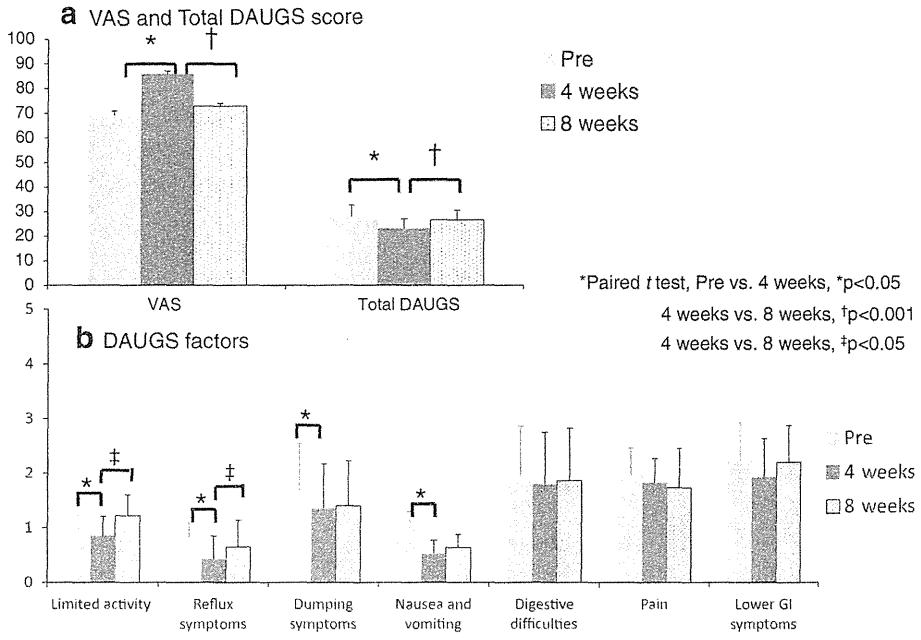

Fig. 3 Appetite visual analog scale (VAS) scores and DAUGS scores before and after rikkunshito administration. Higher VAS values indicate a better status for appetite, while the DAUGS scoring system evaluates better function as a lower score (see "Patients and methods"). *Error bars* represent SDs. *GI* gastrointestinal

rikkunshito administration and they decreased after the drug withdrawal (Pre: 6.6 ± 1.9, 4 weeks: 8.6 ± 1.2 points, $p < 0.05$; 8 weeks: 7.3 ± 1.1 points, $p < 0.05$ compared with the score at 4 weeks). The total DAUGS score and the scores reflecting limited activity due to decreased food consumption, reflux symptoms, dumping symptoms, and nausea and vomiting were significantly improved after rikkunshito administration. Four weeks after the drug withdrawal, the total DAUGS score and the categories of limited activity due to decreased food consumption and reflux symptoms had significantly deteriorated.

The QLQ-C30 scores before rikkunshito administration, after the administration, and after the drug withdrawal are presented in Fig. 4. There were no significant differences in the global health status scores. Among the functional scales, patients scored better after rikkunshito administration and scored worse after the drug withdrawal regarding physical functioning (Pre: 86 ± 11, 4 weeks: 96 ± 7, $p < 0.01$; 8 weeks: 86 ± 9, $p < 0.01$). With respect to the symptom scales and items, there were no significant differences at any time points.

Association between symptomatic improvement and acyl-/total ghrelin ratio

Table 4 lists the associations between symptomatic improvement and the acyl-/total ghrelin ratio after 4 weeks' rikkunshito administration. We divided the subjects into high ($n = 13$) and low ($n = 12$) acyl-/total ghrelin groups using a cut-off value of 9.8 %, representing the ratio of acyl-/total ghrelin after 4 weeks' rikkunshito administration. However, there were no significant differences in the VAS appetite score, DAUGS score, or QoL score between the two groups.

Discussion

Traditional Japanese medicines (Kampo) are widely prescribed in Japan for patients with various gastrointestinal symptoms, and rikkunshito is one of the most popular Kampo medicines for these patients. Some clinical studies have reported that rikkunshito promotes recovery from anorexia in patients with FD [17, 18, 30, 31] and gastroesophageal reflux disease (GERD) symptoms [19, 20, 32]. In another study conducted in patients with advanced breast cancer, concomitant administration of rikkunshito with antiemetics such as granisetron proved to be effective against the anorexia and vomiting induced by anticancer drugs [23]. In a recent report, rikkunshito administration was shown to stimulate peripheral ghrelin secretion in rats with anorexia induced by cisplatin or selective serotonin reuptake inhibitors [16, 33]. Furthermore, oral administration of rikkunshito was shown to stimulate the secretion of ghrelin from the stomach in healthy volunteers. Also, the plasma acylated ghrelin concentration was shown to be significantly increased after rikkunshito treatment [15]. Ghrelin stimulates food intake [9] and triggers a positive energy balance through a central mechanism involving hypothalamic neuropeptides [9–12]. Taken together, these reports suggest that the induction of ghrelin could be

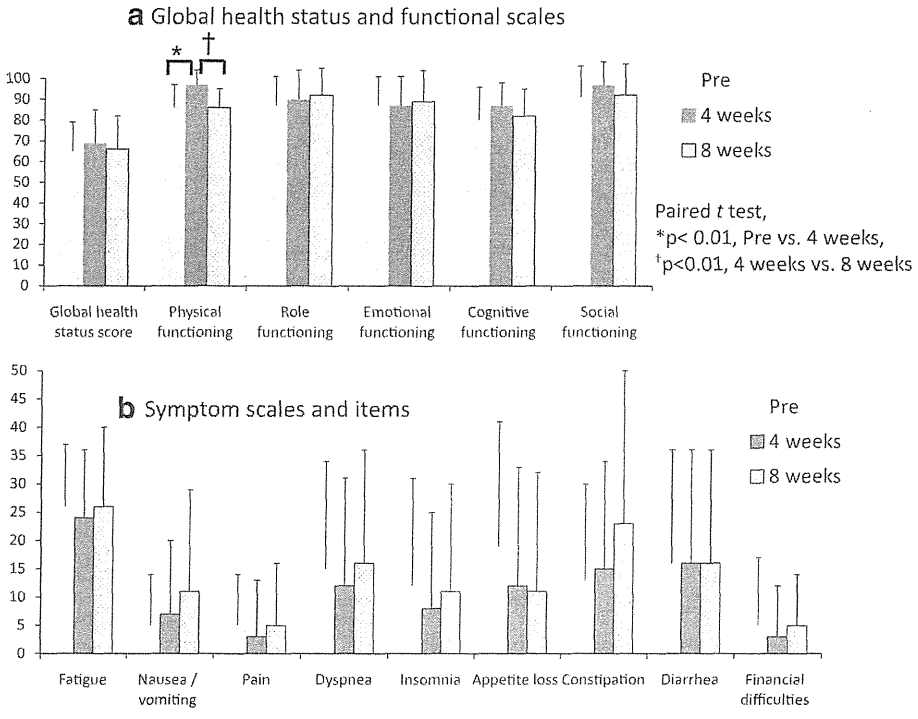

Fig. 4 Quality of life scores before and after rikkunshito administration. High scores for global health status and functional scales represents a higher ("better") level of functioning, whereas a high score for a symptom scale or item represents a higher ("worse") level of symptoms (see "Patients and methods"). *Error bars* represent SDs

Table 4 Associations between symptomatic improvement and acyl-ghrelin/total ghrelin ratios after rikkunshito administration

Parameter	High acyl-/total ghrelin group[a]	Low acyl-/total ghrelin group[b]	*p* value
Appetite (VAS)	8.7 ± 0.37	8.5 ± 0.39	0.87
Total DAUGS	22.7 ± 8.9	23.5 ± 7.4	0.88
Limited activity	0.60 ± 0.52	0.95 ± 0.79	0.24
Reflux symptoms	0.47 ± 0.62	0.52 ± 0.55	0.85
Dumping symptoms	1.30 ± 0.28	1.36 ± 0.27	0.87
Nausea and vomiting	0.38 ± 0.37	0.55 ± 0.49	0.39
Functional scales (QLQ-C30)			
Physical functioning	92.3 ± 7.6	89.4 ± 7.2	0.33

Values are mean ± SD

The DAUGS scoring system evaluates better function as a lower score, while higher values indicate better status for appetite (VAS) and physical functioning in the QLQ-30 (see "Patients and methods")

DAUGS Dysfunction After Upper Gastrointestinal Surgery for Cancer, *VAS* visual analog scale, *QLQ-30* European Organization for Research and Treatment of Cancer quality-of-life instrument for use in international clinical trials in oncology

[a] Acyl-/total ghrelin after rikkunshito administration >9.8 %

[b] Acyl-/total ghrelin after rikkunshito administration <9.8 %

responsible for some of the beneficial clinical effects exerted by rikkunshito.

