclear. It has been thought that multiple factors are involved in this disease and there are several possible etiological mechanisms such as altered brain-gut interaction, abnormal motor function, visceral hypersensitivity, *H. pylori* infection, genetic factor and psychosocial factors. Several peptides are thought to be related with gastrointestinal (GI) motility through brain-gut interaction or local regulatory pathway, and especially ghrelin, leptin which are closely associated with food intake and satiety have been actively evaluated.

Ghrelin, orexigenic hormone which consists of 28 amino acids is produced mainly in X/A-like cells lining gastric body mucosa. It is modified to acylated form by ghrelin O-acyltransfrase (GOAT) but this easily loses the octanoylation of the third amino acid and becomes des-acyl ghrelin (2). Although des-acyl ghrelin accounts for major proportion of total ghrelin, only acyl ghrelin can react with growth hormone

secretagogue receptor (GHSR) and transduce signal to hypothalamus via vagus nerve. It has been presented that decreased acyl ghrelin correlated with impairment of gastric emptying (3), leading to postprandial fullness or vomiting (4, 5). Recently published data has focused on not only acyl-ghrelin but des-acyl ghrelin and suggested the latter also promotes gastric motility (6). However, in spite of numerous studies aiming to find out the relation between ghrelin and FD, the results were still controversial. Furthermore, most of them dealt with only certain form of ghrelin.

On the other hand, leptin is considered as a central peptide which regulates energy expenditure and food intake. Although it is produced mainly by adipose tissue, both leptin and leptin receptors were detected in rat and human gastric mucosa (7). As one of pluripotent roles of leptin, the potential paracrine or autocrine effect for adjusting gastric motility has been presumed. It was also reported that leptin could affect gastric motility through vagus nerve or have a direct influence on satiety center leading to early satiation in PDS patients (8, 9). In line with this, there are some studies that its serum level in FD patients is higher than in control patients (10, 11). However, following data about leptin in FD are very scant and there are few reports handling with both hormones.

Meanwhile, *H. pylori* infection has been suspected to be an influential candidate for pathogenesis of FD (12). However, in spite of several studies which tried to demonstrate the relation between its infection and blood or gastric levels of the aforementioned peptides, the answer is still

debatable, too (13, 14).

From this background, the current study was aimed to elucidate the role of ghrelin and leptin in FD by evaluating acyl, des-acyl ghrelin and leptin according to presence of dyspeptic symptom. Secondly, we tried to determine whether *H. pylori* infection has an influence on ghrelin or leptin by comparing them in plasma and gastric mRNA level according to symptom subtype and *H. pylori* infection.

Materials and Methods

Patient Selection

From January 2011 to March 2013, patients who had epigastric pain and discomfort with normal upper endoscopy and no other explanation for their symptoms were enrolled, prospectively, in Seoul National University Bundang Hospital. All participants completed a self-administered questionnaire, which was translated from original Bowel Disease Questionnaire (BDQ) into Korean (Korean BDQ) and was validated in Korea (15). The questionnaire contained 56 gastrointestinal symptom-related items that corresponded to Rome III criteria (16). Socio-demographic status, past medical history, smoking, alcohol habit and spicy food ingestion were also included in the questionnaires. If the information was not enough to diagnose, electronic medical records were reviewed. The diagnostic criteria of FD include one or more of the

following; bothersome postprandial fullness, early satiation, epigastric pain or epigastric burning. The criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis. Severity of epigastric pain or burning, postprandial fullness and early satiation were scored with a 7 point scale. According to predominant symptom, patients were categorized into either PDS or EPS. Patients on NSAIDs, or with recent medical history of proton pump inhibitors or anticoagulants, patients with concomitant systemic diseases such as diabetes mellitus, renal or liver failure or any malignancy, patients with a body mass index (BMI) of more than 30 kg/m^2, patients with diagnosis of major depression, psychosis, eating disorder, those on regular antipsychotic or antidepressant drugs, patients with prior documented peptic ulcer disease proved by endoscopy, patients with prior history of gastroduodenal surgery and pregnant ladies were excluded. Symptomatic patients whose symptoms did not fulfill the Rome III criteria were also excluded. Since patients with FD could overlap with gastroesophageal reflux disease or IBS (17), patients presenting only typical symptoms due to reflux or IBS were excluded. All subjects had been given their informed consent, and the study protocol was approved by the Ethics Committee at Seoul National University Bundang Hospital.

