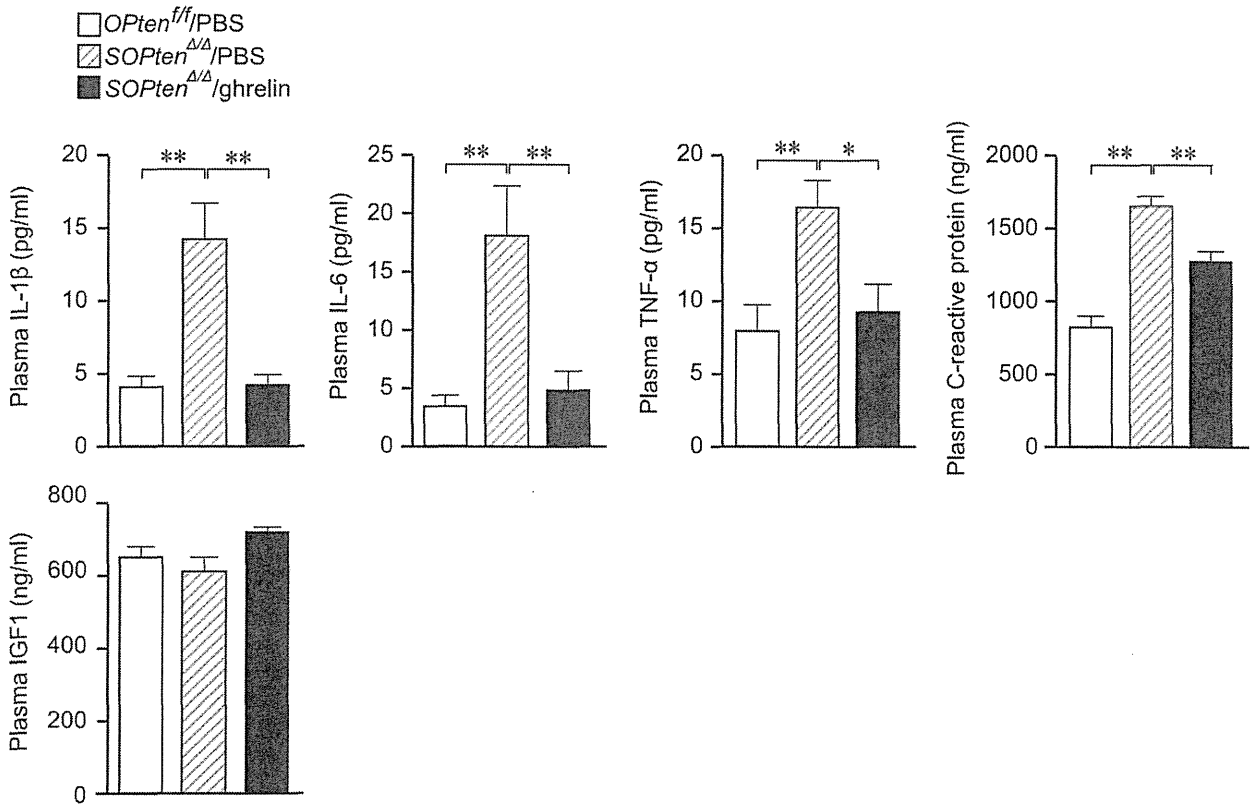

Fig. 5. Effects of ghrelin administration on plasma levels of inflammatory cytokines, C-reactive protein and insulin-like growth factor in urethane-injected SOPten$^{\Delta/\Delta}$ mice. The plasma levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), C-reactive protein, and insulin-like growth factor 1 (IGF1) in the *SOPten*$^{\Delta/\Delta}$/phosphate buffered saline (PBS) group, *SOPten*$^{\Delta/\Delta}$/ghrelin group, and *OPten*$^{f/f}$/PBS group at 28 days after ghrelin or PBS treatment. Data are the mean ± S.E.M. of 8–15 mice per group. $^{*}P < 0.05$. $^{**}P < 0.01$.

pathway and p38 MAPK pathway. Upregulation of Atrogin1 occurs via p38 MAPK activation (Li et al., 2005), and inhibition of the NF-κB pathway is sufficient to decrease tumor-induced muscle loss by inhibiting the upregulation of MuRF1 (Cai et al., 2004; Moore-Carrasco et al., 2007). Ghrelin may therefore exert an anti-catabolic effect through the reduction of systemic proinflammatory cytokines. In addition, Yamamoto et al. (2008) reported that GH-releasing peptide-2, a GHS-receptor agonist, directly attenuated Atrogin1 and MuRF1 mRNA levels through the GHS-receptor in C2C12 myotubes. Taken together, these findings suggest that ghrelin may have an anti-catabolic effect through a direct blockade of the proteolytic pathways in skeletal muscle, in addition to its inhibitory effect on the systemic inflammation.

We also demonstrated that systemic ghrelin administration enhanced the anabolic pathway in skeletal muscle in tumor-bearing mice. While ghrelin administration did not affect plasma IGF1 levels, ghrelin-treated mice showed increases in IGF1 mRNA and phosphorylated-Akt expression in gastrocnemius muscle as compared to their control counterparts. It has been reported that systemic IGF1 infusion did not reverse muscle atrophy (Brink et al., 2001), while muscle-specific overexpression of IGF1 reversed muscle wasting (Song et al., 2005). These findings suggest that the local IGF1 expression induced by ghrelin in the skeletal muscle is important for muscle regeneration and hypertrophy. A previous study showed that transgenic mice in which Akt was inducibly activated in skeletal muscle demonstrated dramatic muscle hypertrophy (Lai et al., 2004), further supporting that this pathway is sufficient to mediate hypertrophy downstream of IGF1 upregulation. Akt induces activation of protein synthesis by upregulation of the mammalian target of rapamycin complex 1 signaling, which in turn activates p70-S6-kinase (Fearon et al., 2012). Akt also mediates FoxO phosphorylation and subsequent inhibition of FoxO transport to the nucleus that is sufficient to block upregulation of the E3 ligases, such as Atrogin1 and MuRF1. Therefore, the activating action of ghrelin on the local IGF1/Akt axis in skeletal muscle might also be involved in the amelioration of muscle wasting in tumor-bearing mice.

In regard to ghrelin's amelioration of skeletal muscle atrophy in tumor-bearing mice, we demonstrated that ghrelin administration retained not only the skeletal muscle mass but also the muscle contraction force in both soleus muscle and tibialis anterior muscle, which are slow-twitch muscle and fast-twitch muscle, respectively (Baldwin and Tipton, 1972; Pullen, 1977). These findings suggested that ghrelin treatment might improve the skeletal muscle function, not just retain the skeletal muscle mass and correct the levels of catabolic factors, in cancer cachexia. In regard to the effects of ghrelin on different types of skeletal muscle, our results differ from the previous study in which ghrelin administration attenuated the atrophy of plantaris muscle (one of the fast-twitch muscles), but did not mitigate the atrophy of soleus muscle in a mouse model of unloading-induced muscle atrophy (Koshinaka et al., 2011). In that study, the dose administered was one-eighth of that used in the present study (approximately 2.5 nmol/mouse/day vs. 20 nmol/mouse/day), and the duration of ghrelin treatment was one-half of that used in the present study (2 weeks vs. 4 weeks) (Koshinaka et al., 2011). Therefore, in addition to the differences in the rodent models, the discrepant finding of our present study that ghrelin affected both slow-twitch muscle and fast-twitch muscle may be related to the experimental dose and/or the duration of ghrelin treatment.

Weight loss is an important prognostic indicator for cancer patients (Tisdale, 2002), and one that has often been ascribed to accompanying anorexia. The pathogenesis of cancer anorexia is multifactorial, and involves the hypothalamic and energy intake-modulating signaling