In the present study, however, the administration of rikkunshito did not increase the ghrelin concentration per se. The difference from the previous findings with healthy volunteers could be attributed to gastrectomy, because ghrelin is predominantly secreted by gastric endocrine cells. In our previous study, a persistent decline of serum ghrelin and body weight was commonly observed after total gastrectomy [34]. In the present study, the plasma ghrelin concentration was indeed higher in the patients who had undergone distal gastrectomy than in those who had undergone total gastrectomy. It is unlikely, therefore, that rikkunshito attenuates post-gastrectomy symptoms through eliciting ghrelin secretion from the stomach.

In contrast to the serum ghrelin findings, the ratio of the acyl-/total ghrelin concentration increased significantly after treatment with rikkunshito and declined 4 weeks after the drug withdrawal. During metabolic processing, a fraction of desacyl-ghrelin is acylated in position 3 (serine) to form variants of acyl-ghrelin. Acyl-ghrelin displays dose-dependent GH-releasing activity [8, 9], stimulates food intake, triggers a positive energy balance [10–12], and is considered to be the active form of ghrelin [11, 35–37]. Actually, several components of rikkunshito demonstrate inhibitory activity against ghrelin desacylating enzymes [38]. In addition, 10-gingerol, a component of rikkunshito,

inhibits exogenous ghrelin desacylation. Thus, rikkunshito may enhance the plasma acyl-ghrelin level, at least in part by inhibiting the circulating ghrelin degrading enzyme [39]. These findings, taken together, suggest that the rise in the ratio of the acyl-/total ghrelin concentration could be responsible for the beneficial effect of rikkunshito in terms of the attenuation of gastrointestinal symptoms.

Rikkunshito was reported to significantly accelerate gastric emptying and reduce gastrointestinal symptoms in adult patients with chronic idiopathic dyspepsia [17]. In another study, it was reported that, postoperatively, dyspeptic pediatric patients exhibited symptomatic relief and a significant reduction in the mean symptom score after treatment with rikkunshito over a 1-month period [21]. In the sphere of gastrointestinal surgery, rikkunshito improved gastric emptying and ameliorated the postoperative symptoms of patients who had undergone a pylorus-preserving gastrectomy (PPG) [22]. Although the attenuation of postoperative symptoms observed in these studies was reproduced in our present study, we failed to detect significant differences in the VAS scores or in any of the DAUGS items between the patient groups with high and low mean ratios of acyl-/total ghrelin concentration. The attenuation of gastrointestinal symptoms and the improvement in appetite after rikkunshito administration may therefore be attributable to other mechanisms associated with the constituents of rikkunshito rather than the one involving ghrelin. Rikkunshito is composed of over 100 compounds, including 8 major constituents, and a prokinetic action via NG-nitro-L-arginine could be considered as another candidate mechanism for the symptom relief [39, 40]. Prokinetic drugs, including erythromycin, cisapride, metoclopramide, and domperidone, may accelerate gastric emptying after gastrectomy and alleviate post-gastrectomy symptoms [40–43]. In adult patients with diabetic gastroparesis, normalization of electrical activity has been seen after domperidone administration [44]. Cisapride has been demonstrated to improve gastric emptying and myoelectrical activity in children with dyspepsia [45]. However, further studies will be needed to reinforce the hypothesis that herbal medicines, such as rikkunshito, act as prokinetic drugs, as there have been few reports objectively evaluating such effects of these agents [17, 46].

Recently, some reports have demonstrated that rikkunshito induces endogenous ghrelin secretion via its flavonoids, which antagonize 5-HT2B and 5-HT2c receptors [16, 33]. Furthermore, these reports demonstrated that the oral administration of rikkunshito ameliorated anorexia and gastrointestinal dysmotility in rats treated with either cisplatin or selective serotonin reuptake inhibitors, suggesting that 5-HT2 receptor antagonism could be at least partially responsible for the effect of rikkunshito in improving appetite loss [16, 33]. Rikkunshito attenuated cisplatin-induced gastrointestinal dysfunction through the improvement of ghrelin release that occurred via the 5-HT2B/2C antagonistic action of flavonoids contained in rikkunshito [16]. In general, herbal medicines such as rikkunshito have not been first-choice candidates for treating various diseases, because they consist of multiple components whose pharmacological function and active ingredients have not been elucidated in detail. Therefore, additional mechanistic studies are needed to conclusively determine the effects of rikkunshito in the near future.

Finally, we note that the differences in the VAS scores, the total DAUGS scores, and scores for various DAUGS items before and after the administration of rikkunshito were significantly different, but perhaps the differences were not spectacular. It is not possible at this stage to deny the possibility that the improvements observed had merely been placebo effects. On the other hand, the statistically significant improvements observed after only 4 weeks of drug intake could indicate the favorable potential of this drug. A prospective placebo-controlled randomized trial testing longer exposure to rikkunshito and including other useful parameters such as body weight, endoscopic findings, and hematological tests as endpoints is needed to further pursue our understanding of this drug.

In conclusion, our study showed, for the first time, that the administration of rikkunshito resulted in the attenuation of post-gastrectomy symptoms, possibly through an increase in the ratio of the acyl-/total ghrelin concentration. Rikkunshito is potentially useful to minimize gastrointestinal symptoms after gastrectomy. A well-designed multicenter randomized trial is warranted to confirm the present findings.

Conflict of interest The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Reduced plasma ghrelin levels on day 1 after esophagectomy: a new predictor of prolonged systemic inflammatory response syndrome

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Abstract
Background and purpose Ghrelin, a stomach-derived hormone, stimulates growth hormone secretion and appetite, and inhibits excessive inflammatory response. Plasma ghrelin might affect the inflammatory response to stressful surgical interventions. The aim of this study was to investigate the relationship between serial changes in plasma ghrelin concentrations and the postoperative clinical course after esophagectomy.
Methods The prospective cohort study subjects were 20 patients with esophageal cancer, who underwent esophagectomy with gastric tube reconstruction. Blood samples were taken six times perioperatively during the course of esophagectomy.
Results The plasma ghrelin level decreased to 33 % (range 15–90 %) on postoperative day (POD) 1, relative to the preoperative level, then recovered to about 50 % by POD 3–10. The duration of systemic inflammatory response syndrome (SIRS) was significantly longer in patients with a marked ghrelin reduction to <33 % on POD 1, than in those with less marked reduction of ≥33 % (6.1 ± 1.3 vs. 2.1 ± 0.6 days, $P = 0.019$). On POD 1, the only inflammatory marker that correlated with the duration of SIRS was the % ghrelin, whereas C-reactive protein, leukocyte count, and IL-6 did not.
Conclusion An early postoperative drop in plasma ghrelin correlated with prolonged SIRS after esophagectomy. Thus, the supplementation of low plasma ghrelin may help minimize excess inflammatory response in these patients.

Keywords Esophagectomy · Ghrelin · Esophageal cancer · Inflammation · SIRS

Introduction

Esophageal cancer is the sixth most common cause of death from cancer [1]. Although it is considered to be associated with poor prognosis, recent improvements in surgical treatment, postoperative intensive care, and perioperative therapy have improved treatment outcomes [2]. However, localized esophageal cancer is often resected by right thoracotomy combined with laparotomy, which represents one of the most invasive gastrointestinal operations, associated with the possible complications of systematic inflammatory response syndrome (SIRS), cardiopulmonary sequela, and stress-induced organ dysfunction [2]. Surgeons and intensive care unit (ICU) specialists have tried to resolve this problem, and several clinical studies have been conducted to investigate how to overcome the excessive inflammatory state, using recombinant interleukin-1 receptor antagonists, antitumor necrosis factor antibodies, polymorphonuclear elastase inhibitors, and corticosteroids [3–8]. Some of the reports on these studies described the clinical benefits of these approaches. The administration of corticosteroids during surgery is widely used and described as "recommended" perioperatively for patients undergoing esophagectomy in the Guideline for Esophageal Cancer

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Treatment [9]. The present study focuses on the role that ghrelin, a gut-hormone, plays in improving the perioperative care of patients undergoing esophagectomy.

