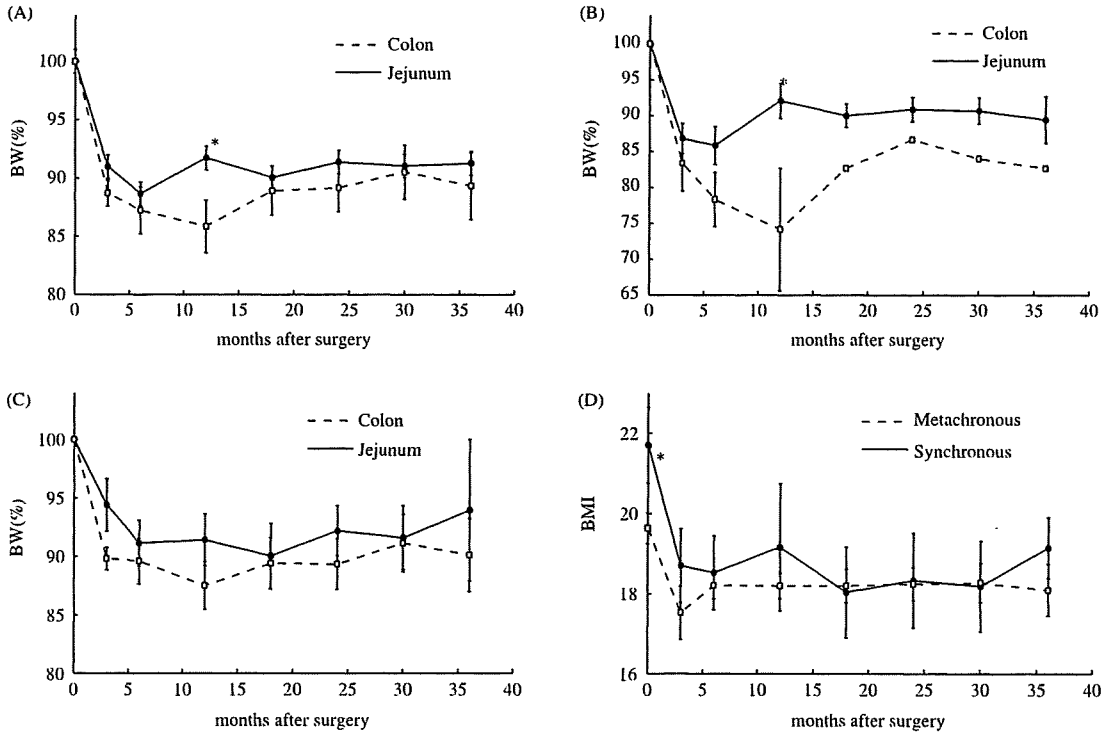

Fig. 2 Post-operative bodyweight alteration in colon and jejunum reconstruction groups of patients. (A) The post-operative bodyweight alteration after colon (dotted line) or jejunum (continuous line) reconstruction is indicated as a proportion of the preoperative value. The values were calculated according to the period of gastrectomy, i.e., either (B) synchronous or (C) metachronous. In (D), the absolute bodyweight (BMI) alteration is indicated for the synchronous (continuous line) and the metachronous (dotted line) gastrectomy. The difference in bodyweight between the two groups was evaluated at each time point using Student's *t*-test, indicating a *P* value smaller than 0.05 with an asterisk.

bodyweight loss was smaller and constant, i.e. 2% to 3% higher in the jejunum group, but not to a statistically significant extent. Since the period of gastrectomy, either metachronous or synchronous, affected bodyweight until 12 months after surgery (Fig. 2d), the bodyweight loss was compared for subgroups based on the period of gastrectomy. In the synchronous gastrectomy group there was a great difference of bodyweight loss at 12 months between the colon and the jejunum group (–7.9% *vs* –25.4%, $P = 0.0105$), while in the metachronous gastrectomy group, the difference between two groups was smaller and not statistically significant (Fig. 2c). With respect to preservation of the remnant stomach, there was no significant difference of bodyweight loss between 15 cases with preservation of the remnant stomach and seven without it in the colon group patients undergoing prior distal gastrectomy.

DISCUSSION

In this retrospective study, the jejunum reconstruction was superior to the colon reconstruction on several points; the anastomotic leakage tended to be less frequent, the hospital stay was shorter and the postoperative bodyweight loss was less in the jejunum reconstruction group. Since surgical procedure generally depends on the surgeon's preference and this type of operation is not frequently performed, this is the first study to compare esophageal reconstruction using the colon and the jejunum.

Various factors are usually implicated in anastomotic leakage, including blood supply, physical tension, surgical procedure (hand or staple sewing) and systemic nutrition.[10–12] In this study, the blood supply seemed to have been sufficient in both groups because of the supercharge anastomosis. There was no difference in surgical procedure and nutrition status. The tensile force to the anastomosis, which should be stronger for the jejunal reconstruction than for the colon type, cannot explain why the leakage was more frequent in the colon group. Another possible factor causing a difference between the two types of reconstruction is the presence of intestinal bacteria, which prosper more in the colon and terminal ileum than in the jejunum[13] and may affect the healing of the anastomotic leakage or expose minor sub-clinical leakage. Minor leakage is usually encapsulated and cured by the surrounding connective tissue. In the absence of such surrounding tissue, the healing of minor leakage may be prolonged with subcutaneous anastomosis. Taken

together, infection of the surrounding tissue by intestinal bacteria may cause or worsen the status of anastomotic leakage in the colon group. A high incidence of anastomotic leakage in the colon reconstruction by the subcutaneous route was also observed in the previous study.[14] In the present study, jejunum reconstruction partly reduced the incidence of leakage, but it was still more frequent than the gastric tube reconstruction by the posterior mediastinal route, accounting for less than 10% in our institution. In future work, the influence of route of reconstruction should be considered.

Bodyweight loss is one of the most serious sequela after esophagectomy; strongly affecting postoperative quality of life as well as immune function, infection and survival.[15-17] Since appetite, emotional and metabolic condition should not be different between the two groups, one possible reason for weight loss is the reduction of oral intake due to early satiety, which is very difficult to evaluate in an objective and reproducible fashion. Patients with a previous history of gastrectomy suffer less from bodyweight loss after esophagectomy than those without it. These patients had already experienced a 5 to 10% bodyweight loss after gastrectomy[18] their intestine had adapted well to the dumping syndrome and they had learned how to eat slowly. In this study, synchronous gastrectomy was more frequent in the jejunum reconstruction group and the difference from the colon group was prominent at 12 months with a synchronous gastrectomy but not with a metachronous gastrectomy. Attempts have been made to produce a pouching space to increase oral intake, but its contribution to bodyweight has not always been successful.[19,20] With respect to subcutaneous reconstruction for esophageal cancer patients, the volume of the lumen was greater in the colon than in the jejunum. In addition, the residual stomach was preserved for many of the colon group but had been removed for those of the jejunum group in which the Roux-en-Y reconstruction method had been used. Thus, reasons other than the reservoir space may be the underlying factors influencing oral intake after esophagectomy. In general, continuous anterograde peristalsis was stronger in the jejunum than in the colon[13] which is well preserved after Roux-en-Y reconstruction.[21,22] This characteristic of the jejunum enables the consumption of more at a slow and continuous pace during a meal, while stasis results in a lesser total oral intake although the reserve space in the colon group is greater than that in the jejunum group. Alternatively, the presence of Bauhin's valve in the terminal ileum may have caused the food stasis in the colon group. Another possibility might be diarrhea, which is often observed along with bodyweight loss after gastrectomy. Although we could not obtain the data of stool frequency in this retrospective study, the colon group might have had more frequent stools because the subcutaneous right colon is not likely to have contributed to water absorption. Further work is needed on this issue.

The length of lifted jejunum is the most practical problem for reconstruction. Since the marginal vessels do not develop well after the fifth jejunal artery and vein, we lifted the jejunum using the fourth jejunal artery and vein as a pedicle, in which the top of the lifted jejunum is located around the sternal notch. We and others have reported that esophageal cancers after distal gastrectomy are frequent in the lower esophagus.[2,23] Therefore, in most cases we can leave enough of the oral esophagus around or below the sternal notch. However, for upper thoracic esophageal cancers in which the oral margin often reaches the cervical esophagus, the colon should be employed for reconstruction. Intraepithelial spread, intramural metastasis and multiple cancers frequently disturb the oral margin of esophageal cancer patients during operation. Thus, the presence or absence of cancers at the oral edge should be checked before deciding on the reconstruction organ. Although no significant difference in operative time was noted, the operative procedure is slightly more complicated for the colon group than the jejunum group. Three anastomoses are necessary for colon reconstruction, while Roux-en-Y reconstruction using the jejunum usually requires two anastomoses or only one anastomosis, when prior Roux-en-Y reconstruction has been done.

