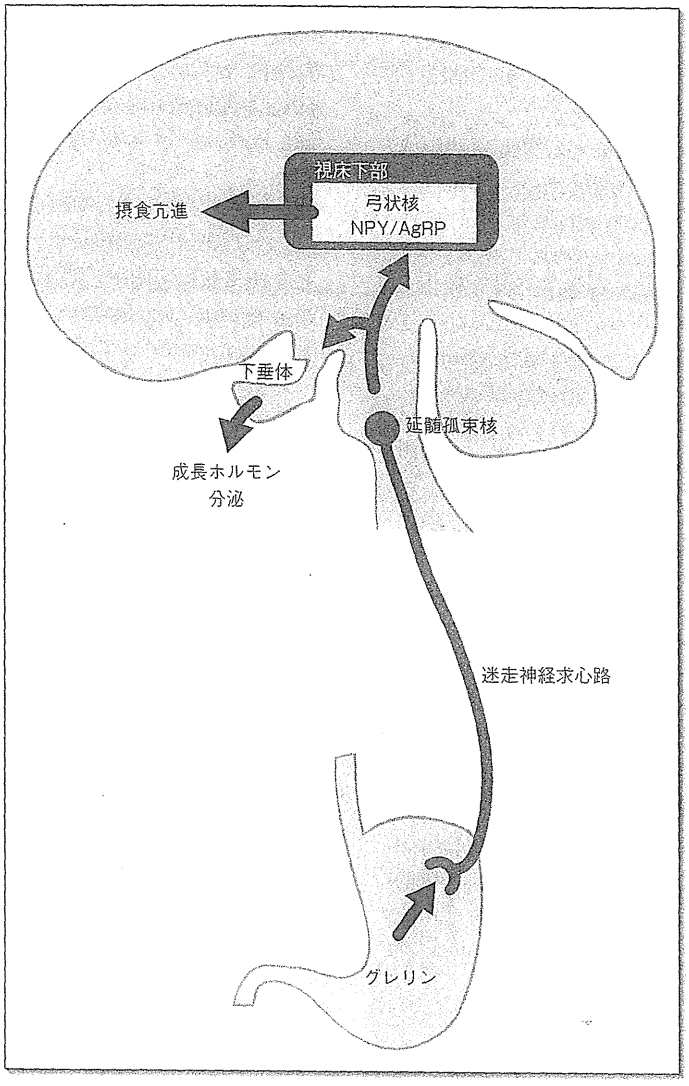


図1．胃で分泌されたグレリンの脳における作用経路

レリン受容体も発現しており，脳室内に投与されたグレリンは視床下部弓状核のNPY/AgRPニューロンを直接に刺激し，強力な摂食亢進作用を示す[2]。NPY/AgRPニューロンにおけるGHSR-1aの刺激はSIRT1/p53経路を活性化，p53とカンナビノイドのシグナルによってAMPキナーゼをリン酸化する。リン酸化AMPキナーゼによりmTORがリン酸化され，リン酸化mTORによってNPY/AgRPが産生されることが知られている[19]。また，POMCニューロンにもグレリン受容体が発現しており，グレリンの刺激は摂食抑制蛋白POMC産生ニューロンを抑制する。さらに，視床下部には摂食行動調節に関わっていると考えられている外側野と室傍核が存在する。外側野においては摂食行動を促進するオレキシンが産生され，室傍核においては摂食抑制作用を有するcorticotropin-releasing hormone（CRH）が産生される。グレリンは視床下部弓状核のNPY/AgRPニューロンを介して，外側野オレキシン産生細胞を刺激し，室傍核のCRH産生細胞を抑制する。

## まとめ

グレリンと摂食調節機構について概説した。グレリンは食欲の調節において中枢性，末梢性に非常に重要な役割を果たしているが，摂食行動を促進または抑制するホルモンや物質は上記以外にも多数知られている。これらが複合的に作用して摂食が調節されていると考えられており，その交絡的な作用の詳細はまだ明らかではない。摂食行動の調節は特定のホルモンや物質のみでは単純には説明できないしくみであり，今後も多くの知見の蓄積が必要な研究領域である。

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REVIEW ARTICLE

# Clinical application of ghrelin administration for gastric cancer patients undergoing gastrectomy

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**Abstract** Loss of body weight is a common (and the most serious) sequela after gastrectomy. It impairs quality of life, increases various diseases including infection, and may affect long-term survival. Ghrelin, an intrinsic ligand of the growth hormone secretagogue receptor, was discovered in the stomach in 1999. In addition to growth hormone secretion, ghrelin has pleiotropic functions including appetite stimulation, increasing bowel movement and absorption, and anti-inflammatory reactions. In consequence, ghrelin comprehensively leads positive energy balance and weight gain. The fundic gland of the stomach produces the majority of ghrelin, and plasma ghrelin declines to 10–30 % of the preoperative level after total gastrectomy and 50–70 % after distal gastrectomy. Although plasma ghrelin is never restored after total gastrectomy, it gradually recovers to the preoperative level within a few years after distal gastrectomy. Chronic gastritis due to *Helicobacter pylori* infection and vagotomy are additional factors that perturb the ghrelin secretion of gastric cancer patients after gastrectomy. A randomized clinical trial that revealed that recombinant ghrelin administration successfully increased both food intake and appetite, and ameliorated weight loss after total gastrectomy. Ghrelin administration could thus be a promising strategy to transiently improve the nutritional status of patients who who have undergone gastrectomy, but its effect in the long term remains unclear. Further studies are warranted to elucidate the mechanism of ghrelin and to create and evaluate the analogs that could be administered orally or subcutaneously.

**Keywords** Ghrelin · Gastrectomy · Gastric cancer · Weight loss

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## Introduction

Loss of body weight is a common, serious outcome in patients with gastric cancer who have undergone gastrectomy. It correlates well with a decline in postoperative quality of life and is the most reliable indicator of malnutrition, which impairs immune function, infection susceptibility, and survival [1–3]. Although various mechanisms have been considered, such as the perturbation of absorption due to reduced pancreatic excretion [4, 5], a decrease in the gastric acid level [6], reflux esophagitis [7], intestinal floral alteration [8], and increased peristalsis and diarrhea [9], reduced food intake [10, 11] is the most conceivable explanation for weight loss after gastrectomy. To combat loss of appetite, surgeons dealing with gastric cancers have tried to increase food intake by producing a gastric substitute, such as a jejunal pouch, with limited success [12]. However, we frequently observe that patients do not exhibit significant weight loss after total gastrectomy when they resort to small but frequent meals. Another study indicated that the majority of patients with total gastrectomy were able to eat as much food as healthy subjects under a regulated program [13].

Taken together, we can conclude that (1) patients who have undergone gastrectomy have the ability to maintain body weight when food intake is adequately performed; (2) only loss of storage volume cannot account for reduced

food intake after gastrectomy; (3) there is a relatively large change in eating behavior after gastrectomy that is controlled by an unknown mechanism. In this review, we discuss ghrelin and research about its clinical applications.

## The discovery of ghrelin and its features

Ghrelin is a peptide hormone that was discovered in 1999 as an endogenous ligand for the growth hormone (GH)-secretagogue receptor (GHS-R). The 28-amino-acid ghrelin peptide is the endogenous ligand for GHS-R1a, which stimulates GH release from the pituitary gland [14]. X/A-like cells of the oxyntic glands in the stomach produce the majority of ghrelin, and smaller amounts are secreted by other organs, such as the intestine, pancreas, kidney, and hypothalamus [15, 16]. Ghrelin has several physiological functions in addition to the secretion of GH, including the promotion of the appetite signal that antagonizes leptin in the hypothalamus [17], stimulation of gastrointestinal activity (e.g., peristalsis, gastric acid secretion, and pancreatic excretion through the vagal nerves) [18], and regulation of fat metabolism [19] (Table 1). Ghrelin also mitigates pro-inflammatory cytokine production and attenuates the stress signal [20]. Ghrelin exists as two major molecular forms: acyl ghrelin and des-acyl ghrelin. Ghrelin is octanoylated at Ser3, an unusual post-translational modification that is catalyzed by the enzyme ghrelin O-acyltransferase (GOAT) [21, 22]. Des-acyl ghrelin, which lacks the Ser3 residue octanoylation, is unable to release GH or bind to the classic GHS-R1a receptor [23]. These characteristics indicate that octanoic acid plays an important role in physiological activity via GHS-R1a, and des-acyl ghrelin has been considered an inactive form of ghrelin.

Ghrelin peptide is the only gastrointestinal hormone known to stimulate appetite. A randomized double-blind study of healthy volunteers demonstrated that ghrelin enhances appetite and increases food intake [24, 25]. Several clinical trials of patients with heart failure [26], pulmonary disease [27], cancer cachexia [28], or undergoing chemotherapy [29] concluded that ghrelin successfully improved their diseases along with increased oral food intake and body weight. In the field of surgical treatment for obesity, reduced ghrelin levels after sleeve gastrectomy are associated with successful weight loss and appetite suppression [30]. Taken together, the discovery of ghrelin allows the proposal of a new concept, body weight regulation by the stomach, which can be applied to various diseases with malnutrition.