Ghrelin is a novel peptide hormone secreted mainly in the stomach [10]. It has a variety of biological functions, including the stimulation of growth hormone secretion from the pituitary gland, with resulting anabolic effects; the control of energy expenditure; stimulation of appetite; and improvement of cardiopulmonary functions [11–15]. It also suppresses excessive inflammatory response by inhibiting proinflammatory cytokine production, mononuclear cell binding, and nuclear factor-κB activation [16]. With regard to ghrelin and surgical stress, we previously reported significant reductions in ghrelin concentrations by up to 50 %, 3 and 7 days after esophagectomy [17]. However, the relationship between the reduction in plasma ghrelin during esophagectomy and experimental evidence that ghrelin attenuates inflammatory response is poorly understood.

Esophagectomy is an aggressive surgical treatment that tends to induce uncontrollable inflammatory response with the overproduction of inflammatory mediators. According to recent reports, some peripheral blood values such as serum transferrin and plasma factor XIII were found to be predictive markers of the postoperative course of esophagectomy [18]. Ghrelin might be one of these predictive markers, especially in relation to inflammatory response. Therefore, we conducted a prospective cohort study and serially monitored changes in plasma ghrelin concentrations perioperatively to analyze the relationship between changes in plasma ghrelin concentrations and the postoperative clinical course. The results showed that ghrelin correlated with surgical stress-induced inflammatory response after esophagectomy, suggesting that this hormone is involved in the postoperative inflammatory response.

Methods

Study population

This prospective observational cohort study began in May, 2008, enrolment ended in July, 2009, and all patients were monitored continuously until July, 2010. The following criteria were used for study entry: thoracic esophageal cancer, treated by radical esophagectomy with gastric tube reconstruction; an age range of 20–80 years; adequate function of major organs; no other active malignancy; provision of written informed consent; and lack of severe co-morbid conditions and infectious diseases.

A registration center at the Department of Gastroenterological Surgery, Osaka University Medical School was responsible for the registration and prospective monitoring of the subjects.

Surgical procedure and postoperative management

All patients underwent surgery at the Department of Gastroenterological Surgery, Osaka University Hospital (Osaka, Japan), immediately prior to which they were given methylprednisolone diluted in 100 ml saline, at a dose of 250 mg/body. The thoracic portion of the esophagus was resected via right-sided thoracotomy, about 10 cm long, along the fourth intercostal space, with video-assisted thoracic surgery. Reconstruction was done using a gastric tube with cervical anastomosis via the posterior mediastinal route. None of the patients received enteral nutrition via a jejunostomy tube. At the end of the operation, all patients were placed on mechanical ventilation and transferred for immediate postoperative care to the ICU of Osaka University Hospital. All patients were weaned off mechanical ventilation on postoperative day (POD) 1 and received systematic and nutritional care via a central venous infusion.

Study protocol

The primary endpoint of this study was the serial changes in plasma ghrelin concentrations perioperatively and the secondary endpoints were the clinical course after surgery, including morbidity, and the duration of SIRS. Blood samples were taken six times during the perioperative course: the day before surgery; 2 h after the start of surgery, during thoracotomy; 4 h after the start of surgery, during laparotomy; and on PODs 1, 3, and 10. SIRS was diagnosed when two or more of the following criteria were met: a body temperature of less than 36 °C or greater than 38 °C; a heart rate greater than 90 beats per min; tachypnea, represented by a respiratory rate of greater than 20 breaths per min or an arterial partial pressure of carbon dioxide of less than 32 mmHg; and a leukocyte count of less than 4,000 cells/mm^3 or greater than 12,000 cells/mm^3 or immature band forms greater than 10 %.

Measurement of plasma ghrelin and serum IL-6 concentrations

Blood samples were immediately transferred into chilled glass tubes containing disodium ethylenediaminetetraacetic acid (EDTA) for plasma sampling, and a separating agent for serum sampling, followed by centrifugation at 4 °C. The plasma samples were mixed with a 10 % volume of 1 N hydrochloric acid (HCl) and stored at −50 °C, while the serum samples were stored at −50 °C. Plasma active- and desacyl-ghrelin, and serum interleukin-6 (IL-6) concentrations were measured with sandwich-type enzyme immunoassay kits according to the protocol supplied by the respective manufacturer (Mitsubishi Kagaku Iatron, Inc.

for ghrelin, and BD Biosciences, San Diego, CA, for IL-6). The total plasma ghrelin concentration was calculated as active-ghrelin plus desacyl-ghrelin concentrations [19]. Other laboratory tests were conducted at the Laboratory of Clinical Investigation, Osaka University Hospital.

Statistical analysis

Continuous variables are expressed as mean ± standard error of the mean (SEM) unless stated otherwise. Statistical differences between groups were calculated by the Student's t test, Mann–Whitney test, or χ^2 test. Statistical significance was set at $p < 0.05$. All calculations were performed using the JMP® software (SAS Institute, Cary, NC, USA).

Results

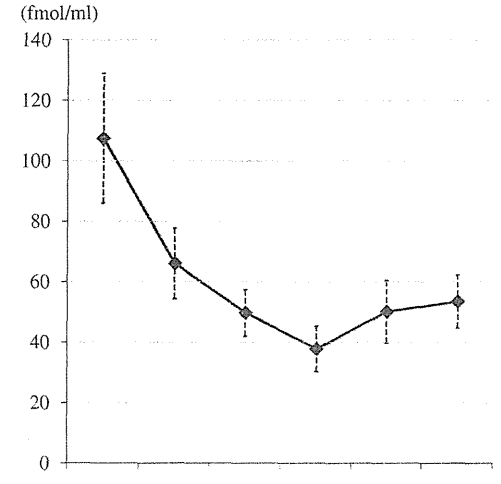
Patient characteristics and clinical course

A total of 20 patients with pathologically proven squamous cell carcinoma were enrolled in this study and none withdrew. They comprised 16 men and 4 women, with a mean age of 64 years, and mean body mass index (BMI) of 20.9 kg/m^2 measured preoperatively. According to preoperative UICC classification, 1 patient had stage I disease, 5 had stage IIA, 3 had stage IIB, and 11 had stage III. Three patients did not receive any preoperative therapy, 16 received preoperative chemotherapy, and 1 received preoperative chemoradiotherapy. Surgery was performed 4–6 weeks after completion of the preoperative treatment. The mean operation time was 464 min and the mean blood loss was 638 ml. Postoperatively, the mean and median (range) period of SIRS was 4 and 3 (0–14) days, respectively. The incidence of postoperative complications more severe than grade 2, according to the CTC-AE version 3.0, was 5 (25 %), including one case of anastomotic leakage and four cases of pneumonia. None of the patients required reoperation and there were no deaths within 30 days after surgery.

Changes in plasma ghrelin concentrations

The mean total plasma ghrelin concentrations measured preoperatively, 2 h after the start of surgery (2H), 4 h after the start of the surgery (4H), and on PODs 1, 3, and 10 were 107.4 ± 21.5, 66.0 ± 11.7, 49.7 ± 7.7, 37.7 ± 7.5, 50.1 ± 10.4, and 53.4 ± 8.8 fmol/ml, respectively (Fig. 1). Based on these results, the plasma ghrelin concentration decreased early, during surgery, with a nadir on POD1, and remained low, at about 50 % of the preoperative ghrelin level, on PODs 3–10.