An esophagectomy, especially one involving construction with an organ other than the stomach, is one of the most difficult types of gastrointestinal surgery with high operative morbidity and mortality. Many factors, including operative time, surgical stress, preoperative complications and cancer curability, are considered in deciding on the operative procedure. The final decision for each operation is left to the surgeon, according to his or her knowledge and experience. In our institution it took three years to change the standard procedure from colon reconstruction to jejunum reconstruction, which was retrospectively analyzed in this study. Although a prospective randomized trial is necessary to prove the true benefit and superiority of jejunum reconstruction, it would require much time and great effort to perform this kind of trial because of the limited number of patients and long follow-up period for quality of life evaluation. We hope our findings can help surgeons who attempt this complicated surgery to make better informed decisions.

References

1 The Japanese Society for Esophageal Diseases. Comprehensive registry of esophageal cancer in Japan, 1999. Esophagus 2001; 2: 43–69.

2 Wada H, Doki Y, Nishioka K *et al.* Clinical outcome of esophageal cancer patients with history of gastrectomy. J Surg Oncol 2005; 89: 67–74.
3 Cerfolio R J, Allen M S, Deschamps C, Trastek V F, Pairolero P C. Esophageal replacement by colon interposition. Ann Thorac Surg 1995; 59: 1382–4.
4 Davis PA, Law S, Wong J. Colonic interposition after esophagectomy for cancer. Arch Surg 2003; 138: 303–8.
5 Fujita H, Yamana H, Sueyoshi S *et al.* Impact on outcome of additional microvascular anastomosis – supercharge – on colon interposition for esophageal replacement: comparative and multivariate analysis. World J Surg 1997; 21: 998–1003.
6 Hirabayashi S, Miyata M, Shoji M, Shibusawa H. Reconstruction of the thoracic esophagus, with extended jejunum used as a substitute, with the aid of microvascular anastomosis. Surgery 1993; 113: 515–19.
7 Sobin LH, Wittekind C. TNM Classification of Malignant Tumours, 6th edn. New York: John Wiley, 2002.
8 Doki Y, Ishikawa O, Takachi K *et al.* Association of the primary tumor location with the site of tumor recurrence after curative resection of thoracic esophageal carcinoma. World J Surg 2005; 29: 700–7.
9 Miyata H, Yano M, Doki Y *et al.* A prospective trial for avoiding cervical lymph node dissection for thoracic esophageal cancers, based on intra-operative genetic diagnosis of micrometastasis in recurrent laryngeal nerve chain nodes. J Surg Oncol 2006; 93: 477–84.
10 Wormuth J K, Heitmiller R F. Esophageal conduit necrosis. Thorac Surg Clin 2006; 16: 11–22.
11 Peracchia A, Bardini R, Ruol A, Asolati M, Scibetta D. Esophagovisceral anastomotic leak. A prospective statistical study of predisposing factors. J Thorac Cardiovasc Surg 1988; 95: 685–91.
12 Swanson S J, Linden P. Esophagectomy for esophageal cancer. Minerva Chir 2002; 57: 795–810.
13 Haubrich W S, Schaffner F, Berk J E, (eds). Bockus Gastroenterology, 5th edn. Philadelphia, PA: W.B. Saunders Company, 1995.
14 Lee Y, Fujita H, Yamana H, Kakegawa T. Factors affecting leakage following esophageal anastomosis. Surg Today 1994; 24: 24–9.
15 Doki Y, Takachi K, Ishikawa O *et al.* Ghrelin reduction after esophageal substitution and its correlation to postoperative body weight loss in esophageal cancer patients. Surgery 2006; 139: 797–805.
16 Marinho L A, Rettori O, Vieira-Matos AN. Body weight loss as an indicator of breast cancer recurrence. Acta Oncol 2001; 40: 832–7.
17 Tsuburaya A, Noguchi Y, Yoshikawa T *et al.* Long-term effect of radical gastrectomy on nutrition and immunity. Surg Today 1993; 23: 320–4.
18 Takachi K, Doki Y, Ishikawa O *et al.* Postoperative ghrelin levels and delayed recovery from body weight loss after distal or total gastrectomy. J Surg Res 2006; 130: 1–7.
19 Nakane Y, Michiura T, Inoue K *et al.* A randomized clinical trial of pouch reconstruction after total gastrectomy for cancer: which is the better technique, Roux-en-Y or interposition? Hepatogastroenterology 2001; 48: 903–7.
20 Fuchs K H, Thiede A, Engemann R, Deltz E, Stremme O, Hamelmann H. Reconstruction of the food passage after total gastrectomy: randomized trial. World J Surg 1995; 19: 698–705.
21 Haglund U, Fork F T, Hogstrom H, Lilja B. Esophageal and jejunal motor function after total gastrectomy and Roux-Y esophagojejunostomy. Am J Surg 1989; 157: 308–11.
22 Coelho J C, Pupo C A, Matias J E, Marchesini J C. Electromyographic evaluation of the gastrointestinal tract in patients with chronic Roux-en-Y limb. Surg Gynecol Obstet 1990; 170: 399–402.
23 Alexandrou A, Davis P A, Law S, Whooley B P, Murthy S C, Wong J. Esophageal cancer in patients with a history of distal gastrectomy. Arch Surg 2002; 137: 1238–42.

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CLINICAL STUDY

Repeated administration of ghrelin to patients with functional dyspepsia: its effects on food intake and appetite

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Abstract

Background: Ghrelin plays a major role in the regulation of food intake (FI), which makes it a strong candidate for the treatment of anorexia.

Objective: We attempted to evaluate the clinical response to repeated ghrelin administration in patients with anorexia caused by functional disorders, such as functional dyspepsia (FD).

Subjects and methods: Subjects included in this study were those who 1) were diagnosed with functional anorexia, including FD and other eating disorders with the exception of anorexia nervosa; 2) were lean (body mass index (BMI) < 22 kg/m^2); and 3) exhibited decreased FI. Subjects received an i.v. infusion of ghrelin (3 μg/kg) for 30 min twice a day (before breakfast and dinner) for 2 weeks. We investigated the effects of ghrelin administration on FI, appetite, hormones, and metabolic parameters.

Results: Six patients with FD were enrolled in this study. Ghrelin administration tended to increase daily FI in comparison with levels before and after completion of treatment, but this difference that was the primary endpoint of this study did not reach statistical significance ($P=0.084$). Hunger sensation was significantly elevated at the end of drip infusion ($P<0.0001$). No severe adverse effects were observed.

Conclusions: These results suggest that ghrelin administration is safe and that this treatment has stimulatory effects on appetite in patients with FD. Further studies are necessary to confirm the efficacy of ghrelin treatment for anorexia-related disorders.

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Introduction

Ghrelin is a gut hormone, which is produced mainly in the stomach and a 28-amino acid peptide with an *n*-octanoylation modification at Ser 3 (1). This peptide increases feeding when administered either peripherally or centrally (2–4). Ghrelin is the only hormone that exhibits an orexigenic effect following peripheral administration. The unique action of ghrelin may therefore be invaluable in the development of novel treatments for anorexia-related disorders. In addition, ghrelin exhibits a variety of actions including stimulation of growth hormone (GH) secretion, gastric motility and gastric acid secretion, and induction of positive energy balance (5, 6). Preliminary clinical trials have begun to assess the utility of ghrelin for the treatment of cachectic conditions (7–9). Our previous examination of the clinical effects and safety of i.v. low- and high-dose (1 and 5 μg/kg respectively) ghrelin in young healthy volunteers demonstrated that ghrelin was safe and tended to increase appetite in a dose-dependent manner (10). These findings suggest that ghrelin may be useful for the treatment of disorders related to appetite.

Anorexia is associated with a variety of functional disorders, including functional dyspepsia (FD), eating disorders (EDs), and depression. FD is a disorder characterized by chronic or recurrent symptoms of upper abdominal pain or discomfort (11). Although no specific organic abnormalities are present, abnormalities in gastrointestinal motility and sensitivity are thought to play a role in the dysmotility type of FD, which comprises a substantial subset of patients. Anorexic ED is characterized by chronically decreased caloric intake, resulting in self-induced starvation. Patients with anorexia nervosa (AN), which is a typical anorexic ED, do not eat because of obsession with obesity and/or a distorted body image. Anorexic ED in the absence of obsession with obesity or distorted body image is categorized as 'other EDs' or 'EDs unspecified'

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according to the International Classification of Diseases (ICD)-10 (12). Some patients with FD also suffer from anorexia with body weight loss and are frequently diagnosed with ED. No treatment guidelines for patients with ED and FD have been established. In this study, we investigated whether repeated ghrelin administration could increase appetite and food intake (FI) in patients with functional anorexia.