## Gastrectomy and ghrelin secretion

Fundic glands in the stomach produce the majority of ghrelin. Patients with resected gastric cancer experience low plasma ghrelin concentrations. Table 2 lists studies of the change in ghrelin concentration after gastrectomies [31–37]. In total gastrectomy patients, ghrelin concentrations were immediately reduced to 12–29 % of the preoperative concentration. In contrast, ghrelin concentrations decreased to 39–71 % of the preoperative concentration immediately after distal gastrectomy. These reductions in ghrelin concentration are a direct result of the fact that most ghrelin is produced by A-like cells in the fundic gland of the stomach. In fact, sleeve gastrectomy for bariatric surgery immediately results in a 67 % reduction in the concentration of ghrelin [38]. *H. pylori* infection also markedly reduces ghrelin-producing cells and plasma ghrelin.

**Table 1** Physiological functions of ghrelin

| |
|---|
| Orexigenic effect via the hypothalamus [17, 58, 59] |
| Ghrelin, which increases c-fos expression in the arcuate nucleus, also activates hypothalamic neuropeptide Y (NPY)/Y1 receptors and agouti-related peptide (AgRP) pathways |
| Stimulation of GH secretion from the pituitary gland [17, 60–62] |
| Ghrelin is involved in GH release in a non-acute setting. GH regulates IGF-I levels, promotes anabolism, and increases muscle strength. Ghrelin enhances lipolysis via GH and stimulates protein synthesis, myoblast differentiation, and muscle growth via IGF-1 |
| Antiinflammatory action [20, 63, 64] |
| Ghrelin inhibits the activation of NF-κB, a transcription factor known to control the production of multiple proinflammatory cytokines during inflammatory insults |
| Stimulation of gastrointestinal peristalsis [18] |
| Ghrelin acts on motor neurons in the myenteric plexus, activates a vago-vagal reflex, or may stimulate central pathways |
| Augmentation of cardiac output and reduction of blood pressure [26] |
| Ghrelin improves myocardial structure and function in chronic heart failure (CHF) via its GH-releasing effects |
| Inhibition of insulin secretion [65, 66] |
| Ghrelin has obesogenic/diabetogenic properties. These properties may be direct effects of ghrelin on pancreatic islet function and/or indirect effects through the modulation of GH secretion |

**Table 2** Representative reports of changes in ghrelin concentration in patients who have undergone gastrectomy

| References | Procedure | Number of cases | Preoperative ghrelin level | % Postoperative decline of ghrelin concentration from baseline | |
|---|---|---|---|---|---|
| | | | | Short term (%) | Long term (%) |
| Jeon et al. [32] | DG | 24 | Active 276 pg/ml (82.7 fmol/ml[a]) | Day 1 51 | |
| | | | | Day 7 88 | |
| Takachi et al. [35] | DG | 38 | Total 95 fmol/ml | Day 3 39 | 3 years 77 |
| Wang et al. [36] | DG (B-I) | 23 | Active 468 pg/ml (138.8 fmol/ml[a]) | Day 1 37 | 1 year 93 |
| | | | | Day 7 51 | |
| Wang et al. [36] | DG (B-II) | 19 | Active 460 pg/ml (136.5 fmol/ml[a]) | Day 1 36 | 1 year 82 |
| | | | | Day 7 51 | |
| Kim et al. [34] | DG | 45 | Total 310 pg/ml (92 fmol/ml[a]) | Day 2 71 | 3 months 81 |
| Kamiji et al. [33] | DG | 14 | Active 993 pg/ml (294.2 fmol/ml[a]) | – | 6 years 77 |
| Jeon et al. [31] | DG | 18 | Active 113 pg/ml (33.5 fmol/ml[a]) | Day 1 50 | 1 year 57 |
| | | | | Day 7 85 | |
| Zub-Pokrowieckae al. [37] | DG | 10 | Active 293 pg/ml (86.9 fmol/ml[a]) | – | 4–5 years 82 |
| Jeon et al. [31] | TG | 12 | Active 390 pg/ml (115.7 fmol/ml[a]) | Day 1 29 | – |
| | | | | Day 7 30 | |
| Takachi et al. [35] | TG | 26 | Total 95 fmol/ml | Day 3 12 | 3 years 20 |
| Kamiji et al. [33] | TG | 7 | Active 993 pg/ml (294 fmol/ml[a]) | – | 3–5 years 51 |
| Zub-Pokrowiecka et al. [37] | TG | 10 | Active 293 pg/ml (86.9 fmol/ml[a]) | – | 4–5 years 46 |
| Jeon et al. [32] | PG | 4 | Active 427 pg/ml(126.7 fmol/ml[a]) | Day 1 25 | – |
| | | | | Day 7 48 | |

*TG* total gastrectomy, *DG* distal gastrectomy, *PG* proximal gastrectomy, *B-I* Billroth-I reconstruction, *B-II* Billroth-II reconstruction

[a] x pg/l was converted to x/3.3709 fmol/ml

Generally, patients with gastric cancer and atrophic gastritis have a low basal level of ghrelin. Therefore, the degree of decline caused by gastrectomy can be considered low. Ghrelin concentrations recover relatively soon after surgery; many studies have shown that at 7 days after surgery, the ghrelin concentrations of patients with distal gastrectomy were 51–88 % of preoperative levels. In the long term, postoperative plasma ghrelin levels sometimes approach preoperative levels in patients who have undergone distal gastrectomy. It has been reported that the number of ghrelin-producing cells does not increase after gastrectomy [39]. Persistent low body weight after gastrectomy might stimulate ghrelin secretion from individual ghrelin-producing cells in a negative feedback manner. In contrast, the plasma ghrelin concentrations of patients who have undergone total gastrectomy do not rebound to normal levels if the patients suffer from continuous malnutrition [35]. Although ghrelin is produced by organs other than the stomach, those sources cannot sufficiently compensate for the disappearance of ghrelin-producing cells in the stomach.

## Vagotomy and ghrelin response

Both anterior and posterior vagal trunks were usually resected during gastrectomy for gastric cancer, especially in order to complete D2 lymph node dissection. Therefore, we should consider the influence of truncal vagotomy on ghrelin signals in both afferent and efferent pathways. In the rodent, vagotomy alone has led to the significant reduction of the baseline of fasting plasma ghrelin [40]. After radical esophagectomy for esophageal cancers (which includes truncal vagotomy and reconstruction of the whole gastric tube), ghrelin secretion in human patients was reduced by one-half compared to preoperative levels and gradually recovered within a few years [41, 42].

Vagotomy also perturbs the normal ghrelin secretion response (i.e., significant decline immediately after oral food intake). Pekic et al. [43] performed an oral glucose tolerance test (OGTT) in gastrectomized/vagotomized patients and BMI-matched control patients. Plasma ghrelin levels decreased significantly during the OGTT in control subjects, while no reduction was detected in gastrectomized-vagotomized patients. We frequently employ distal gastrectomy, which preserves the celiac branch of the vagal nerve. The downregulation of plasma ghrelin by food intake was significantly greater in patients with vagal nerve preservation than in patients with complete vagotomy (unpublished observation).

With respect to the efferent pathway, there is a report that the administration of exogenous ghrelin stimulated GH

secretion in vagotomized patients as much as in normal subjects [44]. Increases in appetite and amount of food intake after ghrelin administration are reportedly less significant in vagotomized patients than in control patients [45]. However, other studies in rats reported that ghrelin successfully stimulated food intake after vagotomy when administered intraperitoneally [46]. Moreover, in our previous study, intravenous administration of exogenous ghrelin successfully stimulated food intake and appetite immediately after total gastrectomy and esophagectomy [47, 48]. Our findings suggested that the administered ghrelin crossed the blood-brain barrier to the central nervous system, likely increasing the appetite signal through both the vagal pathway and the circulatory system.

As a whole, vagotomy definitely damages the normal control of ghrelin secretion. However, the relationship between ghrelin and vagotomy remains poorly defined in the output system of endogenous and exogenous ghrelin. Therefore, we cannot draw conclusions about the influence of vagotomy on the biological effects of ghrelin, although GH secretion and appetite stimulation may be differently involved with the vagal nerve. Further observation and experiments are required to clarify this issue.

## Effects of ghrelin administration after total gastrectomy

Because the anabolic effect of ghrelin is apparent, the possible clinical applications of ghrelin in the context of various cachexic states (e.g., anorexia nervosa, heart failure, chronic obstructive pulmonary disease, and the terminal stage of unresectable cancers) should be considered. These studies have demonstrated increases of oral food intake and body weight in both humans and rats. The two species do differ with regard to body composition. For example, ghrelin administration tended to increase fat volume in the rat, while muscle weight and muscle power have been increased more than fat volume in humans.