To examine the serial changes in ghrelin concentrations in each patient and search for distinct changes in patients with prolonged SIRS, we calculated the % ghrelin concentration at each time-point relative to the preoperative ghrelin concentration in each subject, assigned as 100 %. The data in Fig. 2 show a more profound decrease in ghrelin concentrations, especially on POD1, in patients with prolonged postoperative SIRS for >7 days ($n = 4$, solid line) than in the others ($n = 16$, dotted line).



	pre	2H	4H	POD1	POD3	POD10
Mean	107.4	66.0	49.7	37.7	50.1	53.4
SE	21.5	11.7	7.7	7.5	10.4	8.8

Fig. 1 Serial changes in mean plasma ghrelin concentrations after esophagectomy. Data are expressed as means ± the SEM of 20 patients

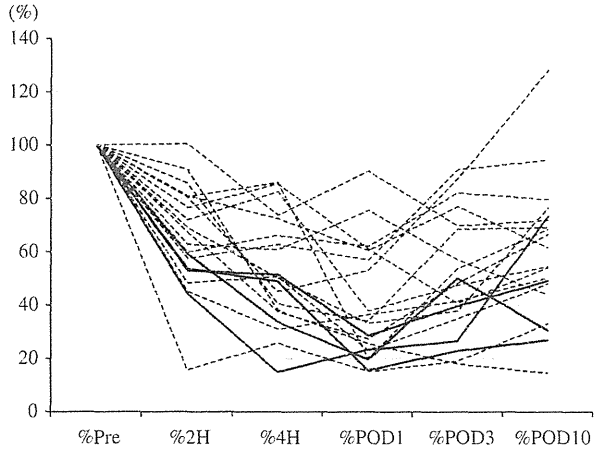


Fig. 2 Serial changes in the % ghrelin concentration for each subject. The % ghrelin concentration represents the percentage at each time-point relative to its concentration before surgery. *Solid lines* patients with SIRS ≥7 days; *dotted lines* patients with SIRS <7 days

Ghrelin deterioration on POD1 and postoperative clinical course

The median % ghrelin concentration on POD1 for the entire group was 33 % (range 15–90 %). We used a cutoff value of 33 % for the % ghrelin concentration on POD1, relative to the preoperative ghrelin concentration, to divide the patients into a high ghrelin concentration group with a ghrelin concentration on POD1 of ≥33 % (HG group, $n = 10$) and a low ghrelin concentration group with a ghrelin concentration on POD1 <33 % (LG group, $n = 10$). There were no differences in the clinical features between the groups (Table 1). However, the duration of SIRS was significantly longer in the LG group than in the HG group (6.0 ± 1.3 days vs. 2.1 ± 0.6 days; $p = 0.019$). Furthermore, the time required to attain a positive nitrogen balance tended to be longer in the LG group than in the HG group (5.8 ± 2.6 days vs. 4.0 ± 0.7; $p = 0.060$; Table 2). With regard to acute-phase parameters, the serum levels of IL-6 and C-reactive protein (CRP) on POD 1 were similar in the two groups, but the serum level of IL-6 on POD3 was significantly higher in the LG group than in the HG group (188.3 ± 48.6 pg/ml vs. 62.5 ± 24.1; $p = 0.037$). The CRP level on POD3 also tended to be higher in the LG group than in the HG group, but the difference did not reach significance (17.4 ± 2.7 mg/dl vs. 13.4 ± 1.8; $p = 0.223$).

Finally, we analyzed the relationship between the duration of SIRS and the various parameters measured on POD1, to find out if any clinical value could be added as a predictor of SIRS or as a therapeutic target(s) against excess inflammatory reaction (Fig. 3). Analysis of the linear regression relationships between the duration of SIRS and CRP, IL-6, leukocyte count, and % ghrelin on POD1 showed the strongest correlation with % ghrelin on POD1; that is, patients with a more profound fall in ghrelin concentration suffered a longer period of SIRS ($p = 0.066$). On the other hand, only the % ghrelin on POD 1, but not age, weight, preoperative therapy, preoperative complications, tumor stage, extent of lymph node retrieval, operative time, or blood loss, correlated with the duration of SIRS (data not shown).

Discussion

Ghrelin is a peptide hormone secreted mainly in the stomach, which was identified in 1999 as an endogenous ligand for the growth hormone secretagogue (GHS) receptor of the pituitary gland [10]. Subsequent studies found that this hormone plays a role in several biological functions, including the regulation of positive energy balance and an increase in appetite [11, 12]. Recent studies have also revealed the clinical impact of ghrelin on patients with cachexia associated with chronic obstructive pulmonary disease (COPD), chronic heart failure, chronic kidney disease, and malignancy, and its use has accomplished good results, not only for increasing appetite and weight, but for improving cardiopulmonary function and/or the primary disease [15, 20–22]. We reported previously that the administration of ghrelin after total gastrectomy for gastric cancer patients and after esophagectomy for esophageal cancer patients stimulated appetite and oral food intake, and attenuated postoperative weight loss, by maintaining lean body weight [23, 24]. It was also reported that ghrelin down-regulates proinflammatory cytokine production by inhibiting nuclear factor-κB activation in vitro, and attenuates sepsis-induced acute lung injury as well as mortality in vivo [16, 25]. We were interested in this function of ghrelin and initially examined the perioperative change in plasma ghrelin concentration after esophagectomy, one of the most invasive gastrointestinal

Table 1 Clinical characteristics of the esophagectomy patients in the high versus low ghrelin groups

	HG ($n = 10$)	LG ($n = 10$)	p value
Gender (male/female)	8/2	8/2	
Age (years)	64 ± 2	65 ± 3	0.661
Preoperative BMI (kg/m^2)	21.7 ± 0.7	20.1 ± 0.9	0.172
Preoperative complications (DM, OMI, COPD)	1	2	0.531
Location (upper/middle/lower thoracic part)	2/6/2	2/3/5	0.309
Clinical stage (I/IIA/IIB/III)	1/4/1/4	0/1/2/7	0.213
Preoperative therapy (none/CT/CRT)	2/7/1	1/9/0	0.453
Preoperative ghrelin concentration (fmol/ml)	94.5 ± 33.3	120.3 ± 28.5	0.5628

Data are expressed as mean ± SEM or the number of patients

HG group patients with a % ghrelin concentration on postoperative day (POD) 1 relative to the preoperative ghrelin concentration of >33 %, *LG group* patients with a % ghrelin concentration on POD1 relative to the preoperative ghrelin concentration of <33 %, *BMI* body mass index; clinical stage was classified according to the UICC classification, *CT* chemotherapy, *CRT* chemoradiotherapy, *DM* diabetes mellitus, *OMI* old myocardial infarction, *COPD* chronic obstructive pulmonary disease

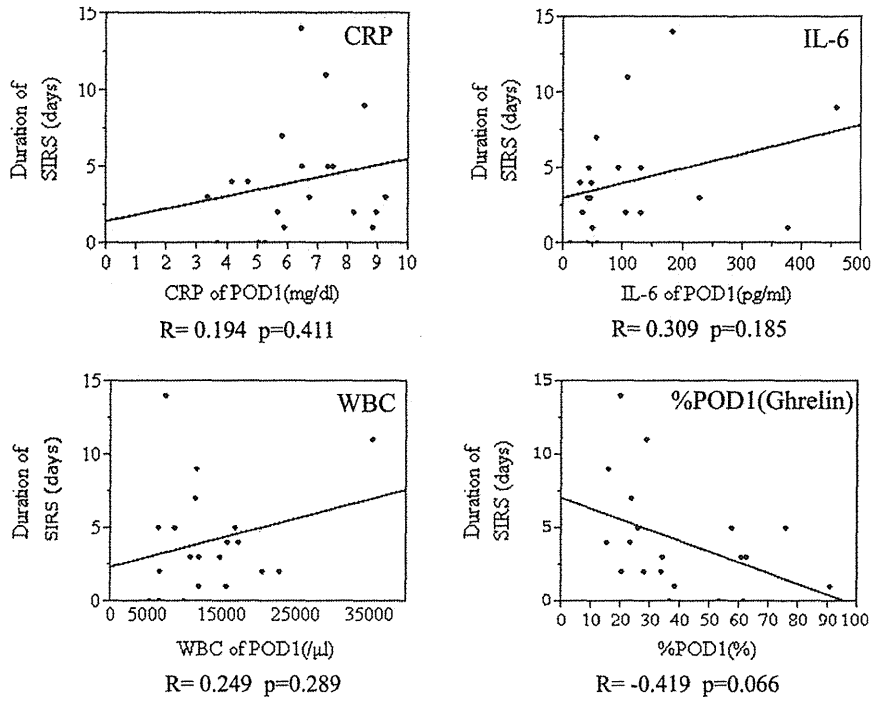


Fig. 3 Relationships between the parameters measured on postoperative day (POD) 1 and the duration of SIRS, evaluated by linear regression analysis

Table 2 Perioperative laboratory data of esophagectomy patients from the high and low ghrelin groups