Subjects and methods

Patients

To be included in this study, subjects had to 1) be diagnosed as having FD, as defined by the Rome II criteria (11), and/or categorized as 'other EDs (F50.8)' or 'EDs unspecified (F50.9)' according to the ICD-10 classification (12); 2) be lean (BMI <22 kg/m^2); and 3) exhibit decreased FI less than the nutritional requirements for Japanese People (13). Exclusion criteria were 1) the presence of organic gastrointestinal disorders, including peptic ulcer disease, erosive gastritis, gastroesophageal reflux disease, or gastric cancer; 2) anorexia due to organic disorders, such as chronic hepatitis, gall bladder stone, chronic pancreatitis, constipation, chronic glomerulonephritis, and chronic heart failure; 3) current usage of prescription proton pump inhibitors and/or antibiotics within 4 weeks of enrolment; 4) classical (F50.0 (12)) or atypical (F50.1 (12)) AN; 5) habitual vomiting; 6) an obsession with obesity or a distorted body image; 7) schizophrenia or severe depression with suicide ideations; 8) systematic autoimmune disease; 9) severe diabetes or cardiac disease; 10) pregnancy; 11) lactation; 12) past history of malignant tumors; and 13) orthostatic hypotension. Medication that had already been started 1 month before the initial enrolment could be continued during this study, unless the drug dose was changed. Within 4 weeks of the initial enrolment and during the entire period of this study, any additional drugs that might influence the study outcome were not allowed, so steroids, analgesics, and antibiotics were not allowed at any period. The initial planned sample size was eight or more. Patient registration lasted from July 2004 to September 2006. 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.

Study design

Enrolment was performed in two steps (Fig. 1). First, eligible volunteers were enrolled after a 2-week screening period (initial enrolment) and hospitalized for 1 week (from days -7 to -1). From days -4 to -2, daily FIs were measured; subjects whose FI (the mean of three daily FI values) met the inclusion criterion were enrolled on day -1 (final enrolment). These patients received an i.v. infusion of ghrelin (3 μg/kg) for 30 min twice a day (before breakfast and dinner) for 14 days (days 1–14). After treatment, subjects were hospitalized for an additional 1 week (days 15–21) to monitor the safety and clinical efficacy of ghrelin treatment. To establish strict quality control, patient enrolment, monitoring, data management, and statistics were conducted independently and separately.

Study drug

Human ghrelin was prepared as described previously (10). Acylated peptide 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.

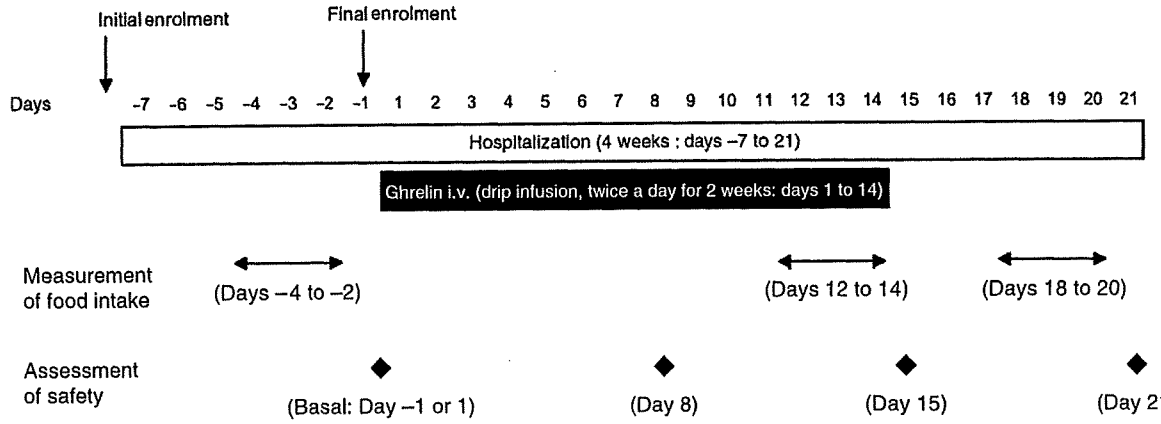

Figure 1 Schematic of the study schedule of enrolment, hospitalization, ghrelin injection, and safety assessment. Day 1 designates the day upon which ghrelin administration was begun.

Assessment of FI and hunger sensation

The primary endpoint of this trial was alteration of FI by ghrelin treatment. Patients were initially served an amount of food equivalent to their recommended dietary allowance (58% carbohydrate, 25% fat, and 17% protein). FI was calculated by dieticians as total caloric intake subtracting the residual FI as in calories. When a subject ate all of the food for two consecutive days, the amount of food was increased by 200 kcal/day. If a subject ate a snack or drink, the calories were added to the measurement of FI. The mean daily FI over 3 days was evaluated. Visual analog scales rating hunger (possible scores 0–10 cm) were completed pre-infusion and immediately after study drug administration (10, 14, 15). The positions on the scale were measured in centimeters.

Measurement of hormone, metabolic parameters, and body composition

Blood samples for the hormone and metabolic parameters were drawn after an overnight fast on days 1, 8, 15, and 21. Plasma levels of active and desacyl ghrelin at the end of injection were also measured to confirm the increases using ELISA kits (Mitsubishi Kagaku Iatron Inc., Tokyo, Japan). GH (normal values: male, <1.46; female, 0.28–8.70 ng/ml) and insulin-like growth factor-I (IGF-I) were measured by IRMA (Mitsubishi Kagaku Bio-Clinical Laboratories Inc., Tokyo, Japan). Body composition was determined using dual energy X-ray absorptiometry (DEXA, QDR-2000; Hologic Inc., Waltham, MA, USA).

Assessment of safety

Vital signs, including blood pressure, pulse rate, and body temperature, were measured every day. Assessments of safety by hematology, blood chemistry, and urine analysis were performed on days 1, 8, 15, and 21. Anxiety levels were evaluated using the Japanese versions of the Self-Rating Anxiety Scale (SAS) (16) and the State-Trait Anxiety Inventory (STAI) (17) tests on days -1, 8, 15, and 21. If the study was interrupted, evaluation was performed when the study was discontinued.

Statistical evaluations

A sample size of eight will have 95% power to detect a difference in means of -300 kcal, i.e., expected relative improvement of 25% (4) (e.g., a baseline mean of 1200 kcal and a post-treatment mean of 1500 kcal), assuming a standard deviation of differences of 200 kcal, using a paired t-test with a 0.05 two-sided significance level. Statistical analyses were performed using SAS version 8 software (SAS Institute Inc., Cary, NC, USA). A two-tailed P value was used, with the required level of significance being set at 0.05.

Results

Baseline characteristics of subjects

Six subjects, three men and three women, were enrolled in this study (Table 1). Although the initial planned sample size was eight or more, enrolment was finished at six because of the end of study term and the difficulties with enrolment. All six subjects were diagnosed with FD (Table 1). Patients 5 and 6 also exhibited mild depression and panic disorder respectively. BMI and FI (% of recommended daily allowance) at final registration were 17.6 ± 1.9 kg/m^2 and 63.2 ± 17.4% (mean $\pm$ S.D.) respectively. Plasma levels of active and desacyl ghrelin appeared to be within normal limits, considering BMI levels and ages (18). Four patients completed the 3-week ghrelin injection course. One patient (patient 5) was dismissed after the final injection and cancelled the examination 1-week post-injection. The last patient (patient 6) discontinued the ghrelin injections on day 6 because he could not continue to be hospitalized.

Clinical effects

Plasma levels of active and desacyl ghrelin at the end of injection at day 1 were 3211 ± 332 (pre-injection: 17.1 ± 10.9) and 993 ± 193.1 (pre-injection: 96.9 ± 29.2) fmol/ml respectively. Ghrelin injection tended to increase daily FI in comparison with levels seen before (days -4 to -2) and after treatment (days 12–14), although this difference was not statistically significant ($P=0.084$; Table 2; Fig. 2A). The statistical power of the study to detect the observed difference (the baseline mean of 1255.9 kcal, the post-treatment mean of 1619.8 kcal and the standard deviation of difference of 355.1 kcal) based on five subjects was 41%. The increase in FI was observed for all three meals (breakfast, lunch, and dinner), but was most pronounced at dinner ($P=0.0504$). The elevated FI appeared to be maintained even 1 week after treatment (days 18–20; Fig. 2A). In individuals, FI increased in four of five subjects, while it decreased in patient 5 (Fig. 2B). FI in patient 1 was significantly elevated at the end of ghrelin treatment (days 12–14) from levels observed before treatment ($P<0.005$). In patients 2 and 4, amounts of food were increased by 200 kcal/day at days 9 and 12 respectively, because they ate all of the food for the previous 2 days.