There are two large differences in the rationale of ghrelin administration with respect to the cachexic states listed above and the post-gastrectomy state. By various means, cachexia has consistently exhibited high plasma ghrelin concentrations combined with weight loss as the result of negative feedback; the effect of exogenous ghrelin may be restricted if the ghrelin signals are already saturated by endogenous ghrelin. In contrast, the post-gastrectomy state is associated with low plasma ghrelin combined with significant weight loss. Therefore, in the latter context it appears reasonable to administer exogenous ghrelin to compensate for reduced endogenous ghrelin. In this respect, we can expect more significant ghrelin effects in gastrectomy patients than in cachexic patients. Another concern is the influence of vagotomy, which, as described in the previous section, might minimize the effect of ghrelin in gastrectomy patients.

There is a randomized, phase II study [47] in which 21 patients undergoing total gastrectomy were assigned to groups receiving ghrelin ($n = 11$) or a placebo ($n = 10$). In the 10 days after starting oral food intake (postoperative days 5–7), an intravenous drip infusion of synthetic human ghrelin (3 μg/kg) or placebo (pure saline) was administered twice daily (before breakfast and before dinner). The mean intake over the 10-day period represented a 32.7 % increase in the ghrelin group compared with the placebo group (13.8 vs. 10.4 kcal/kg/day). At the end of the study period, weight loss was 3.7 % for the placebo group compared with 1.4 % for the ghrelin group. They used dual-energy X-ray absorptiometry to measure body composition. Fat mass, lean body mass and basal metabolic rate decreased significantly in the placebo group; however, the reductions in lean body mass and basal metabolic rate were not significant in the ghrelin group, although the reduction of fat mass was significant. Therefore, exogenous ghrelin lessened weight loss, especially the loss of lean body mass. There were no significant side effects; however, one patient experienced grade 1 diaphoresis. Several months after the trial, there was no between-group difference in weight or appetite. The most critical drawback is that they are currently only able to administer ghrelin intravenously. For long-term administration, another delivery system (e.g., subcutaneous injection or inhalation) should be developed [49]. Oral ghrelin analog, which is already in clinical trials, is a possible ghrelin substitute.

As ghrelin is also a potent GH secretagogue, there are concerns about GH-mediated stimulation of tumor growth, especially regarding treatment of cancer patients. In vitro studies suggest that ghrelin may enhance the proliferation of prostate [50] and pancreatic [51] cancer cells, but not of a lung cancer cell line, where it induced dose-dependent inhibition of cell proliferation and increased apoptosis [52]. Some tumors from archival samples express ghrelin [53], whereas others (gastric cancer and esophageal cancer) do not [54]. According to a review that analyzed ghrelin administration studies, there was no report of anyone suffering from new cancer as an adverse event among 1,850 participants who were registered to 121 studies. [55–57].

## Conclusion

Although our prospective randomized study had a limited number of patients and short-term observation periods, it revealed the beneficial effects of the administration of exogenous ghrelin on body weight and oral intake after total gastrectomy. Although there are issues that must be resolved before clinical application, including elucidation of the

duration of administration and adequate assessment of clinical benefits, surgeons dealing with gastric cancers should be encouraged by the availability of ghrelin. Although decline of ghrelin is certain to play a major role in appetite loss after gastrectomy, it cannot account for all causes that lead to body weight loss. Some patients continue to weigh less even after the amount of food intake has recovered, possibly because of vagotomy, defective fat absorption due to pancreatic insufficiency, bacterial overgrowth, and shortened small bowel transit time [13]. Although surgery is essentially non-physiological and highly invasive, it remains the most reliable therapeutic option to cure cancer. Therefore, it is our obligation to invent new procedures to minimize postoperative side effects.

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# Preservation of the Celiac Branch of the Vagus Nerve during Laparoscopy-assisted Distal Gastrectomy: Impact on Postprandial Changes in Ghrelin Secretion

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**Abstract**

*Background* Ghrelin is a brain-gut peptide with GH-releasing and appetite-inducing properties. Because ghrelin is secreted mainly by the stomach, fasting levels fall after distal gastrectomy. The vagal nerve is responsible for periprandial changes. The presents study investigated the impact of preserving the celiac branch of the vagus nerve during laparoscopy-assisted distal gastrectomy on postoperative ghrelin secretion.

*Method* Between May 2009 and July 2010, 42 consecutive patients who underwent LADG were divided into two groups, the first in which the celiac branch of the vagus was preserved ("Preserved," $n = 21$) and the second in which it was not ("Not Preserved," $n = 21$). Blood samples were collected for assays of several hormones, including ghrelin, leptin, and insulin; these were taken before and 2 h after breakfast on postoperative day 7.

*Results* There were no significant differences in the background characteristics of the two groups. Plasma fasting ghrelin decreased significantly after LADG, by about 50 % of the baseline values in both groups. Postprandial plasma ghrelin levels in the Preserved group were significantly lower than those in the Not Preserved group (23 ± 8 vs 32 ± 9 fmol/ml; $p = 0.0058$). The ratio of the total ghrelin concentration after breakfast to that before was defined as the A/B ratio. The mean preoperative and postoperative A/B ratios were almost the same in the Preserved group (preoperative vs postoperative: 0.41 vs 0.44; $p = 0.52$). On the other hand, the mean A/B ratio in the Not Preserved group increased from 0.41 to 0.61 postoperatively (preoperative vs postoperative; $p = 0.0003$). Preservation of the celiac branch of the vagus nerve during LADG was related to the prandial ghrelin changes.

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## Introduction

Increased detection of early gastric cancer has led to a focus on improving patients' quality of life by preventing or reducing postoperative gastrointestinal dysfunction. To achieve this goal, gastrectomy procedures began to incorporate preservation of the celiac branch of the vagus nerve [1, 2]. Laparoscopic surgery facilitated this preservation due to the magnified view it provides [3, 4]. However, the application of laparoscopic procedures to gastrectomy was controversial because its benefits were not well defined, and more evidence is still required before this procedure gains widespread acceptance. The advantages of nerve preservation, such as regulating gastrointestinal motility and preventing gallstone formation, have been previously reported [4, 5]. A small number of articles have described potential hematological advantages and their clinical significance.

Ghrelin, an endogenous ligand for the growth hormone (GH) secretagogue receptor, displays dose-dependent GH-releasing activity [6, 7]. Ghrelin, which is predominantly secreted by gastric endocrine cells, stimulates food intake and triggers a positive energy balance through a central mechanism involving hypothalamic neuropeptides [8–10]. The function of ghrelin is to stimulate both hypothalamic appetite signals and gastrointestinal activity, such as peristalsis, gastric acid secretion, and pancreatic excretion, through the vagus nerve [11]. Circulating ghrelin levels rise before meals, and rapidly decline after feeding or

gastrointestinal nutrient infusion [12]. The mechanism of daily ghrelin fluctuations is not well known. The postprandial response is known to be influenced by carbohydrates and insulin [12, 13]. The autonomic nervous system, which includes the vagus nerve, also plays an important role in the control of ghrelin secretion [14, 15].

Fasting (baseline) circulating ghrelin levels have been shown to decrease to 56 % of preoperative levels immediately after distal gastrectomy [16]. Postprandial ghrelin changes following distal gastrectomy have not yet been evaluated. In addition, no reports have discussed the impact on ghrelin secretion of distal gastrectomy incorporating preservation of the celiac branch of the vagus nerve. In this report we used a clinical study design to evaluate the influence of celiac branch preservation in laparoscopy-assisted distal gastrectomy (LADG).

## Patients and methods

### Study patients

This prospective observational study focused on 42 patients with gastric cancer who underwent LADG. The study protocol was approved by the Human Ethics Review Committee of the Osaka University School of Medicine. A signed consent form was obtained from each enrolled patient before study entry in accordance with the Declaration of Helsinki. This study began in May 2009 and patient enrollment ended in May 2010. We had another randomized prospective study in this period to investigate the efficacy of preservation of the vagus nerve on gallstone formation (UMIN000003364). The eligibility criteria were as follows: (1) histopathologically proven adenocarcinoma of the stomach, (2) LADG required due to confirmed T1 invasion with submucosal invasion according to UICC (International Union Against Cancer) staging, (3) age: 20–80 years, (4) adequate function of major organs, (5) no other active malignancy, and (6) provision of signed informed consent. Patients ineligible for inclusion were those with severe co-morbidity, infectious disease, or past history of drug allergy. The indication for LADG in our hospital was the confirmation of T1 invasion with submucosal invasion according to UICC staging and tumor management. The indication for laparoscopic D2 lymphadenectomy was tumor with lymph node metastasis or submucosal massive invasion. Bulky node metastasis or para-aortic metastasis was excluded from the criteria.