	HG (n = 10)	LG (n = 10)	p value
Operation time (min)	464 ± 19	464 ± 15	0.987
Operative blood loss (ml)	612 ± 175	665 ± 249	0.592
Postoperative complications	2	3	0.605
Anastomotic leakage	1	0	
Pneumonia	1	3	
Percentage of patients developed SIRS (%)	70	100	0.030
Duration of SIRS (days)	2.1 ± 0.6	6.0 ± 1.3	0.019
Duration required to reach positive NB (days)	4.0 ± 0.7	5.8 ± 2.6	0.060
Serum IL-6			
POD1	106.2 ± 36.0	117.8 ± 41.0	0.834
POD3	62.5 ± 24.1	188.3 ± 48.6	0.037
Serum CRP			
POD1	6.2 ± 0.6	6.7 ± 0.5	0.544
POD3	13.4 ± 1.8	17.4 ± 2.7	0.223
Postoperative hospital stay (days)	28.8 ± 3.9	34.9 ± 6.2	0.414

Data are expressed as mean ± SEM or the number of patients

Abbreviations as in Table 1; *NB* nitrogen balance, *IL-6* Interleukin-6 (pg/ml), *CRP* C-reactive protein (mg/dl)

operations and known to cause excessive inflammatory response. The present study demonstrated a fall in the plasma ghrelin concentration during surgery and the extent of the fall on POD1 correlated with the postoperative duration of SIRS after esophagectomy. Previous studies also identified several parameters that could predict SIRS, such as soluble tumor necrosis factor receptor p 55 after cardiopulmonary bypass, myeloperoxidase in patients with myocardial infarction after primary percutaneous coronary intervention, and the soluble L-selection level in patients with sepsis [26–28]. To our knowledge, no factor has been defined as a predictor of prolonged SIRS during the early postoperative period after esophagectomy. Our results indicated that the % ghrelin on POD1 is a potentially useful predictor of the duration of SIRS after esophagectomy.

Our finding of a significant decrease in ghrelin concentration during the early postoperative period after esophagectomy is in contrast to the reported rise in plasma ghrelin levels 24 h after colon cancer resection and laparotomic cholecystectomy [29]. The exact reason for the decrease in ghrelin secretion from the stomach after esophagectomy is not clear, but it could be attributed in part to the vagotomy or gastric tube reconstruction. However, vagotomy was performed more than 2 h after the start of surgery, whereas the fall in ghrelin concentration started before the vagotomy. Thus, it appears that more than one mechanism is involved in reducing the plasma ghrelin concentration after esophagectomy.

Although prolonged SIRS might cause the plasma ghrelin concentration to drop, the fact that the lowest ghrelin concentration on POD 1 was closely related to the duration of SIRS according to the other parameters measured on POD1, such as the leukocyte count, CRP, and

IL-6 levels, highlights the potential use of ghrelin as a predictor of SIRS as well as a therapeutic target to prevent SIRS. Ghrelin is also known as a suppressor of the excessive production of proinflammatory cytokines and an inhibitor of excessive inflammation-induced organ injury. These properties should enhance research on the anti-inflammatory effects of ghrelin. To our knowledge, there is no information on the clinical effect of administering ghrelin to humans in an acute inflammatory state or shortly after invasive surgery. In rat models of radiation injury and lipopolysaccharide-induced sepsis, intravenous ghrelin attenuated organ injury, reduced cytokine levels, and improved survival. [30, 31] The protective effects of ghrelin on cardiopulmonary function may also be advantageous in the early postoperative period after esophagectomy [14, 15, 20, 21, 32].

In conclusion, the results of this study, in accordance with previous reports, support the next step in investigating the efficacy of ghrelin administration in patients with esophageal cancer to attenuate postoperative inflammatory cytokine production and minimize the duration of SIRS after esophagectomy.

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Conflict of interest We declare no potential conflicts of interest.

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Clinical Significance of Ghrelin Expression in the Gastric Mucosa of Morbidly Obese Patients

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Abstract
Background The concentration of ghrelin, which can affect body weight by influencing appetite, is thought to decrease after sleeve gastrectomy. However, no detailed investigations have examined ghrelin expression in the stomach. The purpose of the present study was to assess localized ghrelin expression and its clinical significance in obese patients.
Methods A total of 52 obese patients who underwent sleeve gastrectomy with or without duodenojejunal bypass were enrolled in the study. The number of ghrelin-positive cells (GPCs) was counted using immunohistochemistry of the gastric mucosa at the fundus. The obese patients were compared with 14 nonobese patients treated for gastric cancer. Ghrelin mRNA expression was also measured in 22 obese patients using a quantitative reverse transcription polymerase chain reaction.
Results The number of GPCs was significantly higher in obese patients than in nonobese controls (33.2 ± 18.3 vs. 14.1 ± 6.1; $p < 0.001$) and correlated with ghrelin mRNA expression. The obese patients were divided into two groups with high and low ghrelin levels based on the number of GPCs. The percent excess body weight loss was significantly greater in the high-ghrelin group, without differences in the patient backgrounds between the two groups ($p = 0.015$).
Conclusions The number of GPCs was higher in obese patients than in nonobese patients and varied individually regardless of body weight. These results suggest that ghrelin expression in gastric mucosa might be a prognostic factor after surgery.

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Introduction

According to the World Health Organization, approximately 2.3 billion adults will be overweight and >700 million obese by 2015. The use of bariatric surgery has thus spread, and its outcomes are continuously improving [1–4]. Restrictive procedures, such as laparoscopic adjustable gastric banding (LAGB) and laparoscopic sleeve gastrectomy (LSG), achieve weight loss by limiting food intake. Other procedures, such as Roux-en-Y gastric bypass (RYGB), combine both malabsorption and a restrictive approach to result in effective weight loss.

Marceau et al. [5] introduced LSG in 1993 as a new type of gastrectomy in conjunction with biliopancreatic diversion. This approach was used initially as a staged laparoscopic intervention performed before biliopancreatic diversion or gastric bypass in cases of extreme morbid obesity [6]. Because the procedure is technically simple and associated with good short-term outcomes regarding weight loss, LSG is being used increasingly as definitive surgery for morbidly obese patients [7–9].

Ghrelin, a gut hormone known to increase appetite and regulate weight and body composition, is considered to play an important role in the good surgical outcomes associated with LSG. Thus, the favorable effects of LSG are derived not only from the combination of volume

restriction and creation of a high-pressure system but also from the reduction in ghrelin concentration [10]. Because the ghrelin-producing area of the stomach is removed during LSG, ghrelin levels have been shown to decrease up to 5 years after surgery [11, 12].

However, many reports have indicated that plasma ghrelin levels negatively correlate with the body mass index (BMI). In other words, the ghrelin concentration is lower in obese patients than in lean subjects [13, 14]. This finding prompts the following questions: (1) Why do obese patients have an enormous appetite despite a low ghrelin concentration? (2) Does the reduction in ghrelin have clinical significance? Currently, basic research evidence is insufficient to resolve these questions, and the regulatory mechanisms underlying ghrelin expression are not fully understood. In addition, ghrelin concentrations are affected by several conditions, including the method of blood collection, mental stress, and diet. The quantification of ghrelin-positive cells (GPCs) can be useful for comprehensively evaluating the ghrelin profile [15]. Thus, the number of GPCs might be an indicator of ghrelin activity, even in obese patients.

Although many studies have addressed perioperative circulating ghrelin levels during the bariatric procedure, the local ghrelin environment, including the number of GPCs, has not been thoroughly investigated. The aim of the present study was to clarify the clinical significance of the number of GPCs in the stomach.

Methods

Patients

A total of 52 obese patients (6 referred to Osaka University Hospital and 46 referred to Yotsuya Medical Cube) were consecutively enrolled in the study from March 2010 to November 2011. Among these patients, 35 underwent LSG and 17 underwent LSG with duodenojejunal bypass (LSGB). All participants met the standard National Institutes of Health criteria for bariatric surgery (BMI > 40 or >35 kg/m^2 with obesity-related co-morbidities). Exclusion criteria included previous bariatric surgery, chronic liver or renal disease, and/or heart failure. The institutional review board approved the study, and all patients provided written informed consent.

Surgical technique

LSG was performed according to the technique described by Regan et al. [16]. Skeletization of the greater curvature of the stomach was initiated 5 cm from the pylorus up to the angle of His using a radiofrequency device. A 36F transoral bougie was positioned from the hiatus to the duodenum along the lesser curvature. The LSG was performed using a linear stapler (EndoGIA; US Surgical, Norwalk, CT, USA) to construct a gastric pouch with 60-ml capacity.