Ghrelin administration significantly increased the hunger sensation experienced at the end of drip infusion ($N=149$, $P<0.0001$; Fig. 3A). This effect was observed at similar levels during both morning ($N=75$, $P<0.0001$) and evening ($N=74$, $P=0.0002$).

Table 1 Baseline characteristics of registered subjects with functional dyspepsia.

Patient	Sex	Age (years)	Associated disorders	BMI (kg/m^2)	Food intake (% of recommended allowance)	Plasma active ghrelin concentration (fmol/ml)	Plasma desacyl ghrelin concentration (fmol/ml)	Ghrelin injection (days)	Period of admission (days)
1	M	30	–	17.1	63.9	10.4	74.2	14	28
2	F	44	–	17.4	70.1	5.3	84.2	14	28
3	F	34	Insomnia	15.9	28.8	36.9	149.5	14	28
4	M	37	–	16.0	75.3	14.0	73.0	14	28
5	F	29	Depression, insomnia, allergy	18.0	73.8	16.0	89.8	14	21[a]
6	M	38	Panic disorder	21.2	67.3	20.2	110.9	5	12[b]
Mean		35.3		17.6	63.2	17.1	96.9		
S.D.		5.6		1.9	17.4	10.9	29.2		

[a]Cancelled the examination after the final ghrelin injection.
[b]Discontinued the ghrelin injections on day 6 because he could not continue to be hospitalized.

Individually, three of the subjects exhibited significantly increased appetites after treatment, whereas the remaining patients did not (Fig. 3B). Interestingly, the former group demonstrated higher hunger scores before treatment than the latter group.

Patient body weight did not significantly change after treatment. At the end of treatment, basal serum GH and IGF levels tended to have decreased ($P=0.080$) and increased ($P=0.090$) respectively, but returned to baseline levels within 1 week (Table 2). Lean body mass tended to increase, while fat mass tended to decrease after treatment (Table 2). There were no significant changes in the blood levels of other hormones or other metabolic parameters, including serum insulin, glucose, adrenocorticotrophin (ACTH), cortisol, thyrotrophin (TSH), free thyroxine, free tri-iodothyronine, prolactin, luteinizing hormone, follicle-stimulating hormone, estrogen, testosterone, glucagon, leptin, albumin, and cholinesterase following ghrelin treatment.

Adverse effects

No serious adverse events were reported. We observed 36 mild and 4 moderate events. Of these, 11 events, all of which were mild, were considered to be associated with ghrelin treatment. These incidences were similar to those that had been previously reported, with eight abdominal events (peristalsis, hunger, discomfort, and pain) and one event each of flushing, somnolence, and hyperhidrosis. All of these complaints were transient and well tolerated. We could not detect any changes in clinical blood chemistries or complete blood counts after ghrelin treatment. Anxiety levels that were evaluated by the SAS and STAI tests did not significantly change with ghrelin treatment. We did not observe any significant effects of ghrelin treatment on sleep or basal levels (pre-infusion) of heart rate and blood pressure.

Discussion

In this study, 3 μg/kg ghrelin was intravenously injected twice a day for 14 days. Although several studies demonstrated that a single injection of 1 μg/kg ghrelin caused hunger sensation in some healthy subjects (19, 20), we observed that ghrelin tended to increase appetite dose dependently (1 vs 5 μg/kg) in a phase I study and was safe at the dose of 5 μg/kg (10). In addition, an i.v. injection of ghrelin twice a day (morning and evening) for 3 weeks to patients with congestive heart failure increased both FI and body weight (7). Furthermore, once a day injection of ghrelin tended to increase energy intake for the rest of the days and/or total 24 h (4, 8). Based on these findings, we adopted the present protocol.

Although ghrelin injection significantly increased appetite and tended to increase daily FI in comparison

Table 2 Summary of clinical effects of ghrelin treatment.

	Before treatment				At the end of treatment					One week after treatment			
	Day(s)	N^a	Mean	S.D.	Day(s)	N^a	Mean	S.D.	P value[b]	Day(s)	N^a	Mean	S.D.
Food intake (kcal/day)	−4 to −2	6	1326.9	493.8	12–14					18 to 20			
		5	1255.9	516.7		5	1619.8	703.1	0.084				
		4	1265.5	596.2		4	1754.3	733.8			4	1692.0	815.4
Weight (kg)	−1	6	48.3	9.0	15					21			
		5	45.5	6.7		5	45.7	6.7	0.339				
		4	45.0	7.6		4	45.3	7.7			4	45.1	7.2
Serum GH (ng/ml)	1	6	0.41	0.37	14					21			
		5	0.48	0.37		5	0.12	0.04	0.080			0.16	0.05
		4	0.35	0.27		4	0.10	0.02			4		
Serum IGF-I (ng/ml)	1	6	193.8	53.4	15					21			
		5	182.5	51.1		5	225.7	92.0	0.090				
		4	171.9	52.3		4	207.6	94.8			4	208.5	84.1
Lean body mass (%)	−5 to −1	6	78.8	6.6	14–16					n.d.			
		5	78.4	7.3		5	79.3	7.9	0.131				
		4	80.4	6.8		4	81.6	6.9					
Fat mass (%)	−5 to −1	6	16.9	6.8	14–16					n.d.			
		5	17.5	7.4		5	16.7	8.0	0.170				
		4	15.6	7.0		4	14.4	7.1					
Serum glucose (mg/dl)	1	6	80.7	10.4	15					21			
		5	84.2	6.5		5	82.8	4.6	0.669				
		4	85.8	6.4		4	82.8	5.3			4	78.0	7.7
Serum insulin (μU/ml)	1	6	4.7	2.9	15					21			
		5	5.2	2.9		5	4.1	0.9	0.502				
		4	5.1	3.3		4	4.2	1.0			4	4.7	2.9
Plasma active ghrelin concentration (fmol/ml)	1	6	17.1	10.9	15					21			
		5	16.5	12.1		5	16.5	8.8	0.985				
		4	16.7	14.0		4	15.8	10.0			4	11.8	5.2
Plasma desacyl ghrelin concentration (fmol/ml)	1	6	96.9	29.2	15					21			
		5	94.1	31.7		5	85.4	37.7	0.463				
		4	95.2	36.5		4	85.6	43.5			4	77.5	46.3

n.d., not done.
[a]N=6, all patients; N=5, excluding patient 6; N=4, excluding patients 5 and 6.
[b]Paired t-test versus levels before treatment.

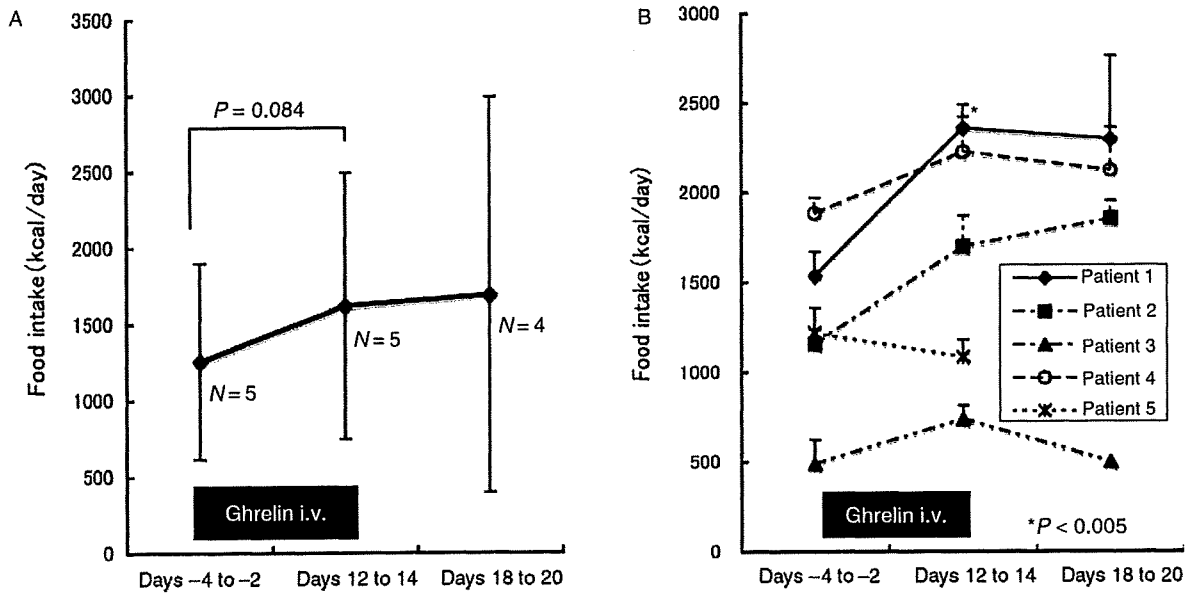

Figure 2 Daily FI was measured before ghrelin injection (days -4 to -2), at the completion of treatment (days 12–14), and 1 week after injection (days 18–20) in five subjects who completed 2 weeks of ghrelin treatment. (A) Mean and 95% confidence interval (CI) for five subjects. (B) Three-day means and standard deviation of daily FI for each subject.

with levels seen before and after treatment (Table 2; Fig. 2A), the difference in FI did not reach statistical significance ($P=0.084$). This may be due to the small number of subjects included in this study. The number of subjects enrolled was six and one of them did not complete ghrelin injections. Indeed, the statistical power of this study was calculated as only 41%. In addition, the study group is heterogeneous as two patients had comorbidity with psychiatric disorders, although all subjects were diagnosed with FD. These factors might have made this study underpowered. Obviously, further studies using more subjects are needed to confirm the effects of ghrelin on FI in these patients.