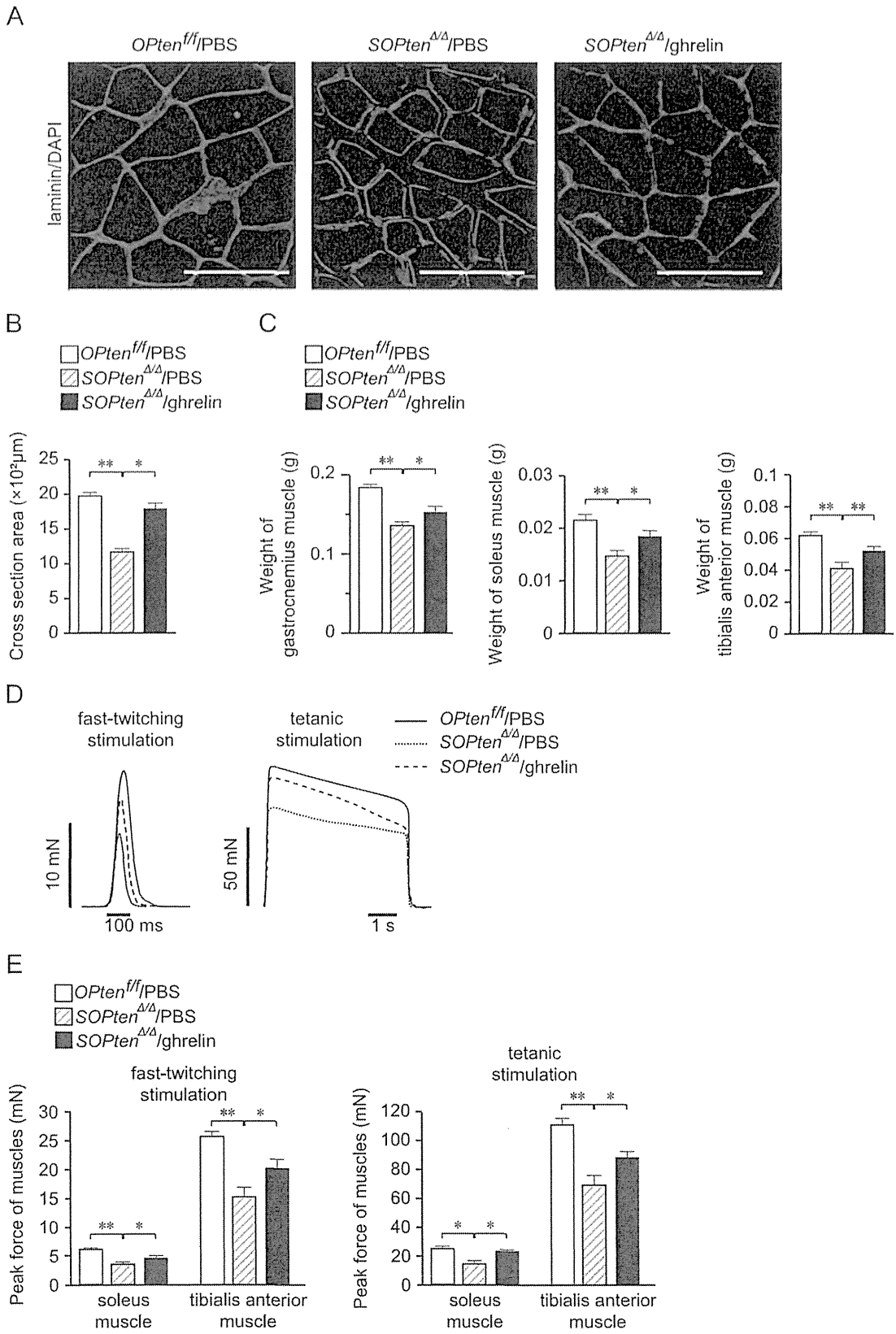

Fig. 6. Effects of ghrelin administration on muscle atrophy in urethane-injected SOPten$^{\Delta/\Delta}$ mice. (A) The sections of gastrocnemius muscles from *SOPten*$^{\Delta/\Delta}$/phosphate buffered saline (PBS) mice, *SOPten*$^{\Delta/\Delta}$/ghrelin mice, and *OPten*$^{f/f}$/PBS mice at 28 days after ghrelin or PBS treatment. The myofiber sizes of lung adenocarcinoma-bearing *SOPten*$^{\Delta/\Delta}$/PBS mice were smaller than those of *OPten*$^{f/f}$/PBS mice, and ghrelin administration suppressed the shrinkage of myofibers in urethane-injected *SOPten*$^{\Delta/\Delta}$ mice. The sections were immunostained with anti-laminin (red) and 4′,6-diamidino-2-phenylindole (DAPI, blue). The images shown are representative gastrocnemius muscle sections from 5 mice per group. Scale bars: 200 μm. The mean cross section areas of gastrocnemius myofibers (B) ($n=5$ per group) and the mean weights of gastrocnemius muscles ($n=12$–23 per group), soleus muscles ($n=12$–13 per group), and tibialis anterior muscles ($n=12$–13 per group) (C) are shown. The cross section areas were measured as described in the Section 2. Data are shown as the mean ± S.E.M. $^{*}P<0.05$. $^{**}P<0.01$. (D) Representative graph of the contraction force trace of tibialis anterior muscles. (E) The peak contraction forces from soleus muscles and tibialis anterior muscles under fast-twitching stimulation (5 mA/1 Hz) or tetanic stimulation (5 mA/75 Hz) in *SOPten*$^{\Delta/\Delta}$/PBS mice, *SOPten*$^{\Delta/\Delta}$/ghrelin mice and *OPten*$^{f/f}$/PBS mice are shown ($n=6$–8 per group). Data are shown as the mean ± S.E.M. $^{*}P<0.05$. $^{**}P<0.01$.

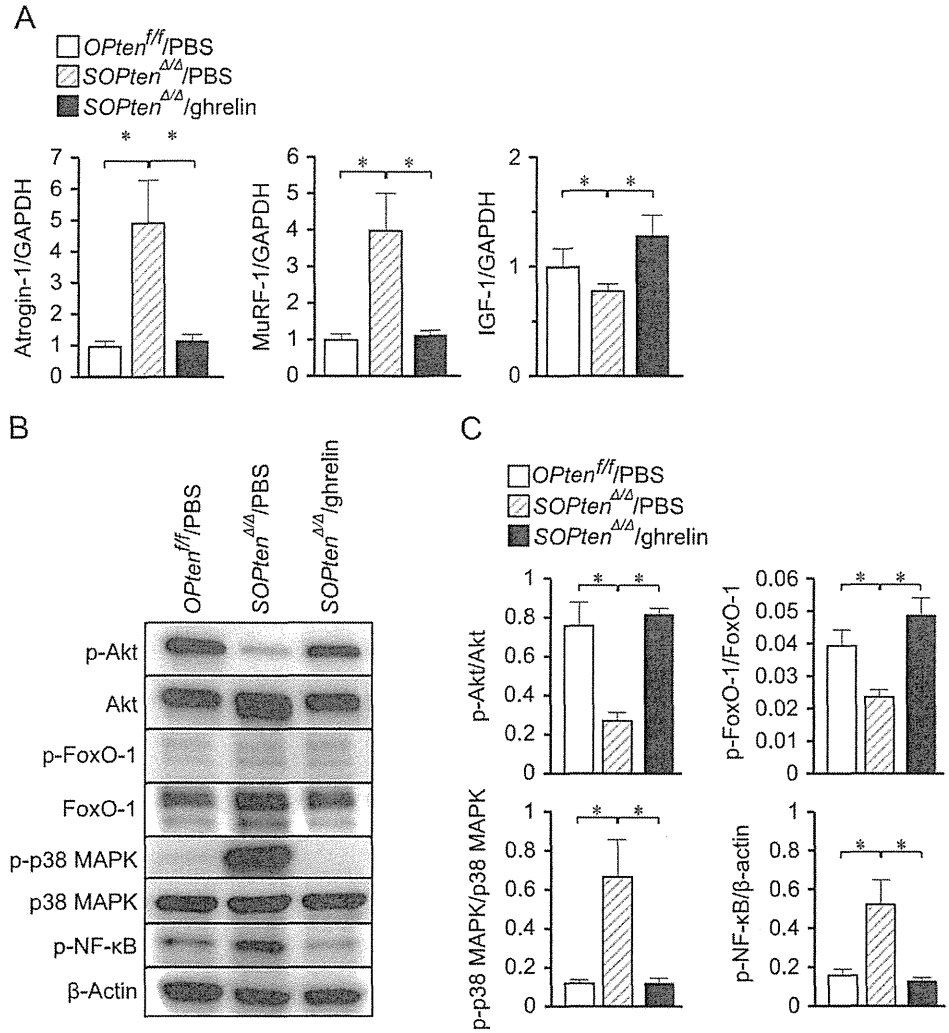

Fig. 7. Effects of ghrelin administration on the expression of catabolic factors and signal transduction molecules in urethane-injected SOPten$^{\Delta/\Delta}$ mice. (A) The mRNA levels of F-box protein-32 (Atrogin1), muscle-specific RING finger protein-1 (MuRF1) and insulin-like growth factor 1 (IGF1) in the lysates of gastrocnemius muscle from *SOPten*$^{\Delta/\Delta}$/phosphate buffered saline (PBS) mice, *SOPten*$^{\Delta/\Delta}$/ghrelin mice, and *OPten*$^{f/f}$/PBS mice at 28 days after ghrelin or PBS treatment. Results are expressed relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Data are the mean ± S.E.M. of 5–7 mice per group. $^{*}P < 0.05$. (B and C) Immunoblots of the phosphorylated forms of Akt, Forkhead box protein O1 (FoxO1), p38 mitogen-activated protein kinase (p38 MAPK), and nuclear factor-kappa beta (NF-κB) protein in the lysates of gastrocnemius muscle from *SOPten*$^{\Delta/\Delta}$/PBS mice, *SOPten*$^{\Delta/\Delta}$/ghrelin mice and *OPten*$^{f/f}$/PBS mice. The quantitative comparisons were performed by using total Akt, total FoxO1, total p38 MAPK, and β-actin as loading controls. Data are the mean ± S.E.M. of 3 mice per group. $^{*}P < 0.05$.

pathways, including hormones, neuropeptides, neurotransmitters and cytokines. These mediators are produced by host immune cells in response to tumor cells or by tumor cells themselves (Fearon et al., 2012; Laviano et al., 2003). Because administration of proinflammatory cytokines (i.e., IL-1β and TNF-α) induces food intake reduction (Bodnar et al., 1989; Ling et al., 1997) and reproduces the characteristic features of the cancer anorexia syndrome (Ling et al., 1997; Mantovani et al., 1998), these cytokines are considered to have a pivotal role in the pathogenesis of cancer anorexia. Ghrelin has been reported to inhibit the expression of proinflammatory cytokines by monocytes (Dixit et al., 2004; Theil et al., 2009) and T cells (Dixit et al., 2004). In this study, we demonstrated not only amelioration of weight loss and food intake reduction but also decreased plasma levels of IL-1β, IL-6, and TNF-α in ghrelin-treated, urethane-injected *SOPten*$^{\Delta/\Delta}$ mice. The inhibitory effect of ghrelin against the expression of proinflammatory cytokines might also attenuate the reduction of food consumption in ghrelin-treated, tumor-bearing mice.