The LSGB procedure has been described previously [17]. Briefly, sleeve gastrectomy was performed using a 36F bougie and linear staplers 5-cm orally from the pyloric ring, similar to LSG. The posterior wall of the duodenum was carefully dissected and the first portion of the duodenum was completely devascularized. The duodenum was divided using a linear stapler. The biliopancreatic limb was measured 50–100 cm from the ligament of Treitz, and the jejunum was divided using a stapler. The alimentary tract was also measured out to 150–200 cm. The jejunojejunostomy was performed using a linear stapler, and an entry hole was closed by hand-sewing. A duodenojejunal end-to-side anastomosis was created using two layers of running suture, including an absorbable one.

Blood sampling and serum ghrelin assay

Because several patients in this study refused additional blood draws, further blood sampling for serum ghrelin levels were performed in only 14 patients. Blood samples were collected before breakfast after an overnight fast, transferred into chilled tubes, stored on ice during collection, and centrifuged. Serum was separated and stored at −50 °C until assay. Serum desacyl ghrelin concentrations, which are almost equivalent to total ghrelin, were measured using sandwich-type enzyme immunoassay kits for ghrelin according to the manufacturer's protocol (Mitsubishi KagakuIatron, Tokyo, Japan) [18].

Immunohistochemistry for GPC counts

Stomach specimens resected during LSG were thoroughly washed in saline immediately after extraction to remove debris (e.g., blood, loose fatty tissue, mucus). The stomach specimens were fixed in buffered 10 % formalin for 24 h. After fixation, six tissue samples were obtained from the fundus. These samples were paraffinized, sectioned, and mounted on slides. Immunohistochemical staining of GPCs was performed using the streptavidin–biotin–peroxidase complex method (HistofineSAB-PO Kit; Nichirei Biosciences, Tokyo, Japan). The following steps were performed at room temperature unless otherwise specified. Paraffin-embedded specimens were sectioned at 3.5 μm thickness, deparaffinized, and dehydrated. To enhance the immunoreactivity of ghrelin, antigens were retrieved at 95 °C for 40 min in citric acid buffer. After blocking endogenous peroxidase activity for 20 min with methanol containing 1 % H_2O_2, the sections were reacted for 15 min with

normal goat serum to prevent nonspecific binding. The sections were then incubated with anti-rat ghrelin polyclonal antibody (Trans Genic, Kumamoto, Japan) at 4 °C overnight. This anti-rat ghrelin antibody specifically recognizes the N-terminal fragment of ghrelin and is able to recognize both rat and human ghrelin [19, 20]. The next day, the sections were washed in 0.01 M phosphate-buffered saline (PBS) and incubated for 20 min with biotinylated goat anti-rabbit immunoglobulin G antibody (10 μg/ml). After washing with PBS, the sections were reincubated for 20 min with peroxidase-conjugated streptavidin (100 μg/ml) and stained with 3,3′-diaminobenzidine tetrahydrochloride in 0.05 M tris–HCl buffer containing H_2O_2. The sections were finally washed in PBS and counterstained with hematoxylin. Negative controls were treated identically but without the primary antibody.

The GPCs in the stomach were small and round or spindle-shaped. GPCs were abundant from the neck to the bottom of the fundic glands. Semiquantitative evaluation of the GPCs was performed by counting the cells under a microscope. At 100× magnification, the microscope field was adjusted to the gastric mucosa area, and the GPCs (which were clearly recognizable as cells with a small amount of cytoplasm and dark brown staining) were counted. Ten fields of mucosa were randomly chosen in each 4 cm long section, and the average number of GPCs was recorded and defined as the GPC count.

As a control, we examined the normal fundic mucosa of 14 patients who underwent total gastrectomy for early gastric cancer. These patients had histologically proven stage IA gastric cancer according to the 7th edition American Joint Committee on Cancer (AJCC)/International Union against Cancer (UICC) tumor–node–metastasis (TNM) staging system. These patients were sex- and age-matched with respect to the obese patients. The GPC counts were determined in the control group using the same method as described for the LSG patients.

Quantitative reverse transcription polymerase chain reaction

Resected samples from 22 patients who consented to the gene analysis were available to evaluate RNA expression. For each patient, two normal mucosa samples (5-mm cubes) were collected from the fornix. The samples were immediately lysed in 175 ml of RNA lysis buffer (4 M GTC, 0.01 M Tris pH 7.5, 0.97 % β-mercaptoethanol; Promega, Southampton, UK). RNA was extracted using the Promega SV Total RNA Isolation kit following the manufacturer's protocol. Contaminating genomic DNA was removed using DNase. The RNA yield and purity were determined using a spectrophotometer at 260 and 280 nm. First strand cDNA synthesis was performed using RNase reverse transcriptase (GIBCO BRL, Paisley, UK). For reverse transcription, the Promega Reverse Transcription System was used according to the manufacturer's protocol. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed using custom oligonucleotide primers and a Light Cycler (Roche Diagnostics, Tokyo, Japan) to calculate ghrelin mRNA expression. Ghrelin expression was normalized relative to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was used as an internal control. The primers were as follows: ghrelin, forward, 5′-TGAGCC CTGAACACCAGAGAG-3′, reverse 5′-AAAGCCAGATGAGCGCTTCTA-3′, expected size 327 bp; GAPDH, forward 5′-CAACTACATGGTTTACA TGTTC-3′, reverse 5′-AAATGAGCCCCAGCCTTC-3′.

Statistical analysis

Data are presented as the median (range) or mean (±SD). The Wilcoxon paired signed rank test was used to compare the clinical and laboratory data between nonobese and obese patients. A similar statistical analysis was applied to compare GPC counts in the stomach. The Pearson correlation coefficient (r) and its significance (p) were calculated between variables. Comparisons of changes in the percent excess body weight loss were tested by two-way repeated measure analysis of variance (ANOVA). Analyses were carried out using JMP version 8.0 (SAS Institute, Cary, NC, USA). A two-sided $p < 0.05$ was considered significant.

Results

GPC counts and serum desacyl ghrelin levels

No significant difference was observed in the demographics of nonobese and obese patients (Table 1). GPC counts tended to vary with the presence or absence of obesity rather than among individuals upon first view of the immunohistochemistry images (Fig. 1).

Figure 2 shows the correlation between BMI and GPC counts. The GPC count varied among individuals in both

Table 1 Patient demographics

Characteristic	Nonobese ($n = 14$)	Obese ($n = 52$)	p
Sex (M/F)	8/6	25/27	0.43
Age (years)	46 (31–68)	43 (23–65)	0.44
Preoperative BMI (kg/m^2)	21.5 ± 3.8	40.6 ± 7.7	<0.001

Data are number of patients, median (range), or mean ± SDs

BMI body mass index

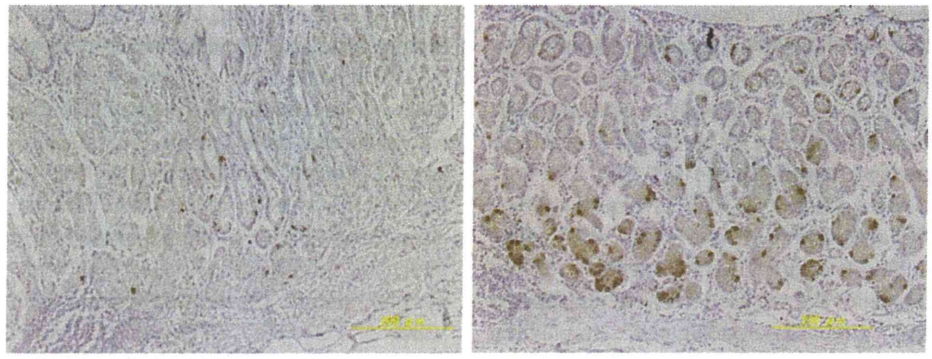

Fig. 1 Representative immunohistochemistry images of GPCs in gastric fundus excised from a nonobese patient and an obese patient

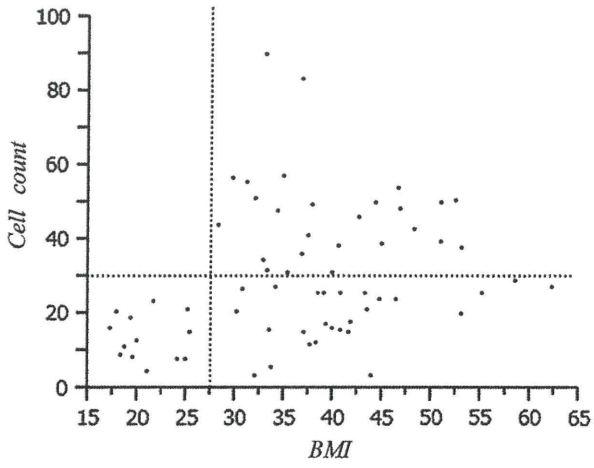

Fig. 2 Correlation between BMI and ghrelin cell count. The *vertical dotted line* represents the border between nonobese and obese patients. The *horizontal dotted line* represents the median GPC count (30 GPCs)