FI increased in four of five subjects after ghrelin treatment, while it decreased in patient 5 (Fig. 2B). The significant increase in appetite after treatment was observed in three patients but not others, although ghrelin injection significantly increased appetite in the whole (Fig. 3). These findings suggest that the effects of ghrelin on FI and appetite differ among individuals. It is

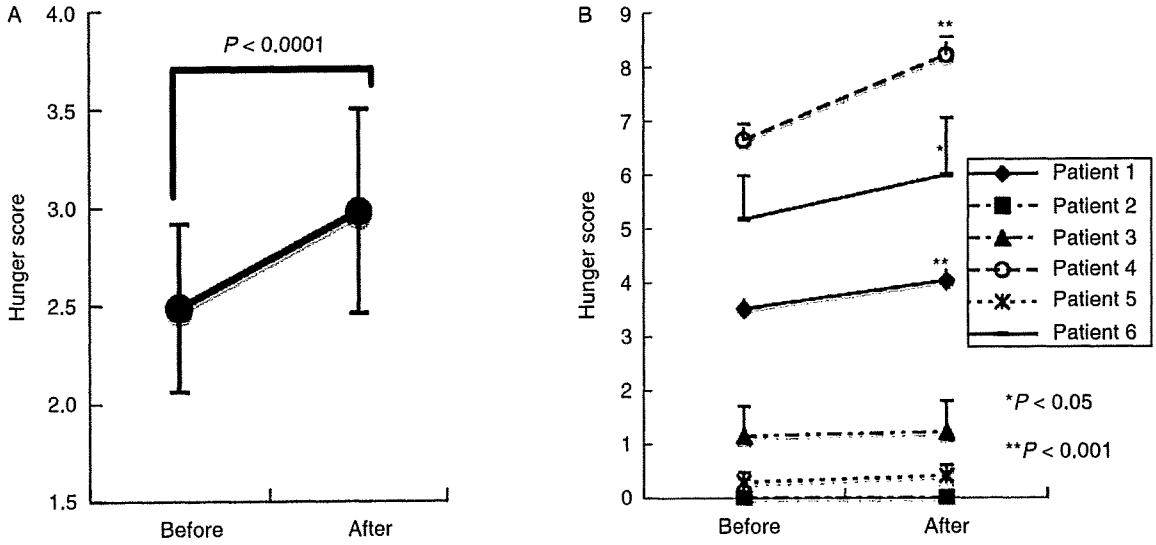

Figure 3 Effect of ghrelin injection on appetite as measured by the VAS hunger score before and after ghrelin injection. The mean and 95% CI for (A) all six subjects ($N=149$) and (B) for each subject ($N=28$ except for patient 6 ($N=9$)) are shown.

unclear why ghrelin did not increase FI in patient 5; this patient also exhibited depression, with high anxiety scores on both the STAI (STAI-state, 75–80 vs 27–47 points for other subjects; STAI-trait, 77–80 vs 29–54 points for other subjects) and SAS (49–55 vs 26–55 points for other subjects) tests throughout the trial. In this patient, ghrelin may have been unable to overcome the anorexia associated with severe anxiety/depression or complex etiology. Further study is needed to better understand these varying responses observed. Interestingly, alterations of appetite did not always parallel changes in FI. The FI of patients 2 and 3 tended to be increased, while their hunger scores did not change (Figs 2B and 3B), suggesting that FI is influenced by mechanisms independent from appetite.

FI tended to remain elevated after completion of ghrelin treatment (days 18–20) in some patients, e.g., patients 1 and 4. Because the reduction of their body weights was not observed at day 15, it is unlikely that acute weight loss caused the persistent elevation of FI in these subjects after cessation of ghrelin treatment. Although the precise mechanism is not known, several possibilities exist. First, acute effects of ghrelin on gastric function may lead to sequential favorable events in gastric mucosa and/or function. Secondly, the improvement in FI may result from an improvement in anxiety concerning FI or a gain of confidence in these patients. Thus, the effect of ghrelin may persist even after the cessation of treatment.

FD is related to disordered gastric emptying, dysregulation of gastroduodenal motility, and visceral hypersensitivity. As ghrelin affects gastric motility and secretion (21, 22), this peptide may affect FI by influencing gastric emptying and motility in patients with FD. A subset of subjects exhibited increased FI after treatment, despite minimal changes in appetite. Conversely, the functional abnormalities in FD may affect the production of ghrelin in the stomach. Plasma ghrelin levels have been reported to be altered (23, 24) and/or correlate with FD symptom score (24, 25). Further studies are necessary to determine the pathophysiological role of ghrelin in FD.

Serum IGF levels tended to increase by the end of treatment, although these changes were not statistically significant. Ghrelin treatment likely stimulates GH secretion (1, 26). The subsequent decrease in basal GH levels may reflect negative feedback from the elevated IGF-I. IGF-I and GH exhibit muscle-enhancing and lipolytic actions respectively, which are compatible with the trend of increased lean body mass and decreased fat mass seen in our patients after treatment. We did not observe any additional changes in serum hormone levels or other metabolic parameters indicative of glucose metabolism, the ACTH-adrenal axis, the TSH-thyroid axis, prolactin, or the gonadotropin-gonadal axis, suggesting that ghrelin minimally affects these systems under these conditions.

No severe adverse effects were observed. All complaints, including mild abdominal discomfort, flushing, perspiration, and somnolence, were transient and well tolerated. Ghrelin did not significantly alter clinical blood chemistries, complete blood counts, and anxiety scores. Thus, ghrelin treatment appears to be safe.

In summary, this is the first report examining the efficacy of ghrelin in the treatment of conditions with functional anorexia, including FD. Although the increase in FI did not reach statistical significance, alterations in appetite and FI seen after treatment appeared to support the therapeutic potential of ghrelin in patients with such disorders. We also confirmed ghrelin's safety. Further studies including placebo-controlled larger ones are awaited to confirm the usefulness of ghrelin in the treatment of FD.

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References

1 Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H & Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999 **402** 656–660.
2 Tschop M, Smiley DL & Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000 **407** 908–913.
3 Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG, Ghatei MA & Bloom SR. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 2000 **141** 4325–4328.
4 Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA & Bloom SR. Ghrelin enhances appetite and increases food intake in humans. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 5992.
5 van der Lely AJ, Tschop M, Heiman ML & Ghigo E. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocrine Reviews* 2004 **25** 426–457.
6 Korbonits M, Goldstone AP, Gueorguiev M & Grossman AB. Ghrelin – a hormone with multiple functions. *Frontiers in Neuroendocrinology* 2004 **25** 27–68.
7 Nagaya N, Moriya J, Yasumura Y, Uematsu M, Ono F, Shimizu W, Ueno K, Kitakaze M, Miyatake K & Kangawa K. Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure. *Circulation* 2004 **110** 3674–3679.