### Surgical procedure

Before LADG, we evaluated tumor location and depth of tumor invasion based on endoscopy and endoscopic ultrasonography results. Surgery consisted of the following procedures, with lymph node dissection performed according to the 2nd Edition of the Japanese Classification of Gastric Cancer: (1) laparoscopic dissection of the greater omentum; (2) division of the left gastroepiploic vein and artery near the spleen, together with lymphadenectomy; (3) division of the right gastroepiploic vein and artery and lymphadenectomy at their origin; (4) transection of the duodenum following its isolation around the pyloric ring; (5) resection of the lesser omentum with preservation of the hepatic branch of the vagus nerve; (6) division of the right gastric vein and artery with lymphadenectomy; (7) taping of the posterior trunk of the vagus nerve at the right dorsal side of the abdominal esophagus. The attachment between the lesser curvature of the stomach and the right crus of the diaphragm was dissected in a cephalad direction, exposing the right and left crura of the diaphragm. The celiac branch of the vagus nerve was usually detected dorsal to the abdominal esophagus, and a piece of tape was looped around it. The left gastric artery and celiac branch of the vagus nerve were revealed after dissection of the surrounding adipose tissue and lymph nodes. The taped vagus nerve was pulled to the right, and the vagus nerve's gastric rami and the surrounding adipose tissue were detached from the nerve with scissors; minor bleeding was ignored (Fig. 1a). Electronic devices such as laparoscopic coagulation shears or monopolar scissors were used only minimally due to possible thermal damage. Small vessels or bleeding was controlled using bipolar coagulation with soft coagulation mode if necessary. The isolated left gastric artery was divided with double clips. Finally, lymph node dissection with nerve preservation was completed (Fig. 1b). (8) Resection of the distal two-thirds of the stomach, depending on the location of the tumor, was followed by reconstruction using the Billroth I or Roux-en-Y method through a 5-cm-long mini-laparotomy incision. At the end of the operation, a drainage tube was left below the liver bed.

### Postoperative course and recorded clinical data

All patients received the same approach to clinical care during their hospitalization. Patients were given an oral diet on postoperative day 3, provided no morbidity was present. The time of hospital discharge was based on the "discharge criteria" used clinically in our hospital. These criteria include the absence of fever (body temperature <37 °C), absence of inflammation as confirmed by a blood test, and the ability to eat half of the normal, solid daily diet. We recorded data concerning patient characteristics (age, gender, body mass index [BMI], and stage), operative parameters (operative time, blood loss, lymphadenectomy, and retrieved lymph nodes), and postoperative clinical course, including complications and duration of hospital stay.

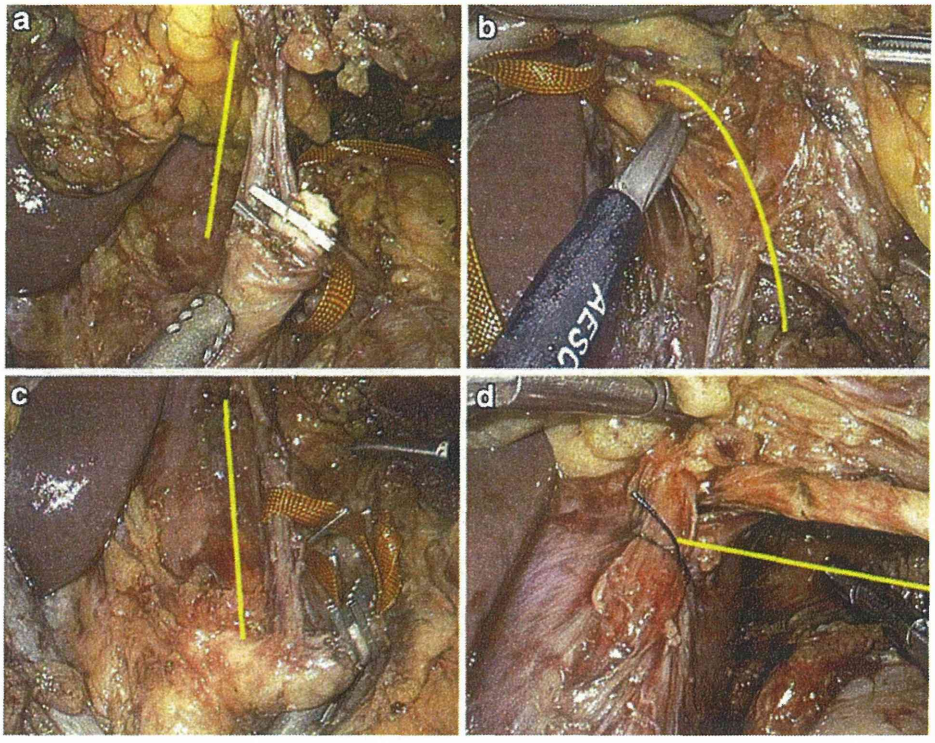



**Fig. 1** Preserving the celiac branch of the vagus nerve with lymphadenectomy (**a**). The taped vagus nerve was pulled to the right, and the vagus nerve gastric rami and surrounding adipose tissue were sharply divided with scissors while minor bleeding was ignored (**b**). Electronic devices such as laparoscopic coagulation shears or monopolar scissors were used only minimally to lessen the risk of thermal damage. If necessary, bleeding from small vessels was controlled with bipolar coagulation in the soft coagulation mode. The isolated left gastric artery was divided with double clips (**c**). Finally, lymph node dissection with nerve preservation was completed (**d**)

### Blood sampling

Blood samples were collected at the same time in the hospital before breakfast after an overnight fast and 2 h after breakfast. The samples were transferred immediately into chilled tubes containing disodium ethylenediaminetetraacetic acid (EDTA) and aprotinin for plasma sampling, centrifuged at 4 °C, separated for serum sampling, and stored at −50 °C. The plasma samples were mixed with a 10 % volume of 1 M hydrochloric acid (HCl) before storage at −50 °C. Plasma acyl- and desacyl-ghrelin concentrations were measured with a sandwich-type enzyme immunoassay kit according to the protocol supplied by the manufacturer (Mitsubishi Kagaku Iatron, Inc). The total plasma ghrelin concentration was defined as the sum of the acyl-ghrelin and desacyl-ghrelin concentrations. Serum growth hormone (GH), insulin, and leptin concentrations were measured with a GH "Daiichi" Kit (TFB, Inc, Tokyo, Japan), chemiluminescent enzyme immunoassay (Fujirebio, Inc, Tokyo, Japan), and Human Leptin RIA Kit (Linco Research Inc, MO), respectively.

### Statistical analysis

Continuous variables were expressed as means ± standard deviations (SD) unless otherwise stated. Statistical differences between groups were calculated with Student's $t$ test, the Mann–Whitney test, or the $\chi^2$ test. Statistical significance was set at $p < 0.05$. All calculations were performed using the JMP (version 9.0) software program (SAS Institute Inc, Cary, NC).

## Results

### Patient characteristics

Table 1 lists patient characteristics and operative records. Forty-two patients with gastric cancer who underwent LADG with (21 patients) or without (21 patients) preservation of the celiac branch of the vagus nerve were enrolled in the study. These two groups were defined as the "Preservation" and "No Preservation" groups, respectively. There were no significant differences in background characteristics, including age, sex, BMI, and clinical cancer staging. Similarly, there were no significant differences in postoperative records, including lymphadenectomy, reconstruction, operative time, blood loss, and retrieved lymph nodes.

### Postoperative course and complications

Table 2 summarizes the study population's postoperative course and complications. With respect to postoperative course, although there were no significant differences in either time until initial oral intake or postoperative hospital

**Table 1** Patient characteristics and operative records

| | Preserved | Not preserved | *p* value |
|---|---|---|---|
| No. of patients | 21 | 21 | |
| Age, years | 67.8 ± 11.0 | 66.0 ± 8.3 | 0.66 |
| Gender (male/female) | 11/10 | 14/7 | 0.35 |
| BMI, kg/m$^2$ | 23.7 ± 0.8 | 23.4 ± 1.6 | 0.76 |
| p-Stage (I/II/III/IV) | 21/0/0/0 | 18/3/0/0 | 0.092 |
| Lymphadenectomy (D1/D2) | 20/1 | 17/4 | 0.15 |
| Reconstruction (B-I/R-Y) | 15/6 | 18/3 | 0.26 |
| Operative time, min | 201 ± 29 | 193 ± 29 | 0.39 |
| Blood loss, ml | 101 ± 89 | 90 ± 83 | 0.68 |
| Retrieved lymph nodes, *n* | 37 ± 12.4 | 33 ± 12.6 | 0.32 |

*BMI* body mass index

stay, the time until start of flatus was shorter in the Preservation group (1.9 ± 0.5 vs 2.7 ± 0.6 days; $p = 0.003$). Postoperative complications (according to the Clavien–Dindo classification [>II]) did not differ significantly between the two groups: in the Preserved group, two patients had anastomotic stricture and one had diarrhea (enterocolitis), whereas in the Not Preserved group, one patient had delayed gastric emptying and one had pancreatic leakage.