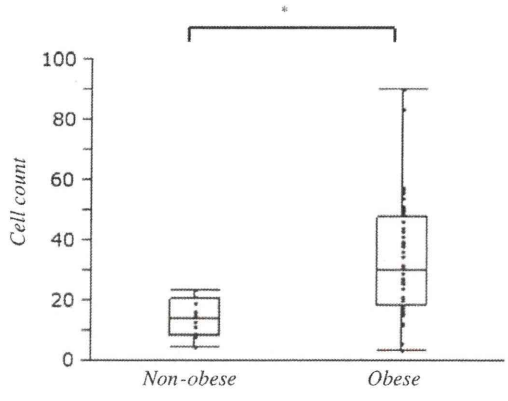

Fig. 3 Comparison of the number of GPCs in nonobese and obese patients. *Lines within the boxes* represent median values; *upper and lower lines of the boxes* represent the 25th and 75th percentiles, respectively; *upper and lower bars outside the boxes* represent the 90th and 10th percentiles, respectively. $*p < 0.05$, by the Wilcoxon paired signed rank test

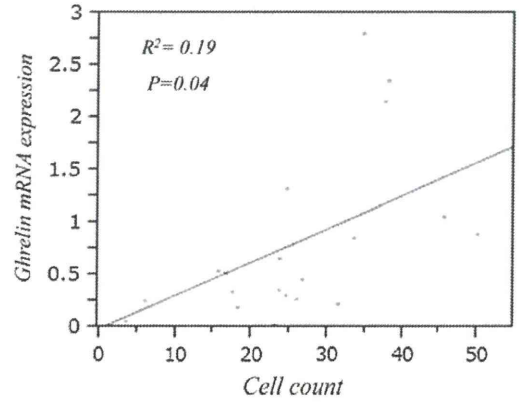

Fig. 4 Correlation between GPC count and mRNA expression; $R^2 = 0.19$, $p = 0.04$

the nonobese and obese groups. The GPC counts were significantly higher in obese patients (33.2 ± 18.3) than in nonobese patients (14.1 ± 6.1) ($p < 0.001$) (Fig. 3). Conversely, serum ghrelin levels were lower in obese patients (90.0 ± 48.7) than in nonobese patients (127.6 ± 70.2), although the difference was not statistically significant.

Quantitative mRNA assessment of ghrelin expression

RNA was extracted from all samples without difficulty, and the level of ghrelin mRNA expression was normalized to the expression of GAPDH. Figure 4 shows a slight correlation between GPC count and ghrelin RNA expression ($R^2 = 0.19$, $p = 0.04$). Three cases exhibited remarkably high expression.

Influence of GPC count and desacyl ghrelin level on surgical outcome

As GPC counts varied among individuals, even in the obese group, we divided the participants into two groups based on high ($n = 26$) and low ($n = 26$) GPC counts

Table 2 Comparison between background data and ghrelin cell count groups

Parameter	Ghrelin cell count		
	High count	Low count	*p*
No. of patients	26	26	
Sex (M/F)	9/17	16/10	0.051
Age (years)	43.0 (23–62)	43.5 (25–65)	0.71
BMI (kg/m^2)	39.7 ± 7.5	41.5 ± 8.0	0.40
Excessive body weight (kg)	47.3 ± 20.3	53.9 ± 23.8	0.28
Infantile obesity (±)	14/12	16/10	0.57
History of DM (±)	15/11	19/7	0.24
History of hyperlipidemia (±)	15/11	21/5	0.07
History of hyperuricemia (±)	11/15	10/16	0.78
LSG/LSGB	18/8	17/9	0.76

Data are number of patients, median (range), or mean ± SD

LSG laparoscopic sleeve gastrectomy, *LSGB* LSG with duodenojejunal bypass, *DM* diabetes mellitus

Table 3 Comparison between preoperative laboratory data and ghrelin cell count groups

Parameter	Ghrelin cell count		
	High count	Low count	*p*
Total cholesterol (mg/dl)	192.5 ± 38.2	190.4 ± 50.5	0.65
Triglycerides (mg/dl)	153.3 ± 95.0	135.2 ± 56.0	0.65
Fasting glucose (mg/dl)	115.8 ± 28.5	124.2 ± 31.0	0.27
HbA1c (%)	6.4 ± 1.2	6.6 ± 1.4	0.52
Uric acid (mg/dl)	6.8 ± 1.3	6.4 ± 1.2	0.16

Data are mean ± SD

HbA1c hemoglobin A1c

using a cutoff value of 30 GPCs. Table 2 compares the patients' backgrounds between the two GPC groups. No significant difference was observed between the high and low GPC groups regarding the preoperative state of the obese patients (Table 3). However, significantly more excess body weight was lost in the high GPC group than in the low GPC group ($p = 0.02$) (Fig. 5a). The percent excess body weight loss 6 months after surgery was 68.8 ± 23.8 % in the high GPC group and 55.1 ± 19.7 % in the low GPC group, respectively. No differences in triglycerides, fasting glucose, hemoglobin A1c (HbA1c), or uric acid were observed, and these obesity-related complications resolved after surgery in each group.

We also divided the patients into two groups based on high ($n = 7$) and low ($n = 7$) evaluated desacyl ghrelin using a cutoff value of 84 fmol/l, which was the median level. Contrary to GPC counts, the desacyl ghrelin level was not significantly correlated with excess body weight loss ($p = 0.36$) (Fig. 5b).

Discussion

This report is the first to focus on the relation between the number of ghrelin cells in the stomach and the effect of LSG on body weight. Ghrelin is the endogenous ligand for the growth hormone (GH) secretagogue receptor 1a, which stimulates GH release from the pituitary gland. Ghrelin is predominantly secreted by gastric endocrine cells. It stimulates food intake and triggers a positive energy balance through a central mechanism involving hypothalamic neuropeptides [21, 22]. The protein also has a variety of other activities, such as activating gut motility [23], improving cardiopulmonary functions [24, 25], and anti-inflammatory effects [26]. The peripheral hormone ghrelin recently drew attention as an important peptide in obesity, even though the appestat is located in the hypothalamus. Also, its secreted hormones or peptides in the central nervous system (e.g., neuropeptide Y, agouti-related

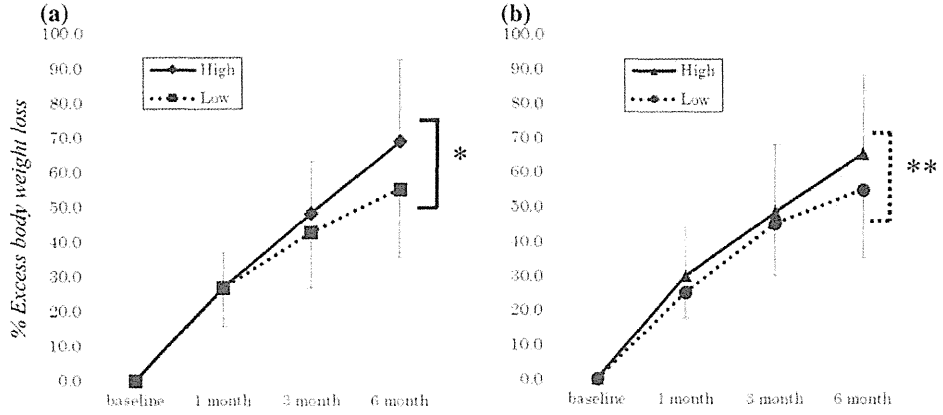

Fig. 5 Changes in percent excess body weight loss from baseline. **a** Each GPC count group. *$p < 0.05$ by ANOVA. **b** Each desacyl ghrelin group. **$p = 0.3877$ by ANOVA

protein homolog, and a melanocyte-stimulating hormone) are directly related to appetite control.