Ghrelin is an endogenous agonist of the GHS-receptor, and exogenous ghrelin administration transiently increases the levels of GH and IGF1 in humans (Nagaya et al., 2001). A previous study demonstrated that high sustained concentrations of IGF1 were associated with an increased risk of various cancers (Renehan et al., 2004). Although we cannot rule out the potential for increased tumor growth after longer-term treatment with ghrelin, there were no significant differences in plasma IGF1 levels or the number and size of the lung tumors between the ghrelin-treated group and PBS-treated group in this study (data not shown). Our data were consistent with a previous study that used a rodent xenograft model (DeBoer et al., 2007; Northrup et al., 2013). Since few studies have examined the effect of ghrelin administration on tumor growth (DeBoer et al., 2007; Northrup et al., 2013), and since IGF1 (Renehan et al., 2004), at least in theory, might stimulate cancer growth, further studies are needed to validate the long-term safety of ghrelin treatment on cancer cachexia.

5. Conclusion

Our results demonstrated the efficacy of ghrelin administration in a rodent model of cancer cachexia. Ghrelin administration ameliorated the body weight loss, suppression of food intake, reduction of fat mass, and skeletal muscle wasting that were

associated with development of lung adenocarcinoma in mice. The pleiotropic effects of ghrelin against cancer cachexia shown in this study may provide relief from the difficult pathological conditions in patients with cancer cachexia.

Acknowledgments

The authors thank Kahori Miyoshi, Koji Toshinai, Sumie Tajiri, and Miki Oshikawa (Miyazaki University) for their technical support. This work was supported in part by a Third Term Comprehensive Control Research for Cancer Grant from the Ministry of Health, Labor and Welfare of Japan (Grant no. 22092501) to M.N.

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Endocrine Journal 2014, 61 (7), 735-742

NOTE

Clinical effects of ghrelin on gastrointestinal involvement in patients with systemic sclerosis

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Abstract. The majority of patients with systemic sclerosis (SSc) have gastrointestinal (GI) tract involvement, but therapies using prokinetic agents are usually unsatisfactory. Ghrelin stimulates gastric motility in healthy human volunteers. In this study, we investigated whether ghrelin could improve gastric emptying in patients with gastrointestinal symptoms due to SSc. The study was performed in a randomized, double-blind, placebo-controlled crossover fashion on two occasions. Ten SSc patients with GI tract involvement received an infusion of either ghrelin (5.0 μg/kg) or saline, and gastric emptying rate was evaluated by ^{13}C-acetic acid breath test. Gastric emptying was significantly accelerated by ghrelin infusion in patients with SSc (ghrelin *vs.* saline: 43.3 ± 11.4 min *vs.* 53.4 ± 5.4 min, P=0.03). No serious adverse effects were observed. Our results suggest that ghrelin might represent a new therapeutic approach for GI tract involvement in patients with SSc.

Key words: Gastric motility, Ghrelin, Systemic sclerosis

SYSTEMIC SCLEROSIS (SSc) is a progressive and multisystem disease characterized by microvascular damage and excess deposition of connective tissue in skin and internal organs, including kidneys, heart, lungs, and gastrointestinal tract. Therefore, patients with SSc have various symptoms such as Raynaud's phenomenon, thickened or hardened skin, and scleroderma renal crisis. In addition to these symptoms, any part of the gastrointestinal (GI) tract can be affected in patients with SSc, and they often have dysphagia, heartburn, bloating, abdominal pain, and diarrhea [1, 2]. Although involvement of the GI tract is not a direct cause of death, it leads to a decline in the quality of life. In an autopsy study, GI muscle atrophy and fibrosis (both of which lead to decreased GI motility) were detected in the esophagus, small intestine, and colon in 74%, 48%, and 39% of patients, respectively [3]. In previous studies, 50–67% of patients with SSc report delayed gastric emptying, which correlates with symptoms of early satiety, bloating, and emesis [4-12]. At present, the cause of scleroderma is still unknown, and there are no effective treatments for SSc. Consequently, the various complications of SSc are treated individually. To treat gastroparesis, prokinetic agents such as metoclopramide, domperidone, erythromycin, mosapride citrate, dinoprost, and octreotide have been used in an attempt to improve GI motility; however, therapies with these agents are usually unsatisfactory [13-17].

Ghrelin, a gut hormone that is produced mainly in the stomach, is a 28–amino acid peptide with an n-oc-

Submitted Feb. 27, 2014; Accepted Mar. 25, 2014 as EJ14-0088
Released online in J-STAGE as advance publication Apr. 17, 2014
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tanoylation modification at Ser^3 [18, 19]. This peptide stimulates gastric motility and accelerates postprandial gastric emptying in human volunteers [20]. Acceleration of gastric emptying by ghrelin has also been observed in patients with diabetic, idiopathic, and post vagotomy gastroparesis, although the numbers of patients enrolled in those studies were small [21-23]. In addition, ghrelin stimulates food intake following peripheral administration [24, 25]. Therefore, we hypothesized that ghrelin might be useful for the treatment of GI disorders related to SSc.

In this study, we investigated whether administration of ghrelin could improve gastric emptying in outpatients with GI symptoms due to SSc. We also evaluated the safety of ghrelin injection to treat GI involvement in patients with SSc.

Subjects and Methods

Patients

To be included in this study, subjects had to have SSc as defined by the American College of Rheumatology (ACR) (Table 1) and exhibit GI involvement. Criteria for GI involvement included gastroesophageal reflux disease (GERD), dysphasia, early satiety, postprandial fullness, bloating, bacterial overgrowth requiring antibiotics, abdominal pain, diarrhea, and/or malabsorption syndrome (Table 2). Exclusion criteria were 1) localized scleroderma; 2) esophageal stenosis; 3) receiving parenteral or enteral nutrition; 4) past history of open-abdominal surgery of the GI tract; 5) allergy against milk or liquid meal (Racol^{TM}); 6) severe hepato-renal or respiratory disorders, severe depression, schizophrenia, mania, severe diabetes, congenital amino-acid metabolic disorder; 7) tendency or past history of suicide; 8) pregnancy; 9) lactation; and 10) past history of malignant tumors. Medication that had already been started before the initial enrolment could be continued during this study. During the entire period of this study, any additional drugs that might influence the study outcome, including prokinetics, antipeptic ulcer agents, and drugs to treat intestine, liver, gallbladder, or pancreatic disease, were not allowed at any time. The initial planned sample size was ten or more. Patient registration lasted from Oct 2010 to Aug 2011. The study protocol was approved by the Ethics Committees on Human Research of the Kyoto University Graduate School of Medicine. We obtained written informed consent from all subjects prior to enrolment. This study was conducted according to the Declaration of Helsinki principals. This trial was registered at the UMIN Clinical Trials Registry as UMIN000003739.

Table 1 Criteria for the Classification of Systemic Sclerosis (Defined by the American College of Rheumatology [ACR])

Major criterion:
• Proximal diffuse (truncal) sclerosis (skin tightness, thickening, non-pitting induration)
Minor criteria:
• Sclerodactyly (only fingers and/or toes)
• Digital pitting scars or loss of substance of the digital finger pads (pulp loss)
• Bilateral basilar pulmonary fibrosis
The patient should fulfill the major criterion or two of the three minor criteria.