8 Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, Frost GS, Ghatei MA, Coombes RC & Bloom SR. Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 2832–2836.
9 Nagaya N, Itoh T, Murakami S, Oya H, Uematsu M, Miyatake K & Kangawa K. Treatment of cachexia with ghrelin in patients with COPD. *Chest* 2005 **128** 1187–1193.
10 Akamizu T, Takaya K, Irako T, Hosoda H, Teramukai S, Matsuyama A, Tada H, Miura K, Shimizu A, Fukushima M, Yokode M, Tanaka K & Kangawa K. Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. *European Journal of Endocrinology* 2004 **150** 447–455.
11 Talley NJ, Stanghellini V, Heading RC, Koch KL, Malagelada JR & Tytgat GN. Functional gastroduodenal disorders. *Gut* 1999 **45** (Suppl 2) II37–II42.
12 World Health Organization. Chapter V. Mental and behavioural disorders. In *International Statistical Classification of Diseases and Related Health Problems 10th Revision*. Geneva: WHO, 1992.
13 Health promotion and Nutrition division, Health service bureau & Ministry of Health and Welfare of Japan. *Recommended Dietary Allowances for the Japanese-5th Revision*. Tokyo: Dai-ichi Shuppan, 1996.
14 Thompson DA & Campbell RG. Hunger in humans induced by 2-deoxy-D-glucose: glucoprivic control of taste preference and food intake. *Science* 1977 **198** 1065–1068.
15 Nakai Y, Kinoshita F, Koh T, Tsujii S & Tsukada T. Perception of hunger and satiety induced by 2-deoxy-D-glucose in anorexia nervosa and bulimia neervosa. *International Journal of Eating Disorders* 1987 **6** 49–57.
16 Zung WW. A rating instrument for anxiety disorders. *Psychosomatics* 1971 **12** 371–379.
17 Iwata N, Mishima N, Shimizu T, Mizoue T, Fukuhara M, Hidano T & Spielberger CD. The Japanese adaptation of the STAI Form Y in Japanese working adults – the presence or absence of anxiety. *Industrial Health* 1998 **36** 8–13.
18 Akamizu T, Shinomiya T, Irako T, Fukunaga M, Nakai Y & Kangawa K. Separate measurement of plasma levels of acylated and desacyl ghrelin in healthy subjects using a new direct ELISA assay. *Journal of Clinical Endocrinology and Metabolism* 2004 **90** 6–9.
19 Arvat E, Di Vito L, Broglio F, Papotti M, Muccioli G, Dieguez C, Casanueva FF, Deghenghi R, Camanni F & Ghigo E. Preliminary evidence that Ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. *Journal of Endocrinological Investigation* 2000 **23** 493–495.
20 Broglio F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, van der Lely AJ, Deghenghi R & Ghigo E. Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 5083–5086.
21 Masuda Y, Tanaka T, Inomata N, Ohnuma N, Tanaka S, Itoh Z, Hosoda H, Kojima M & Kangawa K. Ghrelin stimulates gastric acid secretion and motility in rats. *Biochemical and Biophysical Research Communications* 2000 **276** 905–908.
22 Mori M, Suzuki H, Masaoka T, Imaeda H, Nomoto Y, Hosoda H, Nishizawa T, Kangawa K & Hibi T. Intravenous ghrelin administration enhances gastric acid secretion – evaluation using wireless pH capsule. *Alimentary Pharmacology & Therapeutics* 2006 **24** (Suppl 4) 96–103.
23 Takamori K, Mizuta Y, Takeshima F, Akazawa Y, Isomoto H, Ohnita K, Ohba K, Omagari K, Shikuwa S & Kohno S. Relation among plasma ghrelin level, gastric emptying, and psychologic condition in patients with functional dyspepsia. *Journal of Clinical Gastroenterology* 2007 **41** 477–483.
24 Nishizawa T, Suzuki H, Nomoto Y, Masaoka T, Hosoda H, Mori M, Ohara T, Morishita T, Kangawa K & Hibi T. Enhanced plasma ghrelin levels in patients with functional dyspepsia. *Alimentary Pharmacology & Therapeutics* 2006 **24** (Suppl 4) 104–110.
25 Shinomiya T, Fukunaga M, Akamizu T, Irako T, Yokode M, Kangawa K, Nakai Y & Nakai Y. Plasma acylated ghrelin levels correlate with subjective symptoms of functional dyspepsia in female patients. *Scandinavian Journal of Gastroenterology* 2005 **40** 648–653.
26 Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, Mori K, Komatsu Y, Usui T, Shimatsu A, Ogawa Y, Hosoda K, Akamizu T, Kojima M, Kangawa K & Nakao K. Ghrelin strongly stimulates growth hormone release in humans. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 4908–4911.

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Efficacy of Ghrelin as a Therapeutic Approach for Age-Related Physiological Changes

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Aging is associated with decreases in food intake and GH secretion, termed the anorexia of aging and somatopause, respectively. The mechanisms underlying these phenomena are not fully understood. Although many approaches have attempted to improve these age-related physiological changes, none have achieved satisfactory results. Ghrelin, a 28-amino-acid acylated peptide, was identified as an endogenous ligand for the GH secretagogue receptor. Ghrelin stimulates GH secretion and food intake in animals and humans. Previous studies have demonstrated that the mean plasma concentrations of ghrelin in normal-weight elderly people were lower than those in younger people. We hypothesized that ghrelin administration might improve the metabolic and physiological changes that accompany the anorexia of aging and somatopause. First, 75-wk-old mice fasted for 72 h, after which they resumed feeding with sc administration of ghrelin (360 μg/kg) twice daily for 4 d. Multiple administrations of ghrelin after a 72-h fast increased food intake and hastened body weight recovery with a high lean body mass ratio. Next, 50-wk-old mice were sc injected with rat ghrelin (40 μg/kg) twice weekly from 50–80 wk of age. Long-term administration of ghrelin kept aged mice with low body weight and low adiposity. These results suggest that ghrelin might be a novel approach for the therapy of age-related metabolic and physiological changes. (*Endocrinology* 149: 3722–3728, 2008)

AGING IS ASSOCIATED with progressive decreases in food intake (FI), termed the anorexia of aging (1–3). The physiological causes of the anorexia of aging are largely unknown and likely multifactorial (1, 2). One of the key factors of the anorexia of aging appears to be loss of appetite (4, 5). In comparison with healthy young people, elderly people feel less hunger when fasting and earlier satiety after initiating a meal (5–7). This insensitivity to the signs of appetite can lead to unintentional weight loss and undernutrition in response to acute and chronic illness, resulting in the increasing morbidity and mortality seen in elderly people (1, 8). Our understanding of the control of feeding has increased markedly in recent years. Animal and human studies have examined the roles of orexinergic and anorexinergic hormones, such as neuropeptide Y, orexin, ghrelin, CRH, and cholesystokinin (9–14). Of these, ghrelin has received great interest as a potential therapeutic agent for the anorexia of aging.

Ghrelin, an acylated peptide of 28 amino acids, was identified as an endogenous ligand for the GH secretagogue receptor (15). The major site of endogenous production of ghrelin is the stomach; this peptide is also expressed in the hypothalamus (16–19). Administration of ghrelin stimulates GH secretion and FI in both animals and humans (15, 16, 20–23). Plasma ghrelin levels are regulated by acute feeding states. They rise during fasting and are rapidly suppressed after feeding (12, 17, 24, 25). Ghrelin secretion is also regulated by chronic feeding states. Plasma ghrelin levels are elevated in food-restricted animals and patients with anorexia nervosa and are reduced in obese subjects (12, 17, 24–27). These data suggest a role for ghrelin in energy homeostasis. Previous studies have demonstrated that plasma concentrations of ghrelin in normal-weight elderly individuals were lower than those in younger people (28–30). GH responses to ghrelin administration in elderly people are also lower than those seen in young people (31). It has been speculated that aging is associated with reduced production of ghrelin or attenuation of endogenous ghrelin signaling (32).

Aging is associated with decreases in lean body mass (LBM) and increases in relative fat mass (33, 34). These changes can result in alternations of blood lipid profiles, which favor the development of vascular disease. Decreases in GH secretion are also seen in elderly people (35, 36), termed somatopause, which may contribute to these metabolic and physiological changes. Although the mechanisms inducing somatopause and leading to changes in body composition are not fully understood, clinical studies have attempted GH replacement in elderly persons. Such treatment has only occasionally been effective in increasing muscle mass and strength in elderly subjects (37, 38), and adverse effects, such as glucose intolerance and fluid retention, occurred frequently. Chronic administration of the ghrelin mimetic MK-677 to elderly people, however, restored pulse amplitude of episodic GH secretion and serum IGF-I levels to those seen in young adults (39) and increased bone mineral density (BMD) at the femoral neck with few adverse effects (40). These findings implicated a hypothesis that administration of ghrelin might safely restore intrinsic GH secretion

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Abbreviations: BMD, Bone mineral density; BW, body weight; CT, computed tomography; FI, food intake; LBM, lean body mass.

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and improve age-related metabolic and physiological changes.

Using an animal model of aging, we performed three examinations to evaluate the effectiveness of ghrelin on age-related metabolic and physiological changes. Using an experimental model of physical stress with a 72-h fast established by Wolden-Hanson and colleagues (41–43), we evaluated the influences of aging on FI. Second, we assessed the efficacy of ghrelin on the anorexia of aging; we investigated whether multiple ghrelin administrations can increase FI and hasten the recovery of body weight (BW) and body composition after the physical stressor of a 72-h fast. Third, we assessed the efficacy of long-term ghrelin administration to reverse the age-related metabolic and physiological changes; we investigated whether twice-weekly ghrelin from 50–80 wk of age improved the blood metabolic parameters and body composition.