Gastric Mucosa Specimen

During endoscopy, two pairs of biopsies were taken from both antrum and

body for histological evaluation, determining *H. pylori* infection status and measuring mRNA of ghrelin and leptin. The *H. pylori* infection status was decided by modified Giemsa staining, culture, rapid urease testing (CLOtest, Delta West, Bentley, Australia). If these tests were all negative then ^{13}C-urea breath test (UBiTkit; Otsuka Pharmaceutical, Tokyo, Japan) and serum IgG, specific for *H. pylori*, were measured by an enzyme-linked immunosorbent assay (ELISA) (Genedia *H. pylori* ELISA; Green Cross Medical Science Corp, Eumsung, South Korea); Korean strain was used as antigen in this *H. pylori* antibody test. If one of any these studies except serology showed positive, the patient was judged to be current *H. pylori* infected case.

Measurement of Plasma Acyl, Des-acyl ghrelin and Leptin Levels

We then measured plasma ghrelin levels to evaluate their association with gastric motility. The patient samples were taken right before endoscopy due to avoiding the effect of endoscopy stress. Blood samples were obtained after an overnight fast of 8 h, immediately transferred to chilled polypropylene tubes containing Na 2 EDTA and aprotinin, then centrifuged at 4 ° C. One tenth of the volume of 1 N HCl was immediately added to the separated plasma. The acylated and desacylated forms of ghrelin were measured using commercially available ELISA kits according to the manufacturer's instructions (Active Ghrelin ELISA Kit

and Desacyl-Ghrelin ELISA Kit, SCETI CO., LTD., Tokyo, Japan). The intra-and inter-assay coefficients of variation (CV) were 6.5 and 9.8% for acylated ghrelin, and 3.7 and 8.1% for des-acylated-ghrelin. The Leptin level was assayed using ELISA kits according to the manufacturer's instructions (R&D systems Inc., Minneapolis, MN).

Measurement of mRNA of Ghrelin and Leptin Expression

Biopsied specimen of the gastric fundic mucosa stored in liquid nitrogen. Total RNA was extracted from body specimens (one from the greater curvature and one from the lesser curvature) of the gastric mucosa using Trizol Reagent (Invitrogen, Carlsbad, CA, USA). RNA samples were diluted to a final concentration of 0.5 mg/mL in RNase-free water and stored at -80℃ until use. Synthesis of the cDNA was performed with 1 μg of total RNA with M-MLV reverse transcription reagents (Invitrogen). The thermal cycling parameters for the reverse transcription were 10 min at 65 ℃, 50 min at 37 ℃ and 15 min at 70 ℃. Real time PCR amplification and determination were performed using SYBR Premix Ex TaqTM (Takara Bio, Shiga, Japan) and a StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) according to manufacturer's protocol. The following primers were used: preproghrelin forward, GGG CAG AGG ATG AAC TGG AA; preproghrelin reverse, CCT GGC TGT GCT GCT GGT A; Leptin forward, CCT GAC CTT

ATC CAA GAT GG ; Leptin reverse, GAG TAG CCT GAA GCT TCC AG; GAPDH forward, AGG TGA AGG TCG GAG TCA; and GAPDH reverse, GGT CAT TGA TGG CAACAA. The GAPDH gene was used as an endogenous reference to control for expression independent sample-to sample variability. The amplification protocol consists of an initial denaturation step at 95 ℃ for 10 s followed by 40 cycles of denaturation for 5 s at 95° and annealing/extension of 33 s at 55℃ for GAPDH ; at 58℃ for preproghrelin; at 62℃ for leptin. Relative expressions of target genes were normalized by dividing the target Ct values by the endogenous Ct values.

Statistical Analysis

All peptides values are expressed as median. Mann-Whitney test, Kruskal-Wallis test, Wilcoxon rank sum test and Spearman's correlation test were used for non-parametrical statistical analysis. Student's *t*-test was used for analysis of categorical data. Analyses were performed using the Statistical Package for the Social Sciences (version 19.0; NY, IBM. USA). All statistical tests were two-sided, and a value of $P = .05$ was considered to be statistically significant.

Results

Characteristics of control, PDS, and EPS Patients

One hundred and twenty-nine patients with typical symptom of FD (PDS; n = 88, EPS; n = 41) and one hundred and three healthy control patients without dyspepsia symptom were enrolled. The mean age (± SD) was 51.9 ± 12.1 years (range: 21-76 years). Age in control patients was significantly higher than in PDS patients (control: 54.8 ± 10.7, PDS: 49.0 ± 11.4, and EPS: 50.1 ± 12.2, respectively, P = 0.003). Higher rate of female patients existed in PDS and EPS group compared than in control but there was no statistical significance (57.3% vs. 64.8% and 73.2%, respectively, P = 0.185). However, the BMI scores, smoking, alcohol consumption, regular spicy food ingestion in the three patient groups were not statistically different. In addition, *H. pylori* positivity, neutrophil infiltration grade and presence of intestinal metaplasia and atrophic gastritis did not vary significantly among control, PDS, and EPS patients (Table 1).