Table 2 Grading of Gastrointestinal Involvement in Systemic Sclerosis (Draft Guidelines of Japan)

Upper Digestive Tract
1. Normal
2. Mild: No symptom, but with decreased peristalsis in the lower esophagus
3. Moderate: Gastroesophageal Reflux Disease (GERD)
4. Severe: Dysphagia caused by reflux esophagitis
5. Very Severe: Dysphagia caused by esophageal stenosis
Lower Digestive Tract
1. Normal
2. Mild: Intestinal lesions such as bloating, abdominal pain, diarrhea, etc. with no need for antibiotics
3. Moderate: Overgrowth of enteric bacteria with need for antibiotics
4. Severe: Chronic intestinal pseudo-obstruction or malabsorption syndrome
5. Very Severe: Received intravenous hyperalimentation

Study design

The study was performed in a randomized, double-blind, placebo-controlled two-period crossover fashion on two occasions with a washout interval of at least 2 weeks (Fig. 1). Medication that might affect gastric motility (e.g.: metoclopramide, anticholinergics, calcium channel antagonists, macrolide antibiotics) was discontinued at least 24 hours before ^{13}C-acetic acid breath test. After a 12-hour fast, SSc patients underwent ^{13}C-acetic acid breath test; breath testing started between 8:30 and 9:00 am. Liquid meal (Racol^{TM}, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was used as the test meal. The nutrient composition of 100 mL of liquid meal (100 kcal) is 4.4 g of protein, 15.6 g of carbohydrate, and 2.2 g of fat. ^{13}C was used to label

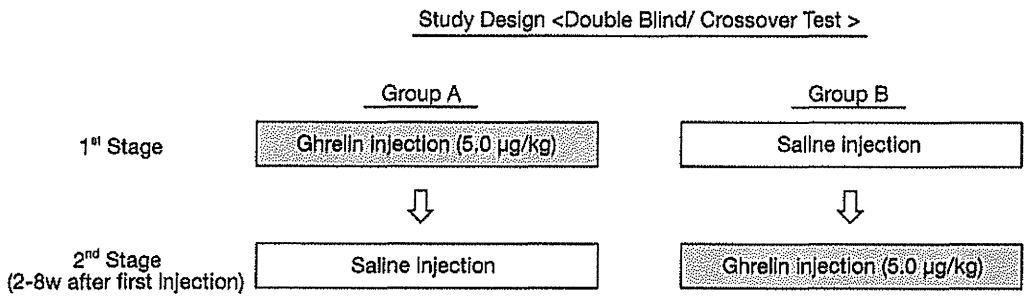

Fig. 1 Schematic of the study schedule.
Ghrelin (5.0 μg/kg) and placebo were administered in a randomized, double-blind, placebo-controlled, and cross-over fashion on 2 separate days at least 2 weeks apart.

acetate (99%; Cambridge Isotope Laboratories, Woburn, MA, USA), which is absorbed in the duodenum but not in the stomach. Liquid meal was mixed with ^{13}C-acetate (100 mg), and all patients ingested 200 mL of test meal within a few minutes. Ghrelin (5.0 μg/kg) or placebo was injected intravenously over 30 seconds immediately after ingestion of the test meal. Breath samples were collected for $^{13}CO_2$ measurement before the meal, and then every 15 minutes for 3.5 hours. During each expiration phase, exhaled air was collected into a bag a total of 15 times. The concentration of $^{13}CO_2$ was measured using a gas chromatograph isotope-ratio mass spectrometer (UBiT-IR300, Ootsuka Electronic Corp), and the measured values were presented as the delta-$^{13}CO_2$ (expressed as a percentage). To evaluate the effect of ghrelin on gastric emptying, T_{max} was defined as the time taken to reach the maximum concentration, calculated using the delta-$^{13}CO_2$ values.

T_{max} assessed by ^{13}C-acetate breath test

The ^{13}C-acetate breath test was developed as a non-radioactive alternative for the measurement of gastric emptying. Braden and colleagues [26] reported that half-emptying times for the ^{13}C-acetate breath test were closely correlated with those measured by radioscintigraphy, using both semisolids and liquids, and that the T_{max} of $^{13}CO_2$ exhalation was itself a reliable parameter compared with the half-emptying times obtained by scintigraphy.

Primary endpoint

The primary endpoint of this trial was T_{max} assessed by ^{13}C-acetic acid breath test.

Secondary endpoint

The secondary endpoints of this trial were alteration in hunger sensation and serum GH levels upon ghrelin or placebo administration.

Assessment of satiety

Visual analogue scale (VAS) scores, 10 cm in length, were used to assess satiety [27-29]. VASs were completed pre-infusion and 15, 30, 60, and 90 min after ghrelin or saline administration. The positions on the scale were measured in centimeters.

Measurement of serum GH concentration

Blood samples for measurement of GH were drawn before ingestion of the test meal and 30 minutes after ghrelin or saline infusion. Serum GH concentrations (normal values: male, <1.46; female, 0.28–8.70 ng/mL) were measured by IRMA (Mitsubishi Kagaku Bio-Clinical Laboratories Inc., Tokyo, Japan).

Measurement of plasma ghrelin levels

Measurement of plasma ghrelin levels was performed as reported previously [30]. Blood samples drawn from a forearm vein were immediately transferred to chilled siliconized glass tubes containing Na_2EDTA (1 mg/mL) and aprotinin (1000 KIU/mL, Ohkura Pharmaceutical, Kyoto, Japan). After centrifugation at 4°C to separate out the plasma, hydrochloric acid was added to samples at a final concentration of 0.1 N. Plasma was immediately frozen and stored at −80°C prior to the assay. Plasma ghrelin concentrations were determined using a ghrelin ELISA kit (Mitsubishi Kagaku Iatron, Tokyo, Japan).

Assessment of safety

Vital signs, including blood pressure, pulse rate, and body temperature, were measured during examination. Changes in physical symptoms were assessed by phone interview 2 weeks after ghrelin or saline injection. If the study was interrupted, assessments of safety by

hematology, blood chemistry, and urine analysis were performed when the study was discontinued.

Ghrelin

Human ghrelin was prepared as described previously [28]. Ghrelin was dissolved in 3.75% D-mannitol to a final concentration of 180 μg/mL. Solutions were filtered and stored at −20°C in sterile vials. Examination by the Japan Food Research Laboratories (Tokyo, Japan) did not find any traces of endotoxin in the ghrelin solutions; a pyrogen test based on the Pharmacopoeia of Japan was also negative.

Statistical evaluations

A sample size of 10 patients was required to provide at least 90% power to detect a T_{max} ratio of 0.70 between treatments with a standard deviation of 30 minutes and an intra-patient correlation coefficient of 0.50 or more. This ratio was based on data from previous studies. All statistical analysis of the two-period cross-over design was performed using a linear mixed-effect model with treatment period as fixed effects and patient as the random effect. The 95% confidence intervals (CIs) for group means and treatment differences were estimated using least-squares means (LS-means) and robust variance. Carry-over effects were assessed using the test for the treatment by period interaction term in linear mixed-effect models. All statistical analyses were performed using the SAS software, version 8 (SAS Institute Inc., Cary, NC, USA). A two-tailed *P*-value was used, with the required level of significance set at 0.05.

Results

Baseline characteristics of subjects

Ten subjects (seven women, median age 67.5 years, range 48–80 years) were enrolled in this study (Table 3). All ten subjects were diagnosed with SSc, as defined by the criteria of the American College of Rheumatology (ACR); all subjects fulfilled the major criterion. Three subjects had diffuse SSc, and seven had limited SSc. Anti–topoisomerase I antibodies were positive in two patients, and anti-centromere antibodies were positive in four patients. As shown in Table 3, all subjects exhibited GI involvement; nine had early satiety and postprandial fullness, seven had heart burn and dysphagia caused by reflux esophagitis, and two had diarrhea. Average BMI at registration was 21.1 ± 4.2 kg/m^2. None took H_2-blockers or antibiotics. One patient (Patient No. 2) discontinued this examination after the first injection (saline) because of a gallbladder stone attack.

Clinical effects

Plasma levels of ghrelin 30 minutes were 999.2 ± 23.7 fmol/mL after ghrelin injection (pre-injection, 20.2 ± 10.3 fmol/mL) and 15.4 ± 6.0 fmol/mL after saline injection (pre-injection, 18.8 ± 7.4 fmol/mL). As shown in Fig. 2, serum GH levels were significantly elevated after ghrelin administration (ghrelin *vs.* saline: 61.9 ± 9.5 *vs.* 1.4 ± 1.2 ng/mL).

Ghrelin shortened gastric emptying time in patients with SSc (Fig. 3). Gastric emptying, as determined evaluated by ^{13}C-acetic acid breath test, signifi-

Table 3 Baseline characteristics

Patient No.	Sex	Age	BMI	subtypes	AT1A	ACA	Gastrointestinal Symptoms			Gastrointestinal Drugs	
							Postprandial Fullness	Dysphagia	Diarrhea	Prokinetics	PPI
1	M	68	26.3	diffuse	-	-	+	-	-	-	-
2	M	48	21.2	diffuse	+	-	+	-	+	+	+
4	F	57	22.2	limited	-	+	+	+	-	+	+
5	F	71	17.4	limited	-	+	+	+	-	+	+
6	M	76	20.2	limited	-	-	+	+	-	-	+
7	F	62	18.8	limited	-	-	-	+	-	-	+
8	F	73	17.7	limited	-	+	+	+	-	+	+
9	F	66	30.1	limited	-	+	+	+	-	+	+
11	F	80	18.1	diffuse	+	-	+	+	+	+	+
12	F	50	18.8	limited	-	-	+	-	-	+	+

ACA, anti-centromere antibody; AT1A, anti–topoisomerase I antibody

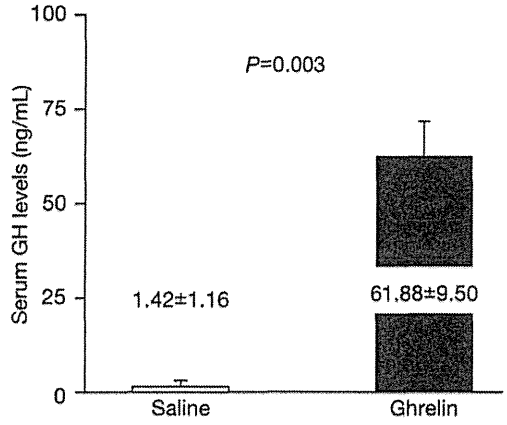

Fig. 2 Serum growth hormone levels 30 minutes after ghrelin or saline injection
Data are shown as means ± SD (n=9).