Materials and Methods

Animals

All procedures using experimental animals were approved by the Kyoto University Graduate School of Medicine committee on animal research. Procedures were performed in accordance with the principles and guidelines established by that committee.

Male C57BL/6 mice were purchased from Japan CLEA (Tokyo, Japan). Animals were housed in air-conditioned animal quarters with lights on from 0800–2000 h. Except where noted, mice were allowed *ad libitum* access to water and standard rat chow (CE-2, 352 kcal/100 g; Japan CLEA).

Experiment 1: influence of aging on recovery of BW after a short-term fast (72-h fast)

Four age groups (n = 10 per age group) of mice (10, 25, 50, and 75 wk old) were analyzed. To evaluate the baseline characteristics of these mice, we measured BW and monitored daily FI for seven consecutive days. After a 7-d baseline period, food was withheld for 72 h. After *ad libitum* feeding was resumed, BW and FI were assessed daily for 5 d. Mice were allowed *ad libitum* access to water throughout the experiment.

Experiment 2: effect of ghrelin on recovery of BW after a short-term fast in aged mice (75 wk old)

Rat ghrelin was purchased from the Peptide Institute, Inc. (Osaka, Japan). After a 7-d baseline period, food was withheld from 75-wk-old mice (n = 30) for 72 h. *Ad libitum* FI was then resumed for 8 d (d 0–7). For the first half of the refeeding period (d 0–3), half of the animals (n = 15) were sc injected twice daily with rat ghrelin (360 μg/kg) at 0900 and 1800 h (ghrelin group), whereas the other half were injected with saline (saline group). BW and FI were measured daily in both groups. During the latter half of the refeeding period (d 4–7), BW and FI were measured in the absence of ghrelin or saline injections. Using computed tomography (CT) (laboratory CT; Lacita, Aloka, Japan), we examined the body compositions of 75-wk-old mice before the 72-h fast (d −3), after the 72-h fast (d 0), and after ghrelin or saline injection (d 4).

Experiment 3: effects of a long-term ghrelin injection on BW and body composition in aged mice (50–80 wk old)

The aim of this experiment was to investigate whether a long-term administration with low-dose ghrelin, which stimulates GH secretion without increase in cumulative FI, may lead to increase in LBM and decrease in fat mass via lipolytic and anabolic effects of elevated GH. We selected a dose and frequency on the basis of three points: 1) a dose that can stimulate GH secretion. 2) a dose that doesn't affect daily FI, and 3) a frequency that can avoid desensitization of GH in response to ghrelin

Because we have previously reported that sc administration of ghrelin at 40 μg/kg stimulated GH secretion and increased FI in 8-wk-old mice (21), we evaluated whether this dose of ghrelin could stimulate GH secretion and FI in 50-wk-old mice. Fifty-week-old mice were sc injected with rat ghrelin (40 μg/kg) or saline at 1000 h under *ad libitum* feeding conditions (n = 8 per group). Blood was collected from the tail veins of mice 15 min after injection. Fifty-week-old mice were sc injected with rat ghrelin (40, 120, and 360 μg/kg) or saline at 1000 h under *ad libitum* feeding conditions; daily FI was then measured. Previous reports have demonstrated that continuous administration of ghrelin with osmotic mini pump desensitizes GH secretion in response to ghrelin (16). To evaluate whether repeated administration of ghrelin desensitizes GH secretion in response to ghrelin, 20 mice were divided into two groups; half of them were sc injected with ghrelin at a dose of 40 μg/kg daily for 15 consecutive days, whereas the other half were injected with ghrelin at the same dose on d 1, 5, 8, 12, and 15 (twice weekly). On d 1 and 15, serum GH levels 15 min after ghrelin injection were measured. Fifty-week-old mice were also sc injected with rat ghrelin (40 μg/kg) at 0900 h twice weekly. Weekly FI was then measured.

Mice were scheduled sc administration of ghrelin twice weekly (Monday and Thursday). Fifty-week-old mice were also examined (n = 40). Half of the animals (n = 20) were sc injected with rat ghrelin (40 μg/kg) at 0900 h twice weekly (Monday and Thursday) from 50–80 wk of age, whereas the other half were injected with saline. BWs of both groups were measured weekly. CT was used to measure body composition before and after the experiment (50 and 80 wk old). After experimentation, blood samples were collected from the tail veins of mice at 1000 h under *ad libitum* feeding conditions for the measurement of blood glucose, serum insulin, triglycerides, total cholesterol, GH, and IGF-I levels. Blood glucose and serum insulin levels were also measured after an overnight fast (1700–0900 h).

Measurement of metabolic parameters and GH/IGF-I axis

Blood glucose was measured using a reflectance glucometer (One Touch II; Lifescan, Milpitas, CA). Serum total protein (Pierce, Rockford, IL), albumin (Albumin-E test; Wako, Osaka, Japan), triglycerides (Triglyceride-E test; Wako), total cholesterol (Amplex Red Cholesterol Assay Kit; Molecular Probes, Eugene, OR), serum GH levels (EIA kit; SPI-BIO, Bonde, France), and serum IGF-I levels (EIA kits; Diagnostic Systems Laboratories Inc., Webster, TX) were measured according to the manufacturer's instructions. Serum was isolated by centrifugation and stored at −20 C until assayed.

Statistical analysis

Results are expressed as the means ± SEM. The statistical significance of the differences in mean values was assessed by two-way ANOVA or Student's *t* test as appropriate. *P* values of <0.05 were considered to be statistically significant.

Results

Experiment 1: recovery of BW after a short-term fast was delayed in aged mice

We assessed the changes in BW and FI of aged mice after a 72-h fast and after refeeding (Fig. 1). The initial BWs of 10-, 25-, 50-, and 75-wk-old mice were 24.7 ± 0.3, 31.1 ± 1.0, 33.2 ± 1.3, and 34.9 ± 1.4 g, respectively. The average daily FI adjusted for BW declined markedly with age ($P < 0.01$). Those of 10-, 25-, 50-, and 75-wk-old mice were 136 ± 2.7, 106.1 ± 5.2, 104.5 ± 2.8, and 93.7 ± 6.3 mg/day·g BW, respectively. With fasting, younger mice lost more weight than older mice, both in absolute values and as percentages of BW before fasting. Ten-week-old mice lost 5.9 ± 0.2 g (23.9 ± 0.7% of prefasting BW), and 25-wk-old mice lost 5.9 ± 0.2 g (18.9 ± 0.6% of prefasting BW), whereas 50- and 75-wk-old mice lost 4.8 ± 0.3 g (14.5 ± 0.9% of prefasting BW) and 4.6 ± 0.2 g (13.1 ± 0.6% of prefasting BW), respectively. Despite a disproportionately increased weight loss, the re-

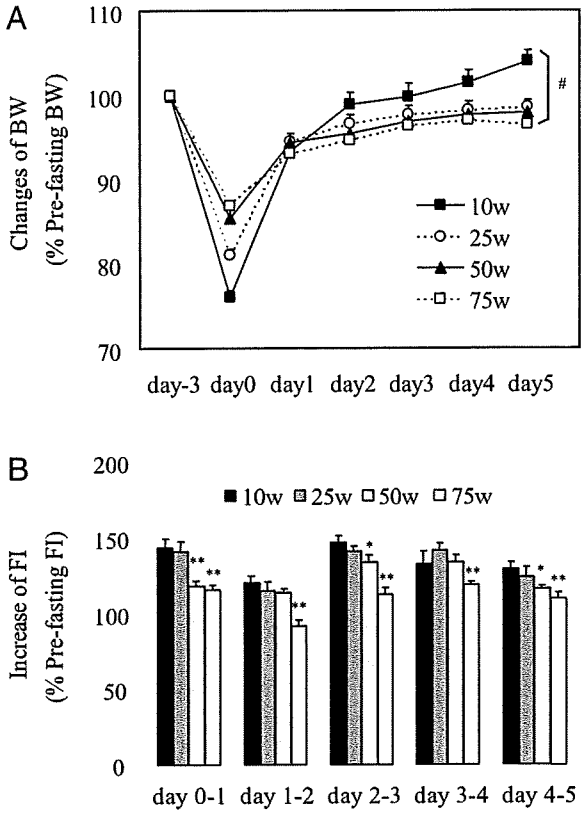

FIG. 1. A, Influence of aging on the recovery of BW after a 72-h fast. The 10-, 25-, 50-, and 75-wk-old mice fasted for 72 h, after which they resumed feeding. Data shown are the means ± SEM. Changes of BW are expressed as a percentage of prefasting weight. #, $P < 0.001$ (10- *vs.* 25-, 50-, and 75-wk-old mice). B, Daily FI for the first 5 d of the refeeding period. Data are expressed as a percentage of the prefasting FI. *, $P < 0.05$, **, $P < 0.01$ (*vs.* 10-wk-old mice on the same day).