Table 1 Baseline characteristics in 103 control and 101 FD patients

	Control (n = 103)	PDS (n = 88)	EPS (n = 41)	*p*- value
Age (yr) (mean±SD)	54.8 ± 10.7	49.0 ± 11.4	50.1± 12.2	0.003
Male/Female (%)	44: 59 (42.7:57.3)	31: 57 (35.2:64.8)	11: 30 (26.8:73.2)	0.185
BMI (mean±SD) (kg/m^2)	22.9 ± 2.5	22.5 ± 3.2	22.8 ± 2.8	0.730
Smoking (%)	15/94 (16.0)	9/66 (13.6)	5/30 (16.7)	0.601
Alcohol consumption (%)	28/93 (30.1)	13/61 (21.3)	11/30 (36.7)	0.250
Regular spicy food ingestion (%)	75/103 (72.8)	58/88 (65.9)	28/41 (68.3)	0.579
H. pylori positivity (%)	67/108 (65.0)	44/86 (50.0)	24/38 (58.5)	0.110
Neutrophil infiltration (%)	56/103 (54.4)	39/81 (48.3)	16/38 (42.1)	0.436
Atrophic gastritis (%)	32/98 (32.7)	26/81 (32.1)	9/37 (24.3)	0.890
Intestinal metaplasia (%)	32/104 (30.8)	16/83 (19.3)	8/38 (21.1)	0.243
Irritable bowel syndrome (%)	17/91 (18.7)	27/82 (32.9)	19/41 (46.3)	0.040
Gastroesphgeal reflux (%)	33/87 (37.9)	34/88 (38.6)	21/41(51.2)	0.440

Comparison of Plasma Acyl, Des-acyl ghrelin of Control and FD Patients

Plasma acyl ghrelin was lower in FD than in control (control: 12.98 fmol/ml, FD: 11.20 fmol/ml, respectively, $P = 0.035$) while plasma des-acyl ghrelin did not show any significant difference (control: 64.70 fmol/ml, FD: 55.64 fmol/ml, respectively, $P = 0.681$). In subgroup analysis of FD, PDS patients had lower plasma acyl ghrelin level than control and EPS patients (control: 12.99 fmol/ml, PDS: 8.63 fmol/ml, EPS: 13.89 fmol/ml, $P < 0.001$, $P < 0.001$) (Figure 1, a). However, there was no significant difference in plasma des-acyl ghrelin level among the three symptom subtype (Figure 1, b). Although female patients are more common than male patients in our outpatient clinic, gender differences did not exist in plasma acyl and des-acyl ghrelin levels in the present study (male: 11.8 fmol/ml, female: 11.8 fmol/ml, $P = 0.482$ for acyl ghrelin; male: 53.1 fmol/ml, female: 62.8 fmol/ml, $P = 0.290$ for des-acyl ghrelin).

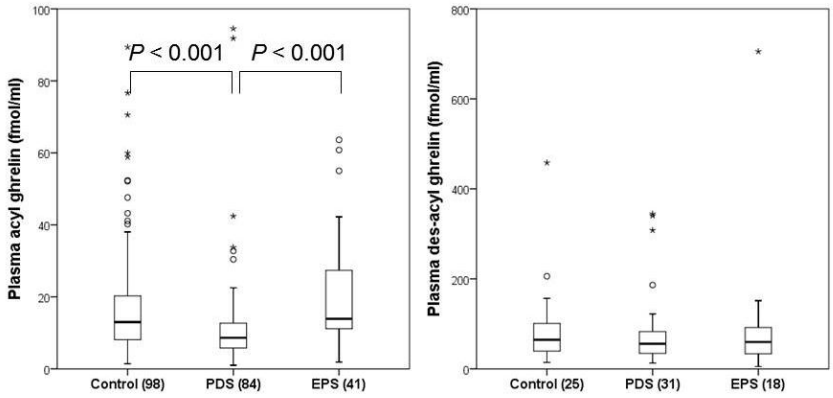

Figure1 Plasma acyl and des-acyl ghrelin in control, PDS and EPS patients. (a) Plasma acyl ghrelin in PDS group was lower than in other two groups ($P < 0.001$, $P < 0.001$). (b) There are no significant differences in plasma des-acyl ghrelin among the three groups. Box represents 27-75% and horizontal bar means median.

Relation between Plasma Acyl Ghrelin and Dyspeptic Symptom

There were significant correlations between plasma acyl ghrelin level and both early satiation score (r = -0.139, P = 0.039) (Figure 2, a) and postprandial fullness score (r = -0.198, P = 0.003) (Figure 2, b). However, epigastric pain or burning symptom score were not significantly correlated with plasma acyl ghrelin level (r = 0.042, P = 0.534) (Figure 2, c).