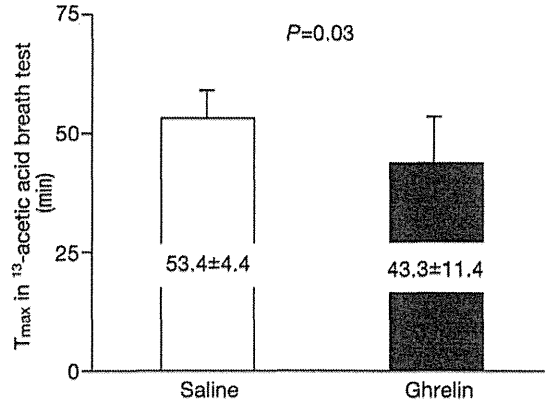

Fig. 3 Gastric emptying of the liquid test meal
Gastric emptying was evaluated by ^{13}C-acetic acid breath test. T_{max} was defined as the time taken to reach the maximum concentration. Data are shown as means ± SD (n=9).

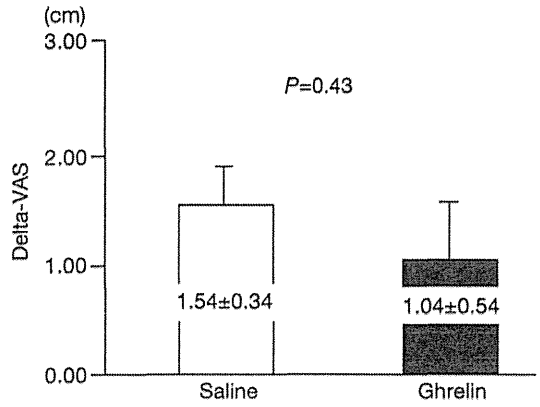

Fig. 4 Effect of ghrelin on appetite in patients with SSc.
The data are shown as changes in the visual analog scores (VAS) after ghrelin or saline injection: maximum value after injection minus value prior to injection (means ± SD; n=9).

cantly accelerated by ghrelin injection (43.3 min, 95% CI 31.8–54.7 min) relative to saline injection (53.4 min, 95% CI 47.4–59.3 min; treatment difference −10.1 min, 95% CI −19.1–−1.2 min, P=0.03). There was no carry-over effect in the primary endpoint (P=0.17 by interaction test). The effects of ghrelin on appetite were assessed by changes in the visual analog scores (VAS) before and after ghrelin or placebo injection. Delta-VAS was calculated as the maximum value after administration minus the value before administration. Thus, the larger amounts of changes in VAS indicated an improvement in early satiety after ghrelin or saline injection. As shown in Fig. 4, however, administration with ghrelin did not improve early satiety. No significant carryover effect or interaction effect was observed (P=0.06).

Adverse effects

No serious adverse effects were observed. We observed two moderate events, flushing and sweating, with incidences similar to those previously reported. These complaints were transient and well tolerated.

Discussion

Administration of ghrelin induces the migrating motor complex in fasting rat and human and accelerates postprandial gastric motility in healthy humans and patients with idiopathic, neurogenic, or diabetic gastroparesis [21-24]. In previous studies, $T_{1/2\ liq}$ (half-emptying time for liquids, in minutes) decreased by 20–30% following injection of 0.7–5.9 μg/kg of ghrelin. We previously reported that ghrelin tended to increase appetite in a dose-dependent manner (i.e., more so at 5.0 than 1.0 μg/kg) in a phase I study, and that it is safe at a dose of 5.0 μg/kg [27, 28, 31]. Based on these findings, we adopted the present protocol.

This study is the first clinical investigation to demonstrate that a single administration of ghrelin significantly accelerated gastric emptying time, relative to placebo, in patients with SSc who had current symptoms suggestive of gastroparesis; the improvement rate was about 23%. This observation is in line with animal studies and with previous reports of a stimulatory effect of a similar dose of ghrelin on gastric motility in humans [20-25]. We initially expected that administration of ghrelin would improve GI motility, result in relief of GI symptoms. However, although the gastric emptying rate was increased following ghrelin

administration, post-prandial satiety did not improved. Franck-Larsson *et al.* reported that delayed gastric emptying in SSc did not relate to gastrointestinal symptoms or myoelectric gastric activity [5]. Taken together, these observations suggest that factors other than gastric motility might contribute to upper gastrointestinal symptoms.

Several prokinetic agents, such as metoclopramide, domperidone, erythromycin, mosapride citrate, and dinoprost, have been used in attempts to improve GI motility in patients with SSc who suffered from gastroparesis; however, therapies with these agents are usually unsatisfactory [13-17]. Soudah *et al.* had reported that octreotide stimulates intestinal motility and reduces bacterial overgrowth, resulting in improvement in abdominal symptoms [15]. Based on that report, we predict that octreotide could be adapted to the treatment of gastric involvement in patients with SSc. Like octreotide, ghrelin may also have therapeutic potential for the treatment of GI dismotility in patients with SSc. Because this study was designed with a single-dose and single-injection protocol, larger-scale and longer-term prospective cohort studies are needed to define the effects of ghrelin on gastric motility in patients with SSc. Also, future studies should investigate whether ghrelin treatment can improve gastrointestinal symptoms.

Cohen *et al.* proposed a two-stage process in the pathophysiology of SSc: a neuropathic phase followed by a myopathic phase [32]. The second phase is characterized by smooth-muscle atrophy and replacement of muscle tissue with fibrosis. From that perspective, it is likely that ghrelin would not be effective for the treatment of SSc patients with second-phase gastroparesis. Although experiments in chemically or surgically vagotomized animals have suggested that the motility effects of ghrelin are caused mainly by activation of vagal afferents [33, 34], the ghrelin receptor is expressed in the enteric nerve system [35, 36]; furthermore, *in vitro*, ghrelin increases electrically induced contraction of rat and mouse muscle strips [37-40]. In addition, in previous studies, injection of ghrelin accelerated gastric motility in patients with neurogenic and diabetic gastroparesis [21-24]. Thus, we expect that ghrelin, at least, is effective for the treatment of SSc patients with first-phase gastroparesis.

There are some limitations in the present study such as small sample size, single dose and single attempt for ghrelin infusion. However, the results of this study suggest that ghrelin may have therapeutic potential for the treatment of GI dismotility in patients with SSc. Further studies using more subjects and with multiple injections of ghrelin will be required in order to confirm the effects of ghrelin on gastric motility and gastrointestinal symptoms in patients with SSc.

Acknowledgements

This study was supported by funds from the Ministry of Education, Science, Culture, Sports and Technology of Japan; the Ministry of Health, Labour and Welfare of Japan; the Tokyo Biochemical Research Foundation; and the Foundation for Growth Science. We would like to thank K. Amemiya for her excellent secretarial assistance, and C. Ishimoto and C. Shiraiwa for their excellent technical assistance. We would also like to thank H. Endo at Toho University for his special technical advice.

Conflict of Interest

The authors have no conflict of interest and nothing to disclose.

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Surg Today (2014) 44:2131–2137
DOI 10.1007/s00595-014-0873-9

ORIGINAL ARTICLE

Serum ghrelin levels partially recover with the recovery of appetite and food intake after total gastrectomy

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Received: 16 July 2012 / Accepted: 3 February 2014 / Published online: 7 March 2014

Abstract

Purpose Ghrelin may lead to weight gain by appetite stimulation. This prospective study investigated the association between weight loss and the ghrelin levels in patients after gastrectomy.

Methods Thirty-three males and eight females were enrolled in the study. The average age was 66 years. Measurements of the serum ghrelin level and an appetite questionnaire were performed preoperatively and at one, three, six and 12 months postoperatively.

Results The preoperative serum total ghrelin level was 51.6 ± 31.9 (fmol/ml ± SD), and that at one, three, six and 12 months postoperatively was 16.9 ± 9.0, 21.2 ± 16.0, 28.0 ± 19.1 and 29.6 ± 20.6 (fmol/ml ± SD), respectively. The appetite score was 2.02 ± 1.09 points at 1 month, and increased significantly to 2.61 ± 1.00 by 12 months.