### Blood tests and hormonal status

Figures 2 and 3 show the effects of food intake on serum glucose and hormonal status. With respect to ghrelin and associated hormones, preprandial plasma total ghrelin (the acyl- plus desacyl- forms) levels decreased significantly after LADG in both groups, by about 50 % of the respective baseline values (before LADG). In addition, postoperative plasma total ghrelin levels 2 h after meals were significantly lower in the Preservation group than in the No Preservation group (23 ± 8 vs 32 ± 9 fmol/ml; $p = 0.0058$; Fig. 2a). There were no significant differences in volume of oral intake at breakfast on postoperative day 7 (205.0 ± 71.3, $n = 16$, vs 181.2 ± 94.7 kcal, $n = 17$; $p = 0.423$). There were no significant differences before LADG in serum glucose or hormonal assays, including those for insulin, ghrelin, GH (a target hormone for ghrelin), and leptin. Serum glucose did not differ significantly between the two groups 2 h after breakfast. We defined the A/B ratio as the ratio of the total ghrelin concentration after breakfast to that before (Fig. 2b). In the Preservation group, the mean A/B ratio was almost the same preoperatively and postoperatively (0.41 vs 0.44, respectively; $p = 0.52$). In contrast, in the No Preservation group, the mean A/B ratio increased from 0.41 preoperatively to 0.61 postoperatively ($p = 0.0003$). There were no significant differences in insulin, GH (a target hormone for ghrelin), or leptin (a hormone with actions that oppose those of ghrelin) between the groups before and after LADG (Fig. 3). There were also no significant differences in the A/B ratio of glucose, insulin, GH, and leptin between the groups.

**Table 2** Postoperative course and complications

| | Preserved | Not preserved | *p* value |
|---|---|---|---|
| Time until start of flatus, days | 1.9 ± 0.5 | 2.7 ± 0.6 | 0.003 |
| Time until start of oral intake, days | 3.7 ± 1.2 | 3.9 ± 1.5 | 0.66 |
| Postoperative hospital stay, days | 13.3 ± 2.8 | 13.9 ± 3.0 | 0.53 |
| Complications Clavien–Dindo classification II | 3 (14.3 %) | 2 (9.5 %) | 0.63 |
| Anastomotic stricture | 2 (9.5 %) | 0 (0 %) | 0.15 |
| Delayed gastric emptying | 0 (0 %) | 1 (4.8 %) | 0.31 |
| Pancreatic leakage | 0 (0 %) | 1 (4.8 %) | 0.31 |
| Diarrhea | 1 (4.4 %) | 0 (0 %) | 0.31 |
| Clavien–Dindo classification > III | 0 (0 %) | 0 (0 %) | 1.00 |

## Discussion

This is the first report to show the influence of gastrectomy on postprandial ghrelin changes, as well as the impact of preserving the celiac branch of the vagus nerve on ghrelin changes 2 h after meals. Postoperative reductions in fasting plasma ghrelin levels due to gastrectomy did not differ between the Preservation and No Preservation groups; however, plasma ghrelin concentrations 2 h after a meal differed between the groups on postoperative day 7. The mean A/B ratio (which compared ghrelin concentrations after and before breakfast) differed preoperatively and postoperatively in the No Preservation group. This ratio did not change postoperatively in the Preservation group. It was interesting that fasting ghrelin levels decreased in both groups, whereas the postprandial reduction in ghrelin levels was maintained in the Preservation group. At the time of this writing there were no reports demonstrating this difference between patients undergoing nerve-preserving and nerve-resection approaches.

Ghrelin levels exhibit diurnal changes, increasing early in the morning until breakfast, decreasing immediately at the time of a meal, and gradually increasing again before the next meal [12, 17]. The mechanism mediating the nutrient-related ghrelin response remains unclear. Two possible factors have been associated with postprandial ghrelin reductions. One is the combination of blood glucose and insulin [18], and the second is the cephalic phase of the gastrointestinal response to nutrient intake [15]. Circulating ghrelin concentrations fall rapidly after nutrient ingestion as well as after oral and intravenous glucose

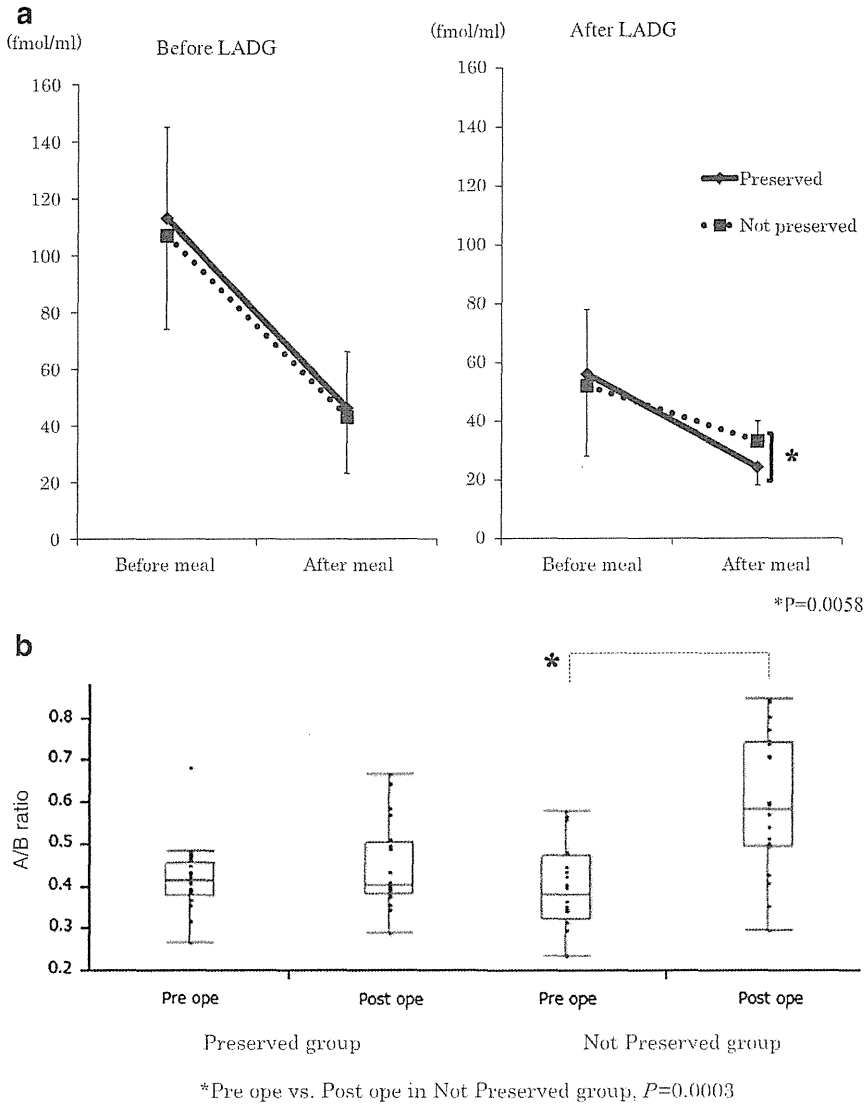



**Fig. 2** **a** Postprandial ghrelin changes before and after LADG. Plasma ghrelin 2 h after a meal in the No Preservation and Preservation groups: 23 ± 8 versus 32 ± 9 fmol/ml; $p = 0.0058$. The *error bar* represents the standard deviation. **b** Differences between the preoperative and postoperative A/B ratios. A/B ratio: preprandial total ghrelin concentration/postprandial total ghrelin concentration The *error bar* represents the standard deviation. Preoperative versus postoperative A/B ratio in the No Preservation group, $p = 0.0003$

challenge. Plasma glucose and insulin concentrations both preoperatively and postoperatively were very similar between the Preserved and Not Preserved groups in this study. Cephalic-vagal stimulation might primarily influence postprandial ghrelin reduction on postoperative day 7.

The vagus nerve consists of both sensory and motor neurons, and 90 % of the subdiaphragmatic vagus nerve consists of afferent fibers [19]. Afferent fibers in the celiac branch first innervate the celiac plexus and then the alimentary canal from the duodenum to the colon, and transmit information from visceral organs to the hypothalamus [20, 21]. The afferent celiac branch of the vagus nerve transmits feedback that downregulates control of ghrelin either directly or indirectly. Afferent signals transmitted through the celiac branch of the vagus nerve may be associated with differences in postprandial ghrelin reduction on postoperative day 7. However, because fasting ghrelin concentrations were almost equal between the Preservation and No Preservation groups, they were not linked to preservation of this vagal branch.