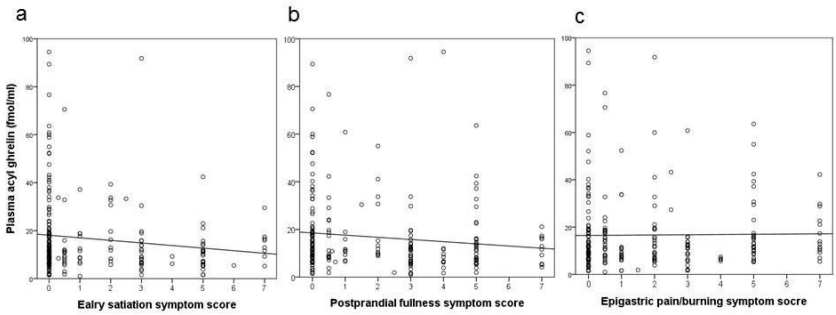

Figure 2 Relationship between plasma acyl level and dyspeptic symptom score. Plasma acyl ghrelin showed negative correlation with (a) early satiation symptom score (r = -0.139, P = 0.039), (b) postprandial fullness symptom score (r = -0.198, P = 0.003). (c) Plasma acyl ghrelin did not correlate with epigastric pain or burning symptom score (r = 0.042, P = 0.534).

Comparison for Alteration of Plasma Acyl Ghrelin Levels depending on Symptom Change

Approximately one year after the first evaluation, fasting plasma acyl ghrelin were measured in twenty-two persons. Median follow-up period was 12.2 months (range: 11.4–16.7 months). There were seventeen patients who presented significantly improved or initially minor symptoms (Figure 3, a) and 5 patients with aggravated or persistent dyspeptic symptoms (Figure 3, b). While plasma acyl ghrelin level of the former participants increased significantly ($P = 0.006$) (Figure 3, c), the 5 latter patients did not show any significant change in their plasma acyl ghrelin level (Figure 3, d). There were no individual changes of BMI during follow-up period.

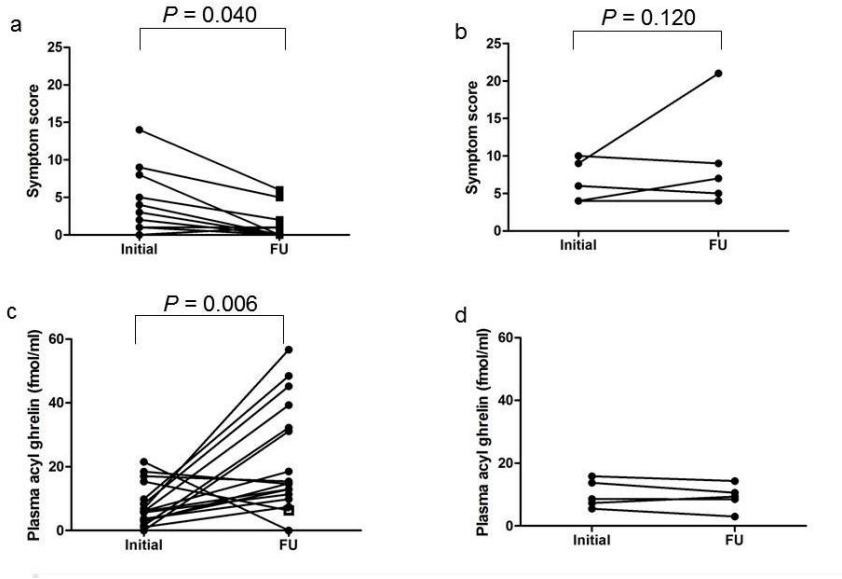

Figure 3 Change of plasma acyl ghrelin level according to symptom variation (one year after the first evaluation, fasting plasma acyl ghrelin were measured again in twenty-two persons). (a) Seventeen patients presented significant symptom improvement (P = 0.040) (c) Their plasma acyl ghrelin level increased significantly at follow-up (P = 0.006) (b) 5 patients had aggravated or persistent dyspeptic symptom. (d) They did not show any significant change in their plasma acyl ghrelin level.