Conclusions The ghrelin levels were reduced after gastrectomy and did not recover by 12 months postoperatively. Further studies are needed to evaluate these results as the basis of a therapeutic trial.

Keywords Ghrelin · Gastrectomy · Weight loss

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Introduction

Weight loss is a common and serious outcome in patients with gastric cancer after total gastrectomy, and this phenomenon correlates with a decline in the postoperative quality of life. Various mechanisms have been considered to be responsible, such as perturbation of absorption due to reduced pancreatic excretion [1], the absence of gastric acid [2], esophageal reflux [3] and increased peristalsis and diarrhea [4]. However, reduced food intake remains the most reasonable explanation for weight loss after total gastrectomy [5, 6]. Therefore, surgeons treating patients with gastric cancer have tried to allow increased food intake by fashioning gastric substitutes, such as a jejunal pouch [7], or performing less invasive surgical procedures, such as laparoscopic resection [8], but such procedures have not always led to increased food intake. Another study indicated that the majority of patients after total gastrectomy could eat as much food as healthy subjects under a regulated program [9]. Our own clinical experience showed that some patients do not have significant body weight loss after total gastrectomy, which they achieve by eating small but frequent meals. These changes suggest that the reduced food intake after total gastrectomy cannot be simply explained by a loss of storage volume due to gastrectomy, but rather reflects a disturbance of eating activity through some other mechanism(s).

Ghrelin was first identified as an intrinsic ligand for the growth hormone secretagogue (GHS) receptor of the pituitary gland in 1999 [10]. However, recent studies have revealed that ghrelin stimulates not only GH secretion from the pituitary gland but also the appetite signal in the hypothalamus, in opposition to leptin [11], a well-known appetite-suppressing hormone derived from fat tissue and gastrointestinal activity, such as peristalsis, gastric acid secretion and pancreatic excretion, through the vagal nerve [12]. Most ghrelin is secreted by the stomach, with only trace amounts from the duodenum, brain and other organs [13]. Therefore, it is reasonable to expect that the ghrelin dynamics will change after total gastrectomy.

The identification of ghrelin led to a novel concept of weight regulation by the stomach, which has been investigated in the surgical field, especially in bariatric surgery and gastrectomy [14–16]. To date, there have been only a few studies that have investigated the ghrelin levels after total gastrectomy. Vagotomy is also expected to affect the ghrelin production from the stomach [17]. The present study was undertaken to prospectively investigate the association between body weight loss and appetite, and the clinico-pathological findings in patients with gastric cancer after total gastrectomy.

Methods

Patients

Patients with gastric cancer who underwent total gastrectomy at Jichi Medical University Hospital from 2004 through 2005 were enrolled in this study. The inclusion criteria were: (1) gastric cancer, (2) total gastrectomy, (3) no serious postoperative complications, (4) no other gastrointestinal diseases, (5) no serious organ dysfunction (white blood cell count: 4,000–12,000/mm^3, platelet count: $\geq$100,000/mm^3, hemoglobin: $\geq$8.0 g/dl, liver function; total bilirubin: $\leq$1.5 mg/dl, GOT: $\leq$80 U/l, GPT: $\leq$80 U/l, ALP: two times the institutional standard or below, renal function; creatinine: $\leq$1.5 mg/dl), and (6) informed consent to participate in this study.

There were 41 patients enrolled in the study who met all the above criteria, including 33 males and eight females. The average age of the patients was 66.0 years (range 45–79).

Surgery and adjuvant chemotherapy

All patients underwent total gastrectomy with an appropriate lymph node dissection. No patient in this series underwent splenectomy as part of the resection. The vagus nerves were divided in every patient in this series. Roux-en-Y reconstruction was performed in all patients. The patients with pathologically demonstrated lymph node metastases underwent adjuvant chemotherapy using TS-1.

Measurement of the serum ghrelin levels

The serum ghrelin levels were measured preoperatively and postoperatively at 1, 3, 6 and 12 months. A 10 ml blood sample was collected from all patients before breakfast on the day of measurement.

Two kinds of rabbit polyclonal antisera were used in this study. One antiserum was raised against the COOH-terminally Cys-extended rat ghrelin (position 1–11) in New Zealand white rabbits (#G606) and was shown to specifically recognize ghrelin with *n*-octanoylated Ser 3 (acylated ghrelin; ghrelin). We performed a radioimmunoassay (RIA) with this antiserum, referred to as NH_2-terminal RIA (N-RIA), to measure the concentration of acylated ghrelin. The other antiserum was raised against the NH_2-terminally Cys-extended rat ghrelin (position 13-28) (#G107) and was shown to recognize both acylated ghrelin and desacyl-ghrelin [18]. A RIA using this antiserum, referred to as COOH-terminal RIA (C-RIA), was used to measure the concentration of acylated ghrelin plus desacyl-ghrelin.

These two ghrelin-specific RIAs were used to measure the acylated ghrelin and desacyl-ghrelin contents in several tissues [18]. The bound and free ligands were separated using a second antibody. "Acylated ghrelin" is referred to as "ghrelin" and "acylated ghrelin plus desacyl-ghrelin" is referred to as "total ghrelin" in this report.

Questionnaire

The appetite, times and amount of meals were scored on a scale from 1 to 5 by the patient using a written questionnaire administered at one, three, six and 12 months after surgery (Table 1). The severity of gastrointestinal symptoms such as dysphagia, abdominal pain and diarrhea were scored by patients, where higher scores indicate more severe dysfunction. The weight (kg) and body mass index (BMI) (kg/m^2) were also measured preoperatively, and were assessed postoperatively at 1, 3, 6 and 12 months.

Statistical analysis

The statistical analysis was performed with the SPSS statistical package, version 18 (SPSS, Chicago, IL, USA). The data are expressed as the mean $\pm$ SD. The differences in the serum ghrelin levels, BMI and questionnaire scores were compared using *t* tests. The correlation between the total ghrelin level and appetite score was analyzed using the Pearson product–moment correlation coefficient. *P* values <0.05 were considered to indicate statistical significance.

Table 1 Postoperative Questionnaire (completed by each patient)

1) How is your present appetite compared to that before surgery (points)?			
Decreased	1	Increased slightly	4
Decreased slightly	2	Increased	5
Same as before	3		
2) How much do you eat now compared to before surgery (points)?			
1/4 or less	1	3/4 to same	4
1/4–1/2	2	Increased	5
1/2–3/4	3		
3) How many times do you eat meals each day?			
Once	4 times		7 times or more
Twice	5 times		
3 times	6 times		
4) Do you have a feeling of fullness after meals compared to before surgery?			
Hungry	1 (point)	Slightly full stomach	4 (points)
Slightly hungry	2	Full stomach	5
Normal	3		
5) Do any of the following symptoms appear within 30 min after a meal?			
Yes or no			
6) Do any of the following symptoms appear 2–3 h after a meal?			
Yes or No			
1) cold sweat	7) fever		13) abdominal pain
2) palpitations	8) weakness		14) diarrhea
3) vertigo	9) sleepiness		15) nausea
4) syncope	10) headache		16) vomiting
5) facial flushing	11) chest discomfort		17) abdominal distention
6) pale skin	12) borborygmy		18) abdominal discomfort

Ethics

This study was approved by the Institutional Review Board of Jichi Medical University (No. 02-38) before beginning the investigation. All patients provided their written informed consent before entering the study.

Results

Serum ghrelin

The preoperative mean concentration of serum total ghrelin was 51.6 ± 31.9 fmol/ml (mean ± SD, normal 80–140 fmol/ml), and that at 1, 3, 6 and 12 months after surgery was 16.9 ± 9.0, 21.2 ± 16.0, 28.0 ± 19.1 and 29.6 ± 20.6 fmol/ml, respectively (Fig. 1a). The preoperative mean concentration of serum ghrelin was 6.2 ± 5.2 fmol/ml (normal 8–14 fmol/ml), and that at 1, 3, 6 and 12 months after surgery was 2.5 ± 2.8, 2.8 ± 4.8, 2.5 ± 3.7 and 2.2 ± 3.1 fmol/ml, respectively (Fig. 1b).

In order to understand the trend in the total ghrelin levels during the postoperative period, patients were divided into a low total ghrelin group (<40 fmol/ml) and a normal total ghrelin level group (>80 fmol/ml). The serum total ghrelin levels for both groups were compared postoperatively and are shown in Fig. 1c. After surgery, there was a significant reduction in the level at 1 month postoperatively compared to the preoperative level for both total ghrelin and ghrelin. Continuous significant increases in the serum levels were observed until 12 months after surgery for total ghrelin, but the concentration of ghrelin was stable over the period from 1 to 12 months postoperatively.

Pathological stage and ghrelin level

The tumor stage using the Japanese classification of gastric carcinoma (version 13) was as follows: stage IA, 16 patients; stage IB, 4 patients; stage II, 7 patients; stage IIIA, 4 patients; stage, IIIB; 6 patients and stage IV, 4 patients.The concentration of serum total ghrelin was not significantly different among the patients with different tumor stages at each time point measured.