Serum ghrelin concentration, which should be measured in the morning after fasting, is determined as the basic level of ghrelin due to circadian variation [14]. The circulating ghrelin level exhibits a significant inverse correlation with the BMI [13], although morbidly obese patients present with an abnormally large appetite and eating disorder despite a low concentration of ghrelin. These conflicting phenomena reflect the uncertainty surrounding circulating ghrelin levels in obese patients and may be related to a disappearance of ghrelin's circadian rhythm. Cummings et al. [27] reported that plasma ghrelin levels in obese patients increase shortly before and decrease shortly after every meal (a "low" concentration circadian rhythm) as in lean subjects. They also found that ghrelin levels after gastric bypass were abnormally low, exhibiting "no circadian rhythm" related to meals, which raises the possibility that this operation reduces weight partly by suppressing ghrelin production [27]. Although the number of patients in that study was small, conflicting information with respect to the plasma ghrelin level and abnormally large appetite in obese patients could not be explained by these results alone. The "low" ghrelin concentration circadian rhythm did not result in a large appetite, and low ghrelin levels without a circadian rhythm after bypass surgery could not be explained because the bypass procedure did not include gastrectomy.

Because Asia has a larger population with gastric cancer compared with Western countries, gastric tissue samples from nonobese patients are not challenging to collect. Therefore, we evaluated local ghrelin expression in the stomach and have already reported the clinical significance of GPC counts using a mapping analysis [15]. The distribution of GPCs in obese patients was similar to that in past reports [28], and the number of GPCs decreased distally from the fundus.

In this study, using a method similar to the ghrelin cell mapping analysis, the number of GPCs at the fundus in morbidly obese patients was estimated semiquantitatively and compared with that in nonobese patients. Surprisingly, the GPC counts were significantly higher in obese patients than in nonobese patients throughout the analysis. Otherwise, the available fasting serum ghrelin levels in 14 obese patients were lower than those of nonobese patients, as had been reported in several published articles [13, 14]. These results have the potential to explain the discrepancy between GPC counts and serum ghrelin levels, although sample number was insufficient to prove this hypothesis. Maksud et al. [29] reported a similar finding of a higher density of ghrelin-immunoreactive cells in the oxyntic mucosa of morbidly obese patients without diabetes mellitus. Although their results were derived from biopsy samples of oxyntic mucosa, the distribution of GPCs was similar in obese and nonobese patients [28], and the number of GPCs decreased distally from the fundus. This finding means that GPC counts using biopsy samples differ for each biopsy site. Therefore, to assess GPC counts of multiple sites, it is considered more appropriate to use resected gastric specimens obtained as close to the fundus as possible, as in our study. Although the mechanism underlying the discrepancy between GPC counts and ghrelin concentration is unknown, our study showed higher GPC counts more accurately in obese patients than in nonobese patients and indicated the individual variability in GPC counts. However, the use of gastric cancer patients as the nonobese control group should be noted because several factors related to gastric cancer, such as cachexia, atrophic gastritis, and *Helicobacter pylori* infection, can potentially reduce ghrelin expression [30, 31]. Among the 14 nonobese patients included in this series, only three had atrophic gastritis and none were cachectic. Data regarding *H. pylori* infection were unavailable. We considered this group suitable as a control population because there were not too many patients with factors that influenced ghrelin expression. In fact, the mean serum ghrelin level in the nonobese patients was 127.6 fmol/l, which was not particularly low. The reason for the discrepancy remains unknown, although it might have been affected by impaired ghrelin secretion. A ghrelin expression level localized to the stomach (as the GPC count is) may be a better indicator of ghrelin activity in obese patients.

Interestingly, the high GPC count group exhibited better excess body weight reduction than the low GPC count group. This difference was not correlated with circulating ghrelin levels. If GPC counts accurately reflect ghrelin activity, ghrelin overproduction may induce abnormal appetite in patients with high GPC counts. Bohdjalian et al. stated that circulating ghrelin decreased significantly after LSG and remained stable for the duration of a 5-year follow-up. These results support the findings of LSG results regarding stable weight loss. As more than 90 % of ghrelin is produced by the stomach, ghrelin production in all patients after LSG is theoretically considered to be reduced to 10 % of preoperative levels [11]. When considering both past reports and the results of this study, the hypothesis may be accepted as follows: the higher the GPC count, the better the surgical outcome of LSG. If sufficient data indicate that ghrelin mRNA expression correlates with the GPC count, preoperative ghrelin mRNA analysis might be a predictor of LSG outcomes, although large-scale studies and precise evaluation are still required.

In addition to several factors that affect ghrelin expression, it should be noted that there is a circadian rhythm of ghrelin secretion [32]. Spiegel et al. [33] reported the relation between ghrelin circadian rhythm and glucose metabolism. In a recent study, Gocl ct al. [34]

demonstrated a reduced amplitude in the circadian rhythms of food intake in obese patients with night eating syndrome, as well as in the levels of ghrelin and other metabolism-related hormones. Considerations from every possible angle regarding the ghrelin profile would be needed to clarify ghrelin involvement more precisely with respect to body weight reduction after LSG.

The ongoing obesity epidemic has worsened during the past decade and become a worldwide public health priority [35]. To solve this problem and establish a better strategy against obesity, obesogenic factors must be identified, especially regarding appetite-related hormones such as ghrelin, and hypothalamic hormones.

Although the present study had several limitations, such as a lack of sufficient samples, follow-up period, and small study population, its results were interesting regarding the relation between localized ghrelin expression and clinical outcomes. Our study may have revealed one reason for significant surgical outcomes after LSG and may implicate LSG as the rational and ideal procedure for bariatric surgery, as almost all of the ghrelin-producing regions of the stomach are resected during the procedure.

Conclusions

The number of GPCs was greater in obese patients than in nonobese patients. In addition, excellent body weight reduction was obtained after sleeve gastrectomy, especially for the high-ghrelin group. Although additional studies are needed, our findings suggest that the GPC count might be a prognostic factor regarding the surgical results of LSG.

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Conflict of interest The authors declare that they have no conflicts of interest.

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FULL PAPER

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Keywords: pancreatic cancer; gemcitabine; interleukin-1β; interleukin-6; chemotherapy

Serum levels of IL-6 and IL-1β can predict the efficacy of gemcitabine in patients with advanced pancreatic cancer

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Background: With this study, we sought to characterise the impact of pro-inflammatory cytokines on the outcomes of gemcitabine monotherapy (GEM) in patients with pancreatic cancer (PC).

Methods: Treatment-naive patients with advanced PC and no obvious infections were eligible for enrolment. All of the patients were scheduled to undergo systemic chemotherapy. Serum pro-inflammatory cytokines were measured using an electro-chemiluminescence assay method before chemotherapy. High cytokine levels were defined as values greater than the median. Clinical data were collected prospectively.

Results: Sixty patients who received GEM were included in the analysis. High IL-6 and IL-1β levels were poor prognostic factors for overall survival in a multivariate analysis ($P = 0.011$ and $P = 0.048$, respectively). Patients with both a high IL-6 level and a high IL-1β level exhibited shortened overall and progression-free survival, a reduction in the tumour control rate, and a high dose intensity of GEM compared with patients with low levels of both IL-6 and IL-1β.

Conclusion: The serum levels of IL-6 and IL-1β predict the efficacy of GEM in patients with advanced PC.

An increase in inflammatory markers is associated with poor prognosis in patients receiving systemic chemotherapy for advanced pancreatic cancer (PC) (Tanaka *et al*, 2008; Morizane *et al*, 2011). C-reactive protein (CRP) is an index of systemic inflammation that is synthesised in hepatocytes by pro-inflammatory cytokines, including IL-1β (Young *et al*, 2008), IL-6 (Morrone *et al*, 1988), IL-8 (Wigmore *et al*, 1997), and TNF-α (Ganapathi *et al*, 1998), via the transcription factor nuclear factor-κB (NF-κB) and the activation of the signal transducer and activator of transcription 3 (STAT3) protein (Nishikawa *et al*, 2008). NF-κB and STAT3 represent major inflammatory pathways for pro-inflammatory cytokines and contribute to the chemoresistance of tumours (Aggarwal *et al*, 2009). An increase in the effects of pro-inflammatory cytokines is believed to attenuate the benefits of chemotherapy and to result in a poor outcome. Recently, the efficacy of anti-inflammatory therapy has been reported in several diseases: with canakinumab as an IL-1β blocker in the cryopyrin-associated periodic syndrome (Kuemmerle-Deschner *et al*, 2011), with tocilizumab as an IL-6 receptor blocker in rheumatoid arthritis (Jones *et al*, 2010), and with siltuximab as an IL-6 blocker in prostate cancer (Dorff *et al*, 2010). In the blockade of intracellular pathways, ruxolitinib is a Janus kinase inhibitor that

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