Relative mRNA Expression of Ghrelin in Gastric Mucosa

No significant differences were shown in gastric mRNA expression of preproghrelin among the three groups (control: 0.96, PDS: 1.19, EPS: 2.27, $P = 0.251$). Furthermore, there was no correlation between relative values of preproghrelin and three subjective dyspeptic symptom score (early satiation: $r = 0.095$, $P = 0.309$; postprandial fullness: $r = 0.130$, $P = 0.162$; epigastric pain or burning: $r = 0.055$, $P = 0.558$). Each plasma acyl and des-acyl ghrelin or summation of them did not significantly correlate with relative expression of ghrelin mRNA (acyl ghrelin: $r = 0.021$, $P = 0.827$; des-acyl ghrelin: $r = -0.061$, $P = 0.666$), while there was strong positive correlation between plasma acyl ghrelin and des-acyl ghrelin ($r = 0.462$, $P < 0.001$).

Comparison of Serum Leptin Level in Control and FD Groups

After subgroup analyses according to gender and BMI, no significant difference in plasma leptin levels among the three symptom groups was found (data not shown). However, in the group with BMI $\geq$ 23 kg/m^2, regardless of gender, persons who have early satiation symptom presented lower serum leptin level than persons without the symptom (early satiety: 2.17 ng/ml, no-early satiety: 2.81 ng/ml, $P = 0.034$).

Relative mRNA Expression of Leptin in Gastric Mucosa

There was no significant difference in leptin mRNA expression level in gastric mucosa among control, PDS and EPS group (control: 1.34, PDS: 1.56, EPS: 1.67, $P = 0.784$). However, relative expression of leptin mRNA in persons with postprandial fullness was significantly higher than those who do not have the symptom (fullness: 1.80 vs. normal: 1.28, respectively, $P = 0.040$). There was also positive correlation between postprandial fullness score and arbitrary level of leptin mRNA ($r = 0.234$, $P = 0.008$).

Level of Gut Hormone according to *H. pylori* Infection

One hundred five persons (58.2%) of all participants were *H. pylori*-positive. There were no significant differences in age, gender, BMI between the *H. pylori*-negative and *H. pylori*-positive groups (data not shown). There was no significant difference in plasma acyl and des-acyl ghrelin and serum leptin level between the two groups (acyl ghrelin: 12.5 fmol/ml vs. 10.7 fmol/ml, des-acyl ghrelin: 51.8 fmol/ml vs. 59.5 fmol/ml, leptin: 2.5 ng/ml vs. 3.0 ng/ml, $P = 0.584$, $P = 0.445$, $P = 0.880$, respectively). Similarly, subgroup analysis among control, PDS and EPS revealed acyl ghrelin in PDS was lower than in control or EPS, regardless of *H. pylori* infection status (Figure 4). Meanwhile, relative expression

levels of preproghrelin mRNA was lower in HP-positive patients than in the other (*H. pylori*-negative: 3.05, *H. pylori*-positive: 0.71, $P < 0.001$). When we analyzed this relative mRNA expression levels of preproghrelin according to presence of active gastritis and atrophic gastritis, patients with active gastritis or atrophy had a significantly lower value of ghrelin mRNA expression than those who did not have them (active gastritis vs. normal: 0.61 vs. 2.30, $P < 0.001$; atrophy vs. non-atrophy: 0.69 vs.1.69, $P = 0.001$). Furthermore, patients with atrophic gastritis presented lower plasma unacylated ghrelin level than the other (atrophy vs. non-atrophy: 53.08 vs. 66.00, $P = 0.033$). However, there were opposite trends in expression of leptin mRNA (HP-negative: 1.32, HP-positive: 1.52, $P = 0.482$; active gastritis: 1.56, normal: 1.26, $P = 0.285$) between the two groups. In subgroup analysis according to gender, women with HP infection had relatively higher expression of leptin mRNA (HP-negative: 0.62, HP-positive: 1.45, $P = 0.044$) than those who without HP infection (Table 2).

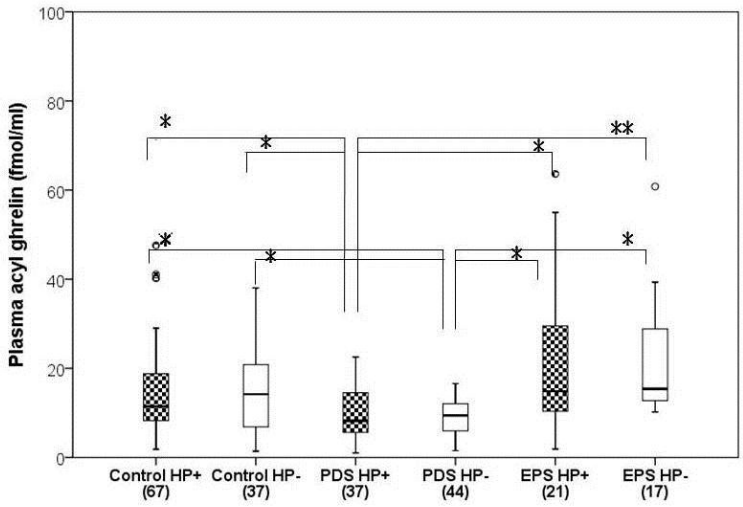

Figure 4 Plasma acyl ghrelin concentrations depending on symptom subtype and *H. pylori* infection. There was no significant difference according to *H. pylori* infection status in the same subtype. PDS groups had lower level of acyl ghrelin than in the other two groups, regardless of *H. pylori* infection status. * $P < 0.05$, ** $P < 0.001$