Adjuvant chemotherapy and ghrelin

Among the patients receiving adjuvant chemotherapy, the preoperative serum total ghrelin level was 44.5 ± 20.9 fmol/ml (AC+), and was 58.0 ± 37.4 fmol/ml in patients who did not receive adjuvant chemotherapy (AC−). There were no significant differences between these two groups at any of the time points measured, but the concentration of total ghrelin increased over the 12 month study period in both groups.

Cancer recurrence and ghrelin

From the viewpoint of recurrence, the preoperative concentration of total ghrelin was 46.7 ± 15.6 fmol/ml in patients who developed recurrent disease and was 53.0 ± 33.8 fmol/ml in patients without recurrence. There were significant differences between these two groups at 1 and 6 months postoperatively. There were no significant differences between these two groups at 3 months and at 12 months. The concentration of total ghrelin showed an increase over the study period in both patient groups.

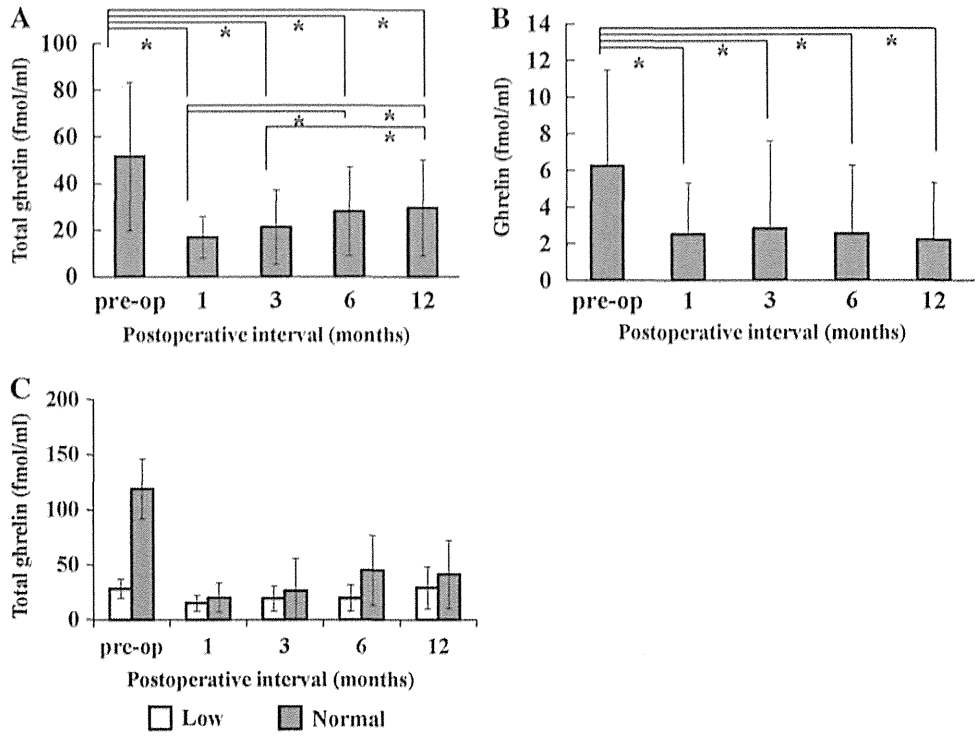

Fig. 1 **a** The concentration of serum total ghrelin preoperatively and at 1, 3, 6 and 12 months after surgery. **b** The concentration of serum ghrelin preoperatively and at 1, 3, 6 and 12 months after surgery. **c** The concentration of serum total ghrelin preoperatively and at 1, 3, 6 and 12 months after surgery in the low and normal ghrelin level patients. The *error bars* indicate the standard deviation in each group. $^*P < 0.05$

Body mass index and ghrelin

Compared with the preoperative BMI, there was a BMI decrease (Δ BMI) of 2.7 ± 1.4, 3.1 ± 1.5, 3.6 ± 1.7 and 3.9 ± 2.0 at 1, 3, 6 and 12 months after surgery, respectively (Fig. 2). After surgery, there was a significant decrease in the BMI when comparing 1 month to 6 and 12 months, and between 3 months and 12 months after resection. The BMI decreased significantly over the 12-month study period.

As a parameter of the overall nutritional status, the serum albumin level was 3.77 ± 0.45, 3.89 ± 0.33, 3.95 ± 0.35, 4.04 ± 0.41 and 4.07 ± 0.46 mg/dl preoperatively and at 1, 3, 6 and 12 months after surgery, respectively. There was a significant increase compared to the preoperative levels at 3, 6 and 12 months after surgery, and the level was significantly increased at 12 months after surgery compared to 1 month postoperatively.

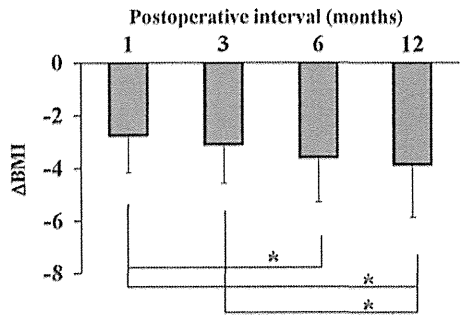

Fig. 2 The ΔBMI is shown at 1, 3, 6 and 12 months postoperatively. There was a significant difference in levels at 1 and 3 months after resection. $^*P < 0.05$

Appetite score

The appetite and symptom scores were based on a questionnaire (Table 1). The average appetite score was 2.02 ± 1.09 at 1 month; it increased gradually to 2.18 ± 0.95 at 3 months, 2.73 ± 1.10 at 6 months and 2.61 ± 1.00 at 12 months after surgery. There were significant changes between 1 and 6 months postoperatively, between 1 and 12 months, and between 3 and 6 months. There were no significant changes in patient satisfaction with meals after surgery (Fig. 3a). The amount of each meal decreased at 1 month, but slightly increased later. There were significant changes in meal volume between 1 month and 6 months, and between 3 and 6 months (Fig. 3b). There was a significant decrease in the frequency of meals, but it returned to the normal range after surgery.

Both the appetite and the ghrelin levels recovered chronologically, but there was only a weak positive correlation (linear regression: $R^2 = 0.047$) between the ghrelin level and the appetite score (Fig. 3c). The Pearson product–moment correlation coefficient was 0.216 ($P = 0.006$). Recovery of the serum concentration of total ghrelin weakly correlated with an improved appetite score.

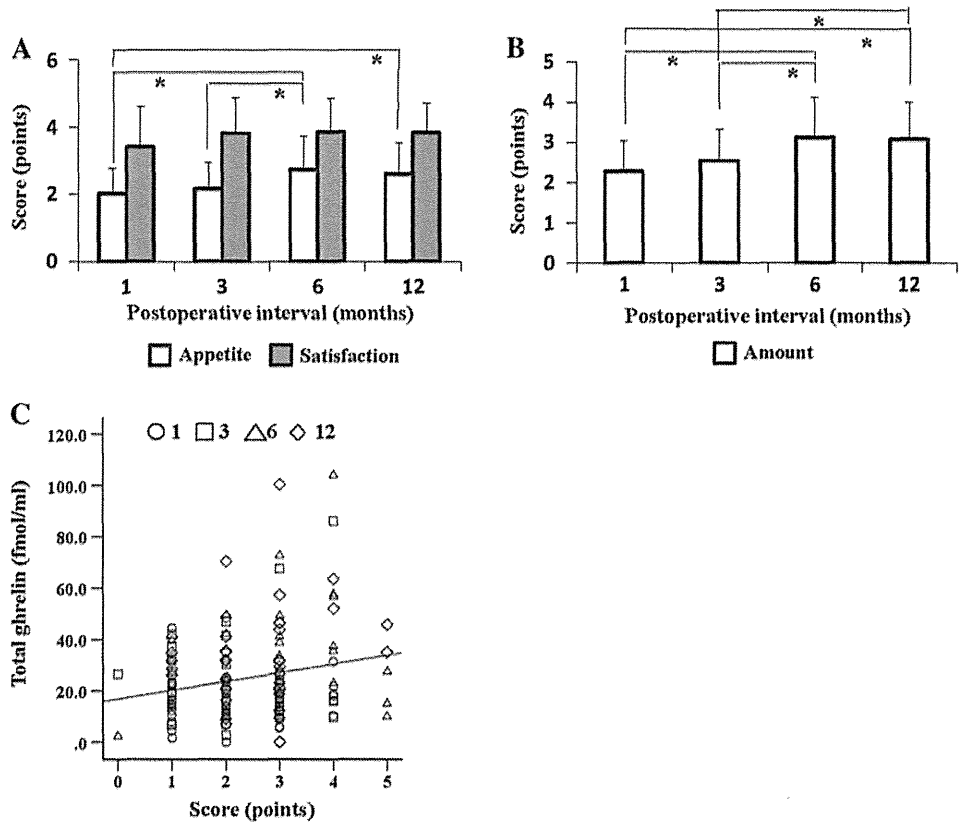

Fig. 3 **a** The appetite and satisfaction scores after surgery (scored as shown in Table 1). **b** The amount eaten at each meal after surgery (scored as shown in Table 1). **c** The correlation between the total ghrelin level and appetite score at 1, 3, 6 and 12 months after surgery. $*P < 0.05$

Dumping syndrome and total ghrelin

Dumping syndrome was defined for patient responses as early (within 30 min of eating) or delayed (at 2–3 h after eating) by the wording in the questionnaire. There were no significant differences in patients with early dumping symptoms and those without early dumping syndrome with regard to the concentration of total ghrelin. The same trend was observed for symptoms of delayed dumping syndrome (at 2–3 h after eating).