Both autonomic nervous system stimulation and hormone secretion are involved in controlling gastrointestinal responses to nutrient intake; these responses are usually subdivided into cephalic, gastric, and intestinal phases. The intestinal phase accounts for changes in postprandial ghrelin levels. Although vagal efferent stimulation to the stomach is lost following LADG, we found differences in postprandial ghrelin changes depending on whether the celiac branch of the vagus nerve was preserved. We hypothesized that this phenomenon was due to stimulation of the stomach by the intraesophageal branch of the vagus. Esophagectomy and proximal gastrectomy, in which the

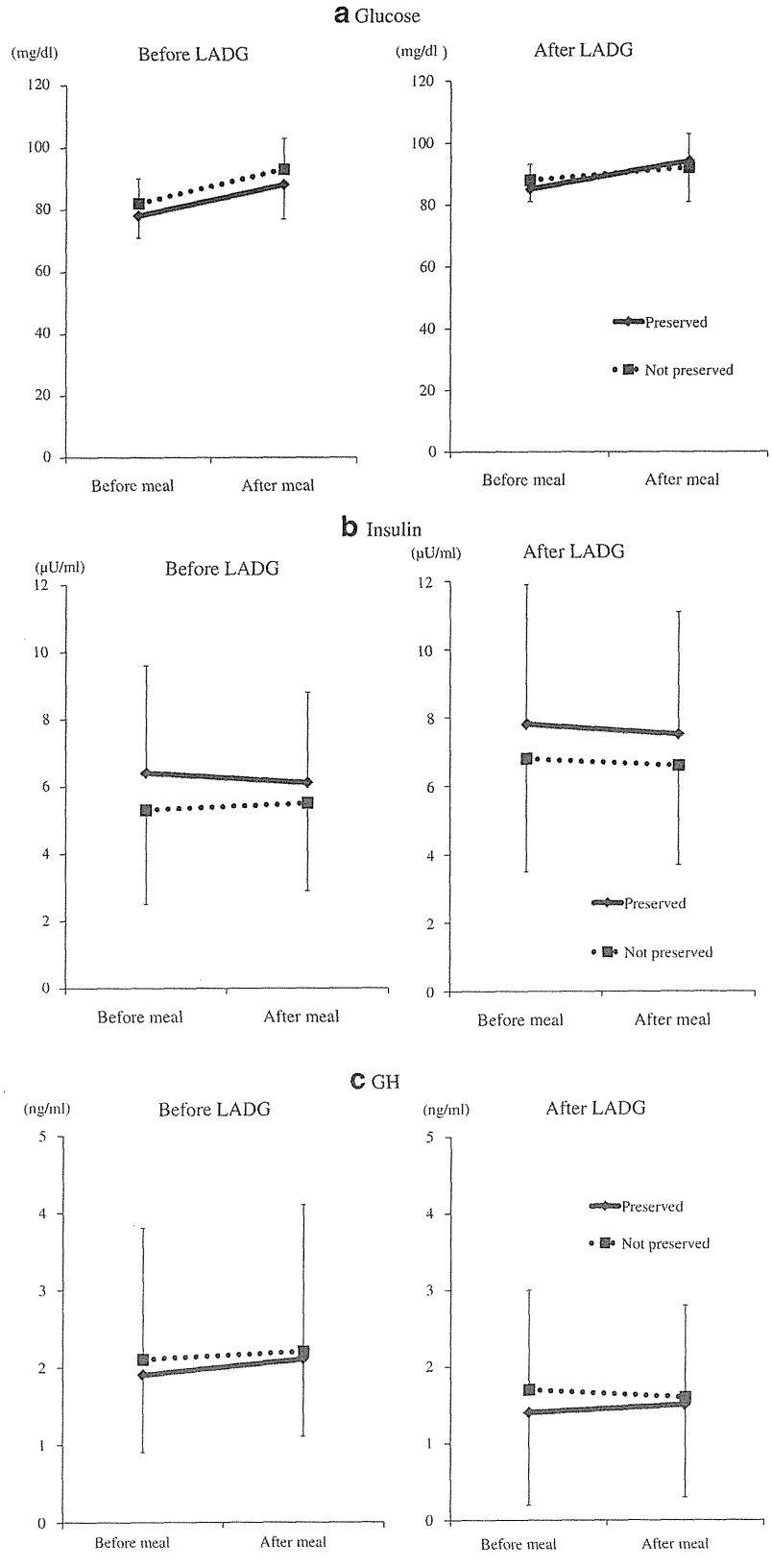
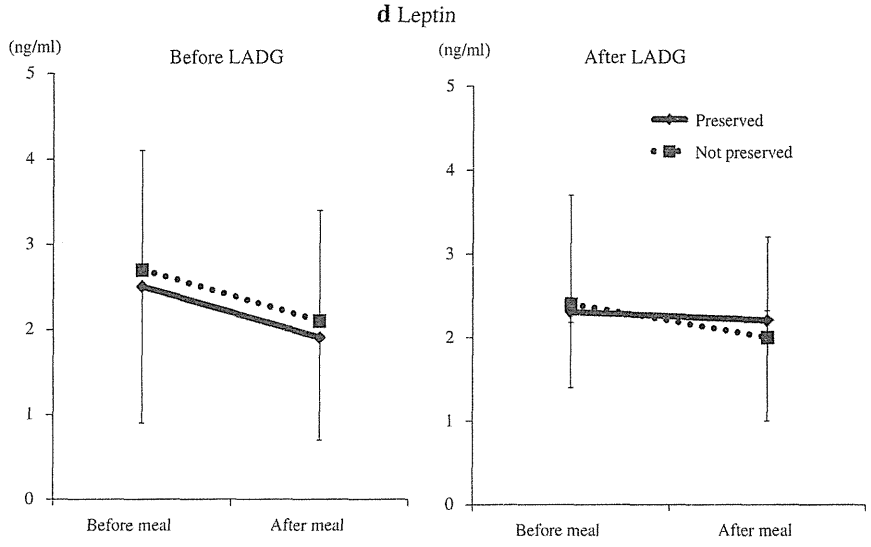



**Fig. 3** Pre- and postprandial glucose and hormonal changes before and after LADG. The *error bar* represents the standard deviation. There were no significant differences in glucose, growth hormone, insulin, and leptin levels measured in the Preserved and Not Preserved group at each time point. There were also no significant differences in the A/B ratio of glucose, insulin, GH, and leptin between the groups

**Fig. 3** continued




stomach is partially or entirely retained without vagus nerve gastric regulation, should be investigated to clarify the relation between the vagus nerve system and postprandial ghrelin changes.

Injury to the vagus nerve during gastrectomy in patients with gastric or duodenal ulcers has been reported to cause diarrhea and gallstone formation due to truncal or selective vagotomy [22]. To prevent these disorders after distal gastrectomy, vagus nerve–preserving techniques were incorporated into the open distal gastrectomy procedure. Uyama et al. [3] and Kojima et al. [4] reported that LADG with vagus nerve sparing was helpful in preventing gallstone formation and diarrhea. Yamada et al. [5] reported that the incidence of early dumping syndrome was reduced and time to first flatus was earlier when using nerve-preserving LADG as opposed to procedures involving nerve resection [22]. In our study, time to first flatus in the Preservation group was also shorter than that in the No Preservation group. Kinami et al. [2] reported that the celiac branch of the vagus nerve was involved in the control of pancreatic insulin release [13]. The above findings might relate to the efferent pathway of the vagal nerve.

Postoperative body weight loss usually occurs in gastrectomy patients. To prevent this, the effects of ghrelin should outweigh those of intestinal hormone antagonists because of ghrelin's pleiotropic functions, including increasing appetite, metabolic rate, and GH release. Ghrelin stimulates food intake through a central mechanism involving hypothalamic neuropeptides. In our previous study, intravenous administration of ghrelin to cancer patients enhanced oral feeding and was effective against weight loss after total gastrectomy [23] and esophagectomy [24]. Postoperative preprandial ghrelin concentration is an important factor influencing food intake. Unfortunately, no differences in postoperative fasting ghrelin concentrations were seen between the Preservation and No Preservation groups in the study reported here. Postprandial ghrelin reduction has been thought to be necessary for maintaining homeostasis and controlling energy balance. Long-term follow-up is necessary to investigate the clinical benefits of maintaining postprandial ghrelin changes following nerve-preserving LADG. We have investigated this issue only one week postoperatively. It is of interest whether the same postprandial hormone differences continue to be present after 1 year.

Laparoscopic surgery has the advantage of providing a magnified view that allows precise manipulation of blood vessels and nerves [3, 4]. However, complete preservation of the celiac branch of the vagus nerve requires excellent control of bleeding. In the present series, electronic devices were not used around the nerve during LADG to avoid thermal damage. Sharp dissection with endoscopic scissors often produces some bleeding, which we controlled using bipolar coagulation. Laparoscopic coagulation scissors are useful, but thermal damage was a problem. The observation of differences in ghrelin concentration patterns confirmed the success of preservation of the celiac branch of the vagus nerve. The postoperative A/B ratio of ghrelin concentration would be the index of the confirmation that the celiac vagus nerve was functionally preserved. At the same time, postoperative neural palsy caused by pulling with tape or thermal injury is concerning issue. For example, once palsy of the recurrent nerve occurred following esophagectomy, the nerve injury might not heal until postoperative day 7. Study over a longer postoperative period is necessary to grasp the precise impact of preservation of this nerve.

In conclusion, preservation of the celiac branch of the vagus nerve during LADG was related to prandial ghrelin changes.