Table 2 Level of ghrelin and leptin according to HP infection and gastritis

	Preproghrelin mRNA	Leptin mRNA	Plasma acyl ghrelin (fmol/ml)	Plasma des-acyl ghrelin (fmol/ml)	Serum Leptin (ng/ml)
HP (-)	2.9 (0.02-63.0) **	1.3 (0.04-10.7) M: 2.1 (0.3-10.7) F: 0.6 (0.04-3.7)*	12.5(1.5-37.2)	51.8 (5.17-344.3)	2.5 (0.7-17.9)
HP (+)	0.72 (0.01-13.9)	1.5 (0.07-10.9) M: 1.7 (0.1-8.8) F: 1.5 (0.1-10.9)	10.7 (1.0-91.8)	59.5 (12.9-705.3)	3.0 (0.1-17.7)
Gastritis (-)	3.2 (0.02-63.0)**	1.3 (0.04-10.7) M: 2.1 (0.3-10.7) F: 0.7 (0.04-3.7)*	11.8 (1.5-37.2)	53.1 (5.2-344.3)	2.5 (0.2-15.2)
Gastritis (+)	0.5 (0.01-13.9)	1.5 (0.08-10.9) M:1.6 (0.08-4.9) F:1.5 (0.2-10.9)	9.0 (1.0-63.6)	59.5 (12.9-458.1)	2.7 (0.1-17.9)
Atrophy (-)	2.5 (0.01-63.0)*	1.45 (0.04-7.6)	11.8 (1.0-63.6)	66.0(5.2-458.1)*	2.4 (0.1-7.1)
Atrophy (+)	1.0 (0.01-13.9)	1.2 (0.1-10.9)	9.5 (3.7-32.8)	50.5 (12.9-88.0)	2.3 (0.7-17.9)

Median values are presented being followed by range in the parenthesis.

M, male; F, female, * $P < 0.05$, ** $P < 0.001$

DISCUSSION

FD is diagnosed depending on mostly subjective symptom, so finding biomarker is important. Gut hormones, especially ghrelin and leptin have been etiological candidate for FD (18). In the present study, significantly lower concentration of plasma acyl ghrelin was presented in PDS patients than control and EPS patients. This is in accordance with Shindo et al (3), demonstrating that PDS patients showed reduced acyl ghrelin level and it was correlated with delayed gastric emptying. We also demonstrated that 17 patients with improvement of symptom showed significant increasing acyl ghrelin level mostly, while the other 5 patients without reliving of discomfort did not show any significant change of the hormone. Taken together, above results support that acyl ghrelin is likely to enhance gastric motility. Furthermore, fasting plasma acyl ghrelin also inversely correlated with not epigastric pain but early satiation and fullness score in our study, this indicates that different pathophysiology could work in different subtype of FD , supporting the ROME III criteria. However, neither plasma des-acyl nor sum of both forms of ghrelin presented significant differences in our study.

Contrary to this, Nishizawa et al. presented both plasma total and active ghrelin in FD were higher than in control and explained this might result from compensational secretion (19). The reason for these inconsistent data may stem from different protocols for measuring peptides. We used as

same ELISA kit as Shindo et al did, but most of other studies measured ghrelin with radioimmunoassay. Another possibility is that an inaccurate patient's classification from insufficient self-reported questionnaire, different diagnostic criteria, ROME II or III, and different application even in the same criteria can lead to conflicting results. Furthermore, in this study we did not consider that menstrual cycle could have an effect on fasting plasma ghrelin level (20).

Whereas some previous study demonstrated stepwise correlations among mRNA, peptide and plasma ghrelin (21), arbitrary value of mRNA of ghrelin in our study didn't show any correlation with sum of circulating acyl and des-acyl ghrelin. This discrepancy between circulating and gastric hormone level has been often reported (14, 22) and might result from the fact that region biopsied could not be representative for the whole hormone synthesis. Another explanation is post-translational procedure, for example, acylation process via GOAT or obestatin-ghrelin balance may act more importantly than preproghrelin mRNA synthesis itself for the determination of circulating acyl ghrelin concentration (23). Finally, releasing from other organs, intestines, lung, kidney and hypothalamus (24), or existence of other forms of circulating ghrelin which were undetectable with current method could be considered (25).