Discussion

Ghrelin, which is secreted mainly from A-like cells in the fundus of the stomach, causes increased weight by stimulating appetite and GH secretion [10, 13]. In general, if the stomach is not impaired, ghrelin functions in a negative feedback loop in relation to body weight. Following surgery that affects gastric function, the reduction of ghrelin may be related to postoperative weight loss. Gastric bypass surgery in morbidly obese patients successfully reduces both the body weight and ghrelin concentration, and probably appetite as well, while starvation reduces the body weight but increases the ghrelin concentration with a sensation of hunger [14, 16]. Patients with gastric cancer generally have an up to 80 % decrease in the serum ghrelin levels and a loss of up to 20 % of their weight after undergoing a total gastrectomy [19, 20].

Transection of the vagus nerve has been reported to abolish the orexigenic effect of ghrelin in rodents [21, 22]. However, there have been some reports that there is no relationship between the ghrelin concentration and vagotomy following gastrectomy or esophagectomy [23].

"Appetite" was scored based on the subjective feelings of the patient. An objective assessment of appetite is difficult because it is a subjective symptom. A recently developed scoring system has been used as a tool for evaluating postoperative gastrointestinal dysfunctions after upper gastrointestinal resections for malignancy [24, 25]. The dysfunction after total gastrectomy was found to be more severe than after distal gastrectomy or pylorus-preserving gastrectomy.

These present results showed that the use of adjuvant chemotherapy, the cancer stage and recurrence are not related to the ghrelin levels in this study. In the questionnaire, the appetite score recovered chronologically in parallel with the change in the concentration of serum ghrelin. Although most ghrelin is secreted by the stomach, with only trace amounts from the duodenum, brain and other organs, it is considered that other organs may secrete ghrelin to compensate after total gastrectomy [13]. The serum ghrelin concentration increased in the early

postoperative period, but did not synchronize with weight recovery and the scores on the subjective questionnaire. The concentration of serum ghrelin decreased significantly immediately after the operation, and increased chronologically in patients after total gastrectomy. Although the total ghrelin level increased chronologically (Fig. 1a), the ghrelin levels did not recover completely after surgery (Fig. 1b). The increase in appetite score may reflect a number of subjective factors, such as a desire to eat after surgery, satisfaction from eating food, etc. While both the ghrelin levels and the appetite score recovered over time, there was only a weak positive correlation between the ghrelin level and appetite score.

Ghrelin enhances appetite and increases food intake in humans. Ghrelin increases the energy intake in patients with cancer and an impaired appetite [26]. Roux et al. [27] reported that ghrelin does not stimulate food intake in patients after an upper gastrointestinal surgical procedure involving vagotomy (three cases of total gastrectomy, one partial gastrectomy and three cases of esophagectomy). Other investigators found no change in energy intake despite an infusion of exogenous ghrelin. This double-blind and placebo-controlled trial supports the conclusion of the current study that ghrelin does not play an important role in food intake or the weight loss in patients after total gastrectomy.

An effect of esophagectomy on ghrelin and weight loss has been reported. For example, Doki and colleagues [17] reported a correlation between the ghrelin level and postoperative weight loss in patients after esophagectomy. They observed that the ghrelin levels did not return to normal in patients within 1 year after surgery. However, this analysis did not study the same group of patients longitudinally. In a prospective study of the same patients over time, our group found that the serum ghrelin levels did return to the preoperative levels following esophagectomy [28]. This result is expected, since most ghrelin is produced in the stomach, which is unaffected by an esophagectomy, although the vagal influence is lost. In a Phase II clinical trial of ghrelin administration after esophagectomy, Yamamoto and colleagues [29] found significantly increased food intake in patients who received exogenous ghrelin. This suggests that clinical trials in patients after gastrectomy should be performed to improve the understanding of this complex physiology.

Although the ghrelin levels increased after surgery in this study, they did not return to the preoperative levels. Given the persistently low levels of ghrelin, if the postoperative weight loss in patients after total gastrectomy is improved by higher ghrelin levels, then supplemental therapy with ghrelin may lead to a reversal of appetite loss and weight loss in the early postoperative period. It has been demonstrated that peripheral ghrelin administration leads to increased food intake and GH release in humans [30]. The safety and efficacy of recombinant ghrelin have been confirmed in clinical trials involving patients with heart failure [31]. The role of ghrelin in the control of appetite remains an area of active investigation. In particular, the effects of surgical resection on the ghrelin levels may provide some valuable insight into its physiological role. This study is associated with several limitations, including the heterogeneous group of patients, with malignancies in various stages, and the fact that chemotherapy was given to select patients. The nature of the questions asked in the survey instrument can also be a potential source of bias. Despite these limitations, the present results suggest the need for further evaluation of the effects of surgical resection on ghrelin.

Considering that the weight loss was not recovered 12 months after surgery, the role of ghrelin in the recovery from weight loss after total gastrectomy remains unclear. Severe weight loss and difficulty recovering the lost weight may depend on other factors, such as dysphagia and diarrhea, which are common after resections for gastric cancer. The present study suggests that further investigations of exogenous recombinant ghrelin therapy are needed to evaluate the possible effects on patients after gastrectomy.

Conflict of interest None of the authors has any conflict of interest to declare.

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J Pharmacol Sci 124, 31 – 39 (2014)

Journal of Pharmacological Sciences

Full Paper

Organ-Specific Activation of the Gastric Branch of the Efferent Vagus Nerve by Ghrelin in Urethane-Anesthetized Rats

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Received September 25, 2013; Accepted October 31, 2013

Abstract. Ghrelin plays multiple physiological roles such as growth hormone secretion and exerting orexigenic actions; however, its physiological roles in the electrical activity of autonomic nerves remain unclear. Here, we investigated the effects of human ghrelin on several autonomic nerve activities in urethane-anesthetized rats using an electrophysiological method. Intravenous injection of ghrelin at 3 μg/kg significantly and transiently potentiated the efferent activity of the gastric vagus nerve; however, it did not affect the efferent activity of the hepatic vagus nerve. The activated response to ghrelin in the gastric efferent vagus nerve was not affected by the gastric afferent vagotomy, suggesting that this effect was not induced via the gastric afferent vagus nerve. Ghrelin did not affect the efferent activity of the brown adipose tissue, adrenal gland sympathetic nerve, and the renal sympathetic nerve. In addition, rectal temperature and the plasma concentrations of norepinephrine, corticosterone, and renin were also not changed by ghrelin. These findings demonstrate that ghrelin stimulates the gastric efferent vagus nerve in an organ-specific manner without affecting the gastric afferent vagus nerve and that ghrelin does not acutely affect the efferent basal activity of the sympathetic nerve in rats.

Keywords: ghrelin, vagus nerve, sympathetic nerve, gastric, vagotomy

Introduction

Ghrelin is a peptide hormone consisting of 28 amino acid residues with an octanoyl modification at Ser^3. It was discovered in the stomach as an endogenous ligand of an orphan G-protein–coupled receptor, the growth hormone secretagogue receptor (1). Stimulation of growth hormone secretion from the pituitary gland and food intake are well known and representative physiological actions of ghrelin (2).

Ghrelin, which is mainly produced in the stomach and inhibits the neural activity of the gastric branch of the afferent vagus nerve, is a unique humoral factor that transmits the fasting signal to the brain (2 – 4). We have previously reported that intravenous injection of ghrelin has stimulatory effects on gastric motility and gastric acid secretion in rats, which were abolished by pretreatment with either atropine or bilateral cervical vagotomy (5). These results indicate that ghrelin activates the vagal gastric efferent nerve, but no report has shown it directly. In contrast, it has also been reported that intravenous injection of ghrelin increases growth hormone secretion and food intake via the gastric branch of the afferent vagus nerve, and these actions were abolished by blockade of the vagal gastric afferent nerve (2, 3). In addition, the inhibition of the sympathetic nerve system by peripherally or centrally applied ghrelin has been reported (6 – 9). However, higher doses than that necessary to stimulate growth hormone secretion were used in studies performed using peripheral injection; thus, the effects of lower doses of ghrelin on sympathetic nerve activity remain unclear.

As mentioned above, the effects of peripheral injection of ghrelin on efferent vagus and sympathetic nerve

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Published online in J-STAGE on December 21, 2013
doi: 10.1254/jphs.13180FP