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ORIGINAL ARTICLE

# Effect of rikkunshito, a Japanese herbal medicine, on gastrointestinal symptoms and ghrelin levels in gastric cancer patients after gastrectomy

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**Abstract**
*Background* Gastric cancer patients who undergo gastrectomy suffer from a post-gastrectomy syndrome that includes weight loss, dumping syndrome, reflux esophagitis, alkaline gastritis, and finally malnutrition. It is important to ameliorate the post-gastrectomy symptoms to restore postoperative quality of life (QoL). The aim of this study was to investigate the effect of rikkunshito, a Japanese herbal medicine, on postoperative symptoms and ghrelin levels in gastric cancer patients after gastrectomy.
*Methods* Twenty-five patients who had undergone gastrectomy received 2.5 g of rikkunshito before every meal for 4 weeks, and a drug withdrawal period was established for the next 4 weeks. Changes in gastrointestinal hormones, including ghrelin, and appetite visual analog scale scores were measured, and QoL was estimated by using the European Organization for Research and Treatment of Cancer core questionnaire QLQ-C30. The Dysfunction After Upper Gastrointestinal Surgery for Cancer (DAUGS) scoring system was used to evaluate gastrointestinal symptoms after gastrectomy.
*Results* Sixteen men and nine women (mean age 61.9 years) were enrolled in the study. All patients had either stage I ($n = 24$) or II ($n = 1$) disease and had undergone either distal gastrectomy ($n = 17$) or total gastrectomy ($n = 8$) by a laparoscopy-assisted approach. The mean ratio of the acyl-/total ghrelin concentration increased significantly after rikkunshito administration (Pre: 7.8 ± 2.1, 4 weeks: 10.5 ± 1.7 %, $p = 0.0026$). The total DAUGS score, as well as the scores reflecting limited activity due to decreased food consumption, reflux symptoms, dumping symptoms, and nausea and vomiting significantly improved after rikkunshito administration.
*Conclusions* The present study demonstrated a significant attenuation of gastrointestinal symptoms after gastrectomy by treatment with rikkunshito. Rikkunshito is potentially useful to minimize gastrointestinal symptoms after gastrectomy.

**Keywords** Rikkunshito · Ghrelin · Gastric cancer · Gastrectomy

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## Introduction

In Japan, the number of long-term survivors after radical surgery for gastric cancer has been increasing as a result of early detection and improved surgical techniques [1, 2]. Although survivors may be rendered free of disease by surgery, they may suffer from post-gastrectomy syndrome, which includes weight loss, dumping syndrome, stasis syndrome, reflux esophagitis, alkaline gastritis, and, finally, malnutrition [3, 4]. Because of the improved prognosis of patients with gastric cancer, it is important to ameliorate post-gastrectomy symptoms to restore postoperative quality of life (QoL) [5–7].

Our group has focused on ghrelin, a gut hormone known to increase appetite [8]. Ghrelin, an endogenous ligand for the growth hormone (GH) secretagogue receptor, displays

dose-dependent GH-releasing activity [8, 9]. Ghrelin, which is predominantly secreted by gastric endocrine cells, stimulates food intake and triggers a positive energy balance through a central mechanism involving hypothalamic neuropeptides [10–12]. In our previous study, we reported that intravenous administration of ghrelin enhanced oral feeding and was effective against weight loss after total gastrectomy [13] and esophagectomy [14] in cancer patients.

Recently, it has been reported that rikkunshito, one of the traditional Japanese medicines, increased the plasma acylated ghrelin level in healthy volunteers and in normal mice [15]. In rats, a flavonoid in rikkunshito suppressed cisplatin-induced decreases in the plasma acylated ghrelin level and increased food intake mediated by 5-HT2B/2C receptors [16]. In general, rikkunshito is used to treat various gastrointestinal tract disorders such as functional dyspepsia (FD) [17, 18], gastro-esophageal reflux [19, 20], dyspeptic symptoms of post-gastrointestinal surgery [21, 22], and chemotherapy-induced nausea [23]. In animal experiments, rikkunshito is reported to ameliorate gastric distension via a nitric oxide-mediated pathway, and it has also been shown to improve delayed gastric emptying [24]. These results indicate that rikkunshito may decrease postoperative symptoms after gastrectomy. To tackle these issues, we conducted a prospective observational study in patients with gastric cancer after they had undergone laparoscopy-assisted gastrectomy. The aim of this study was to investigate the effect of rikkunshito on postoperative symptoms and ghrelin levels in gastric cancer patients after gastrectomy.

## Patients and methods

### Patient eligibility

Patients with histologically confirmed gastric cancer who had undergone gastrectomy and lymph node dissection with curative intent were eligible for participation in the study. Further criteria were: enrollment 6 months to 5 years following the surgery, ability to take solid foods, Eastern Cooperative Oncology Group performance status (PS) of $\leq 1$, age between 20 and 80 years, and adequate function of major organs. Patients were excluded from the study if they were pregnant or desired to become pregnant, undergoing chemotherapy, burdened with other active malignancy, or otherwise considered to be ineligible by the investigator. The study protocol was approved by the Human Ethics Review Committee of Osaka University School of Medicine. A signed consent form was obtained from each enrolled patient before study entry in accordance with the Declaration of Helsinki. This study was registered in the University Hospital Medical Information Network (UMIN R000006959).

Following gastrectomy, prominent postoperative symptoms and complaints tend to resolve naturally after the first 6 months. Our previous randomized study comparing dysfunction after Billroth I and Roux-en-Y reconstruction after distal gastrectomy actually revealed little difference in QoL scores and Dysfunction After Upper Gastrointestinal Surgery for Cancer (DAUGS) scores between the treatment arms after the first 6 months [25]. In order to explicitly evaluate the value of rikkunshito, therefore, all patients in the present study were recruited more than 6 months after the surgery, when various symptoms due to the gastrectomy would have more or less stabilized.

### Rikkunshito administration

The study protocol is summarized in Fig. 1. Rikkunshito, which was obtained from Tsumura (Tokyo, Japan), has 8 main constituents: Glycyrrhizae radix (4.7 %), Zingiberis rhizoma (2.3 %), Atractylodis lanceae rhizoma (18.6 %), Zizyphi fructus (9.3 %), Aurantii nobilis pericarpium (9.3 %), Ginseng radix (18.6 %), Pinelliae tuber (18.6 %), and Hoelen (18.6 %). More detailed descriptions of all the substances that are known to be included in rikkunshito are found in a previous report [26]. Patients who had undergone gastrectomy invariably received 2.5 g of rikkunshito before each meal (7.5 g/day) for 4 weeks. After this administration period, the drug was withdrawn for the next 4 weeks.

### Assessment of gastrointestinal symptoms, appetite, and QoL after gastrectomy

To evaluate gastrointestinal symptoms after the surgical resection of gastric cancer, we used the DAUGS scoring system [27]. Patients rated items related to postoperative dysfunction using a scale ranging from 1 ('not at all') to 5

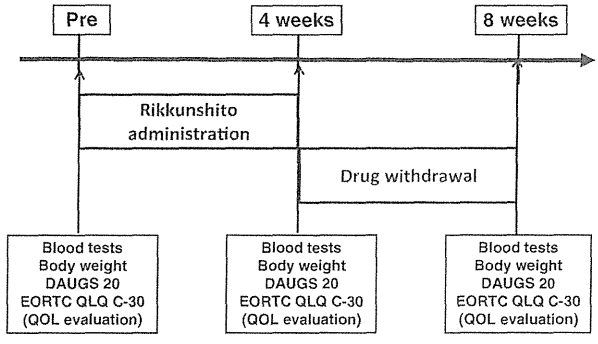


**Fig. 1** Study protocol. *DAUGS* Dysfunction After Upper Gastrointestinal Surgery for Cancer, *EORTC* European Organization for Research and Treatment of Cancer, *QOL* quality of life

('very severe'). The items were divided into 7 categories: (1) limited activity due to decreased food consumption, (2) reflux, (3) gastric dumping, (4) nausea and vomiting, (5) digestive difficulties, (6) pain, and (7) lower gastrointestinal (GI) symptoms [27].

We administered the European Organization for Research and Treatment of Cancer core questionnaire (QLQ-C30) [28] before and after rikkunshito administration, and after the drug withdrawal. The QLQ-C30 contains five functional scales (physical, role, cognitive, emotional, and social), three symptom scales (fatigue, pain, and nausea/vomiting), a global health/QoL scale, and six single items (dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties). All scale and single items scores range from 0 to 100. A high score for a functional scale represents a higher ("better") level of functioning, whereas a high score for a symptom scale or item represents a higher ("worse") level of symptoms [28].

Patients were instructed to rate themselves by selecting the scale at three time points; once prior to rikkunshito administration, once after the administration for 4 weeks, and once 4 weeks after the drug withdrawal.

Appetite profile was measured using a 100-mm visual analog scale (VAS), with the questions 'How hungry are you?' and 'How full do you feel?' that were anchored with scores of '0-not at all' and '100-extremely'. Patients were instructed to rate their appetite by selecting the scale that was closest to their feeling before each of three meals at three time points noted by the investigator: once prior to rikkunshito administration, once after the administration for 4 weeks, and once 4 weeks after drug withdrawal. The mean VAS score was calculated for each of the three measurement days.

To minimize bias, patients were provided with the questionnaire sheets to be filled in at home and were asked to mail them back to the data center using the specific envelopes provided.