Regarding leptin, in the present study, the difference in serum leptin was not found and this is different from previous study which reported serum leptin level in FD was higher than in control (10). However, patients who

display early satiation and BMI $\geq$ 23Kg/m^2 have lower serum leptin level than those who have no early satiation. Since serum leptin is known to be closely related to satiety center in systemic level, whether it is a coincidence or result from complex feedback between hormone and its receptor, this result is worthy of further investigation.

Moreover, there was a significant positive correlation between gastric leptin mRNA level and after-meal fullness symptoms. Considering that stomach accounts for relatively minor part of entire leptin production, its short half-life and existence of the leptin receptor in stomach, local gastric leptin may affect gastric motility. This is at least in part, consistent with previous data which showed its lower level in persons with dyspeptic symptoms than the others (22). Although serum leptin may have a direct influence on satiety center leading to early satiation (8, 9), there was no correlation between serum leptin level and its mRNA expression in gastric mucosa might be natural because its predominant source is adipose tissue.

Numerous researchers have paid attention to the effect of *H. pylori* on leptin or ghrelin and evaluated their changes after eradication, but the data was also still conflicting; increased, or not changed (26-28). First we compared circulating and mRNA levels of two hormones between HP-negative and HP-positive groups. As a result, ghrelin mRNA in the HP-positive group expressed lower than the other group and this is in accordance with previous data (22). At the same time, the presence of neutrophil infiltration and pepsinogen I/II ratio < 4.1 was higher in HP-

positive patients than HP-negative patients (neutrophil infiltration: 19.4% vs. 65.9%, atrophy: 15.6% vs. 42.1%, pepsinogen I/II < 4.1: 51.8% vs. 75.6%, respectively, $P < 0.001$, $P < 0.001$, $P = 0.003$) (29). Since presence of neutrophil infiltration is closely related with *H. pylori* infection, this implies that *H. pylori* infection can cause reducing mRNA expression of gastric ghrelin via gastritis or atrophic change (30). On the contrary to preproghrelin mRNA, leptin mRNA of *H. pylori* positive patients tended to be higher than the others. This trend presented statistical significance only in women group (HP - negative: 0.62, HP - positive: 1.45, $P = 0.044$; Gastritis: 0.70, without gastritis: 1.45, respectively, $P = 0.043$). These are also in favor of previous data (26, 31), and they explained that leptin may be associated with immune or inflammatory response to *H. pylori* infection. However, there were no significant differences in plasma acyl, des-acyl ghrelin and serum leptin levels according to *H. pylori* infection. Only plasma des-acyl ghrelin in patients with atrophic gastritis was lower than in patients without atrophy. Although a few researchers reported des-acyl ghrelin also enhance gastric motility, its clinical significance should be more studied. Nevertheless, one thing remarkable is that in subgroup analysis according to *H. pylori* infection status and symptom subtype, the PDS group showed consistently lower plasma acyl ghrelin level than in control and EPS groups.

Collectively, although HP-induced gastritis could change mRNA expression of preproghrelin or leptin in gastric mucosa, it appears that this

may not lead to straightforward change in circulating peptide's levels. However, increased leptin mRNA expression itself might have influence on gastric motility and the possibility that reduced preproghrelin mRNA might be connected to decrease in plasma level in some condition. Control of gastric secretion of ghrelin and determination of plasma active hormone level need to be investigated subsequently.

There were several limitation in the present study. First of all, we didn't measure objective gastric motility index so that we can't confirm that patients with dyspepsia symptom have real motility disorder. As a result, our study cannot conclude that reduced acyl ghrelin in FD patients has an effect on gastric delayed emptying. Secondly, only small number of persons were evaluated at 12 months-follow up. Thirdly, we diagnosed subtype of FD by predominant symptom, we cannot exclude the possibility that both subtype was mixed in individual person. However, this is the one of a few studies that evaluate FD according to ROME III classification and demonstrated lowered plasma acyl ghrelin is more likely to associated PDS than EPS. Both ghrelin and leptin, from both blood and gastric mucosa and even both major forms of ghrelin; acyl and des-acyl form were measured. We also attempt to find out whether *H. pylori* affects circulating or gastric ghrelin level and eventually functional dyspepsia. Finally, this is the almost the first study to follow up change of acyl ghrelin level in relation to change of dyspepsia symptom.

In summary, in the present study, PDS showed lower level of acyl

ghrelin compared to control and EPS regardless of *H. pylori* infection, but ghrelin mRNA expression was not different between FD and control. HP-positive patients presented lower preproghrelin mRNA expression but this is not directly connected to reducing plasma acyl or des-acyl ghrelin. Serum leptin level was neither significantly different between FD and control group nor affected by *H. pylori* infection. However, patients with *H. pylori* infection tend to show increased level of gastric leptin mRNA.

In Conclusion, circulating acyl ghrelin level can be closely related to development of PDS, which might be determined by not reducing mRNA expression but using more complex interaction in a systemic or posttranslational level. Regarding to leptin, serum leptin didn't show any significant difference between FD and control and *H. pylori* infection may enhance gastric leptin mRNA expression. Further study is needed to elucidate how plasma acyl ghrelin level was regulated.

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요 약

서론: 기능성 소화불량증의 원인으로 그렐린과 렙팁이 지목되어 왔다. 그러나, 많은 연구에도 불구하고 혈장 그렐린과 기능성 소화불량증의 관계에 대해서는 일치된 결론이 없다. 게다가 기능성 소화불량증과 연관하여 렙틴을 다룬 논문은 매우 소수이다. 이번 연구에서는 주요한 두 형태의 혈장 그렐린, 혈청 렙틴 그리고 그렐린과 렙틴의 위점막 mRNA 발현량을 기능성 소화불량증 환자와 대조군에서 비교해 봄으로 써 기능성 소화불량증의 병태생리를 이해해 보고자 한다.

방법: 2011년 1월부터 2013년 3월까지 분당 서울대학교 병원을 방문하여 내시경을 시행한 환자 중 총 129명의 기능성 소화불량증 환자와 103명의 증상이 없는 대조군이 분석되었다. 기능성 소화불량증 환자는 ROME III 진단기준에 따라 88명의 식후불편감 증후군 (PDS) 환자와 41명의 심와부 동통 증후군 (EPS) 환자로 분류되었다. 등록된 모든 환자를 대상으로 기능성 소화불량증상과 위식도 역류증상, 과민성 장 증후군의 증상 등을 묻는 자가 설문 조사지가 실시되었다. 아실화 그렐린과 탈아실화 그렐린 그리고 렙틴은 내시경 직전 공복상태에서 채혈된 혈액에서 ELISA (enzyme-linked immunosorbent assay)를 이용하여 측정하였으며 그렐린과 렙틴의 mRNA는 내시경 중에 얻은 중부체부의 대만곡의 점막에서 Real-time quantitative PCR을 이용하여 측정하였다.

결과: 혈장 아실화 그렐린은 식후불편감 증후군 그룹에서 대조군이나 심와부 동통 증후군 그룹보다 통계적으로 유의하게 낮은

반면 (대조군: 13.0 fmol/ml, PDS: 8.6 fmol/ml, EPS: 13.9 fmol/ml, $P < 0.001$), 혈장 탈아실화 그렐린, 렙틴, 위점막 그렐린과 렙틴의 상대적인 mRNA 발현양은 유의한 차이를 보이지 않았다. 그러나 위점막 렙틴의 상대적인 mRNA 발현양은 식후 팽만감을 가진 그룹에서 그렇지 않은 그룹보다 통계적으로 유의하게 높았다 (1.28 vs. 1.80 $P = 0.040$). 헬리코박터 파일로리균 (HP)에 감염된 위점막 그렐린 mRNA의 발현양은 이 균이 감염되지 않은 위점막 그렐린 mRNA의 발현양보다 통계적으로 유의하게 낮았는데 (HP 음성: 3.05, HP 양성: 0.70, $P < 0.001$), 혈장 아실화, 탈아실화 그렐린의 양은 헬리코박터균 유무에 따른 차이가 나지 않았다. 여성의 경우, 헬리코박터 파일로리균에 감염된 위점막의 렙틴 mRNA의 발현은 비감염 위점막보다 유의하게 높은 수치를 나타내었다 (HP 음성: 0.62, HP 양성: 1.45, $P = 0.044$).

결론: 혈장 아실화 그렐린은 식후불편감 증후군의 발생에 관련이 있을 것으로 사료되나 그 조절은 mRNA를 줄이는 방법이 아니라 번역 후 과정과 같은 것이 관여할 것으로 생각된다. 한편, 렙틴 mRNA의 상승은 위장 이상운동을 일으켜 식후 팽만감을 느끼게 하는데 관련이 있을 수 도 있다. 헬리코박터 균의 감염은 급성위염 이나 위축성 위염을 일으켜 그렐린 mRNA발현을 감소시키고, 특히 여성의 렙틴 mRNA 발현은 증가시킨다.

주요어: 기능성 소화불량증, 그렐린, 아실화 그렐린, 탈아실화 그렐린, 렙틴

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