### Blood sampling

Blood samples were collected before breakfast after an overnight fastbefore and after rikkunshito administration, and after the drug withdrawal. The samples were transferred immediately into chilled tubes containing disodium ethylenediamine tetra-acetic acid (EDTA) and aprotinin for plasma sampling, centrifuged at 4 °C, separated for serum sampling, and stored at −50 °C. The plasma samples were mixed with a 10 % volume of 1 M hydrochloric acid (HCl) before storing at −50 °C. Plasma acyl- and desacyl-ghrelin concentrations were measured with a sandwich-type enzyme immunoassay kit according to the protocol supplied by the manufacturer (Mitsubishi Kagaku Iatron, Tokyo, Japan) [29]. The total plasma ghrelin concentration was calculated as the acyl-ghrelin concentration plus the desacyl-ghrelin concentration. Serum GH, insulin, and leptin concentrations were measured using a GH "Daiichi" Kit (TFB, Tokyo, Japan), a chemiluminescence enzyme immunoassay (Fujirebio, Tokyo, Japan), and a Human Leptin RIA Kit (Linco Research, St Charles, MO, USA), respectively. Serum insulin-like growth factor-1 (IGF-1) levels were measured by radioimmunoassay (RIA) (SRL,Tokyo, Japan).

### Statistical analysis

Continuous variables were expressed as mean ± SD unless otherwise stated. Statistically significant differences between the periods were calculated by paired *t*-test, Fisher's exact test, or the Mann–Whitney test. Statistical significance was set at $p < 0.05$. All calculations were performed using the JMP (version 9.0) software program (SAS Institute, Cary, NC, USA).

## Results

### Patient characteristics

This prospective observational study focused on 25 patients with gastric cancer who had undergone gastrectomy and were enrolled between December 2010 and May 2011. Table 1 lists the demographic and clinical characteristics of the patients in this study. Sixteen men and nine women were enrolled (mean age 61.9 years). The mean body mass index (BMI) was 21.0 ± 2.7 kg/m$^2$. All patients had either stage I ($n = 24$) or II ($n = 1$) disease and had undergone either distal gastrectomy ($n = 17$) or total gastrectomy ($n = 8$). Because early-stage cancers are indicated for a laparoscopy-assisted approach at our institution, all surgeries for patients enrolled in the study were performed by the minimally invasive approach. The mean time elapsed between surgery and enrollment was 890 ± 578 days.

### Blood tests and hormonal assays

Table 2 summarizes the results of laboratory tests, tests of nutritional status, and hormonal assays. None of the indicators of nutritional status changed significantly after rikkunshito administration. With regard to the hormonal assays, GH (a target hormone for ghrelin), IGF-1 (a mediator of GH), insulin, and leptin also did not change significantly after rikkunshito administration. There were no significant differences in the results of other laboratory tests after rikkunshito administration compared with the results 4 weeks after drug withdrawal.

**Table 1** Patients' characteristics

| *n* | 25 |
|---|---|
| Age (years) | 61.9 ± 10.9 |
| Gender (male/female) | 16/9 |
| BMI (kg/m$^2$) | 21.0 ± 2.7 |
| Operative procedure (LADG/LATG) | 17/8 |
| Reconstruction (B-I/R-Y) | 15/10 |
| Postoperative days (days) | 890 ± 578 |
| p-Stage (I/II/III/IV) | 24/1/0/0 |

Values are mean ± SD

*LADG* laparoscopy-assisted distal gastrectomy, *LATG* laparoscopy-assisted total gastrectomy, *BMI* body mass index, *B-I/R-Y* Bismuth-I/Roux-en-Y

**Table 2** Results of laboratory tests, tests of nutritional status, and hormonal assays

| | Pre | 4 Weeks | 8 Weeks |
|---|---|---|---|
| Hemoglobin (g/dl) | 11.2 ± 0.8 | 11.5 ± 1.3 | 11.3 ± 1.1 |
| Albumin (g/dl) | 4.0 ± 0.4 | 3.9 ± 0.3 | 3.8 ± 0.3 |
| Lymphocytes (/μl) | 1580 ± 400 | 1780 ± 580 | 1620 ± 430 |
| Cholinesterase (IU/l) | 274 ± 81 | 275 ± 84 | 266 ± 74 |
| Triglyceride (mg/dl) | 102 ± 58 | 92 ± 42 | 98 ± 31 |
| Total cholesterol (mg/dl) | 197 ± 28 | 198 ± 27 | 202 ± 30 |
| Rapid turnover proteins | | | |
| Pre-albumin (mg/dl) | 26.5 ± 7.3 | 26.8 ± 7.8 | 25.8 ± 6.4 |
| Retinol binding protein (mg/dl) | 4.3 ± 1.2 | 4.3 ± 1.2 | 4.1 ± 1.1 |
| Transferrin (mg/dl) | 244 ± 35 | 243 ± 34 | 229 ± 30 |
| Hormonal assays | | | |
| Growth hormone (ng/ml) | 1.8 ± 1.6 | 1.6 ± 0.8 | 1.7 ± 0.9 |
| Leptin (ng/ml) | 2.8 ± 1.8 | 2.5 ± 1.5 | 2.6 ± 1.4 |
| Insulin-like growth factor-1 (ng/ml) | | | |
| Insulin (μIU/ml) | 6.4 ± 3.2 | 7.2 ± 4.1 | 6.8 ± 4.3 |

Values are mean ± SD

## Ratio of acyl-/total ghrelin concentration and plasma acyl- and desacyl-ghrelin concentration

Figure 2 shows serial changes in the ratio of the acyl-/total ghrelin concentration after rikkunshito administration. The mean ratio of the acyl-/total ghrelin concentration increased significantly after rikkunshito administration (Pre: 7.8 ± 2.1, 4 weeks: 10.5 ± 1.7 %, $p = 0.0026$). Four weeks after the drug withdrawal, the mean ratio of the acyl-/total ghrelin concentration had decreased significantly (4 weeks: 10.5 ± 1.7, 8 weeks: 7.6 ± 2.3 %, $p = 0.0015$). Table 3 shows the serial changes in plasma ghrelin concentrations. The administration and withdrawal of rikkunshito had no significant influence on the concentrations of acyl- and desacyl-ghrelin. Although the difference did not reach statistical

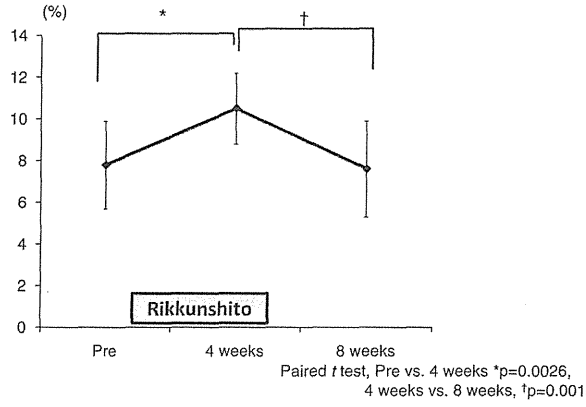


**Fig. 2** Serial changes in the ratios of acyl-/total ghrelin concentration. *Error bars* represent SDs

**Table 3** Plasma ghrelin concentrations before and after rikkunshito administration

| | Pre | 4 Weeks | 8 Weeks |
|---|---|---|---|
| LADG | | | |
| Acyl-ghrelin (fmol/ml) | 5.0 ± 2.8 | 5.7 ± 2.2 | 4.9 ± 2.4 |
| Desacyl-ghrelin (fmol/ml) | 60.8 ± 34.5 | 51.2 ± 35.1 | 53.9 ± 29.8 |
| Acyl-/total ghrelin (%) | 7.6 ± 3.4 | 10.0 ± 2.3 | 8.2 ± 3.4$^\dagger$ |
| LATG | | | |
| Acyl-ghrelin (fmol/ml) | 0.89 ± 0.49 | 1.1 ± 0.60 | 0.74 ± 0.42 |
| Desacyl-ghrelin (fmol/ml) | 12.3 ± 3.1 | 10.1 ± 3.8 | 12.4 ± 3.5 |
| Acyl-/total ghrelin (%) | 6.8 ± 2.2 | 9.8 ± 2.4* | 6.3 ± 2.8$^\dagger$ |

Values are mean ± SD

* Paired *t*-test, $p < 0.05$; Pre versus 4 weeks, $^\dagger$ $p < 0.05$; 4 weeks versus 8 weeks

significance, the mean ratio of the acyl-/total ghrelin concentration was higher after rikkunshito administration in the patients who had undergone distal gastrectomy (Pre: 7.6 ± 3.4, 4 weeks: 10.0 ± 2.3 %, $p = 0.075$). In the patients who had undergone total gastrectomy, the mean ratio of the acyl-/total ghrelin concentration was significantly increased after rikkunshito administration (Pre: 6.8 ± 2.2, 4 weeks: 9.8 ± 2.4 %, $p = 0.011$). After the drug withdrawal, the mean ratio of the acyl-/total ghrelin concentration decreased significantly in all the patients (those who had had distal gastrectomy: 4 weeks: 10.0 ± 2.3, 8 weeks: 8.2 ± 3.4 %, $p = 0.049$; those who had had total gastrectomy: 4 weeks: 9.8 ± 2.4, 8 weeks: 6.8 ± 2.8 %, $p = 0.009$, respectively).

## Effect of rikkunshito on DAUGS score, appetite, and QoL

The VAS scores and DAUGS scores are presented in Fig. 3. The VAS scores were significantly increased after