covery of BW in 10-wk-old mice during refeeding was more rapid (on d 3) than that seen in all other age groups; the recovery of BW in 25-, 50-, and 75-wk-old mice was delayed. These animals did not recover to their average prefasting BW during the first 5 d of refeeding, reaching 98.6 ± 0.9, 98.0 ± 0.5, and 96.5 ± 0.9% of prefasting BW for 25-, 50-, and 75-wk-old mice, respectively. The difference in BW on d 5 between 10-wk-old mice and 25-, 50-, and 75-wk-old mice was significant ($P < 0.001$ for each group). The FI of 10-, 25-, 50-, and 75-wk-old mice in the first day of refeeding increased by 144 ± 5.7, 141.1 ± 6.6, 118.3 ± 3.2, and 116.2 ± 5.7% of prefasting FI, respectively. After adjustment for BW, the overall FI in 75-wk-old mice was significantly lower than that seen for 10-wk-old mice during the refeeding period ($P < 0.01$).

Experiment 2: multiple ghrelin injections restored BW loss after a short-term fast in aged mice

We monitored the changes in BW during experiment 2 (Fig. 2A). During the first half of the refeeding period (ghrelin or saline treatment period), BW recovery was more rapid in the ghrelin-treated subgroup in comparison with that seen in the saline-treated subgroup ($P < 0.01$). BW recovered fully by d 3 in the ghrelin-treated subgroup (100.6 ± 0.6% of

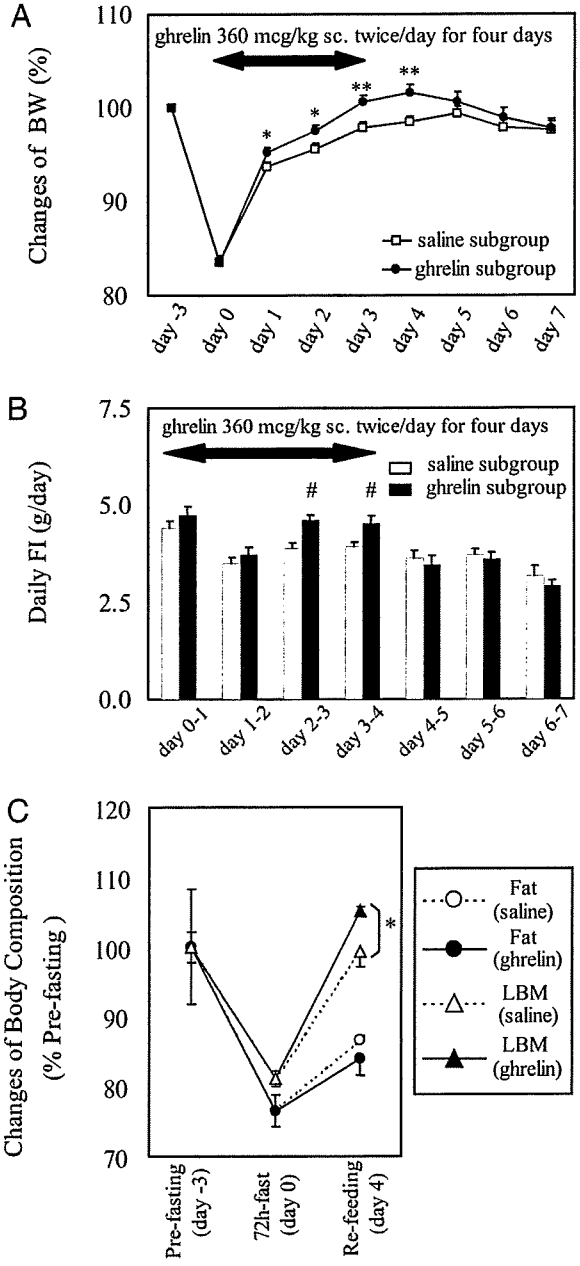

FIG. 2. Effect of repeated ghrelin administration on the recovery of BW and FI after a 72-h fast in aged mice. Seventy-five-week-old mice fasted for 72 h, after which they resumed feeding while being administered saline or ghrelin (360 μg/kg twice daily for 4 d). Data shown are the means ± SEM. Changes in BW (expressed as a percentage of the baseline BW) (A), daily FI (grams per day) (B), and CT-assessed body composition (expressed as a percentage of baseline fat mass or LBM) (C) were assessed in ghrelin-treated animals. *, $P < 0.05$; **, $P < 0.005$; #, $P < 0.001$ (*vs.* saline subgroup on the same day).

prefasting BW), whereas that of the saline-treated subgroup was 97.9 ± 0.6% of prefasting BW on d 3. After discontinuing the ghrelin injections, however, BW in the ghrelin subgroup decreased to levels equal to those of the saline subgroup. On d 7, the average BW of the ghrelin- and saline-treated subgroups were 97.8 ± 1.1 and 97.6 ± 1.0% of prefasting levels, respectively.

We evaluated daily FI during the refeeding period (Fig.

2B). During the first half of the refeeding period (ghrelin or saline injection period, d 0–4), daily FI increased in the ghrelin-treated subgroup in comparison with that seen in the saline-treated subgroup. The cumulative FI in the ghrelin-treated subgroup was 17.6 ± 0.6 g, whereas it was 15.7 ± 0.4 g in the saline-treated subgroup ($P < 0.005$). Daily FI during the latter half of the refeeding period (no-injection period, d 4–7), however, was similar between both groups. During this period, cumulative FI values in ghrelin and saline subgroups were 11.0 ± 0.5 g and 11.2 ± 0.6 g, respectively, which were not significantly different.

CT was used to measure body composition (Fig. 2C). As expected, adiposity and LBM decreased significantly after a 72-h fast (76.5 ± 2.2 and 81.1 ± 1.1% of prefasting levels, $P < 0.001$, respectively). After 4 d of refeeding and treatment, adiposity in the ghrelin-treated subgroup recovered to 83.9 ± 2.3% of prefasting levels and 86.6 ± 0.6% in the saline-treated subgroup; there were no significant differences between these values. LBM in the ghrelin-treated subgroup increased, surpassing the prefasting levels (105.1 ± 0.7%). This was significantly different ($P < 0.05$) from the saline-treated subgroup that returned only to prefasting levels (99.3 ± 2.2%).

Experiment 3: a long-term ghrelin injection decreased fat mass in aged mice

GH secretions of 50-wk-old mice were stimulated by sc ghrelin administration with a dose of 40 μg/kg. Fifteen minutes after ghrelin administration, serum GH levels were significantly higher than those seen after saline injection, 28.1 ± 7.6 and 8.4 ± 0.6 ng/ml, respectively ($P < 0.05$). Administrations of ghrelin with a dose of 40 μg/kg did not affect daily FI, 3.62 ± 0.18 g and 3.55 ± 0.25 g, respectively. Although those of 120 μg/kg or 360 μg/kg increased daily FI: 116.2 and 125.8% compared with control mice, respectively.

Repeated administration of ghrelin markedly attenuated the GH response to injected ghrelin; serum GH levels on d 1 and 15 were 20.4 ± 4.6 ng/ml and 5.8 ± 0.4 ng/ml, respectively (decrease to 28.6%) ($P < 0.05$). Although administration of ghrelin twice weekly did not attenuate the GH response to ghrelin; serum GH levels on d 1 and 15 were 21.6 ± 4.3 ng/ml and 22.3 ± 5.3 ng/ml, respectively. When mice were administrated ghrelin with a dose of 40 μg/kg twice weekly, weekly cumulative FI was equivalent for the saline-treated subgroups, 243.3 ± 2.5 g and 24.5 ± 2.3 g, respectively.

We followed the changes in BW from 50 to 80 wk old (Fig. 3A). The initial BWs of the saline- and ghrelin-treated subgroups were 33.2 ± 0.4 g and 32.8 ± 0.5 g, respectively. There were no significant differences between these values. Body weight increased gradually in both groups. Ghrelin-treated mice exhibited significantly lighter BW than the saline-injected mice throughout the course of the experiment ($P < 0.01$). By the end of the experiment, the average BW of the ghrelin-treated subgroup was 34.9 ± 0.9 g, whereas that of the saline-treated subgroup was 37.7 ± 1.0 g.

Body composition was assessed by CT before (50 wk old) and after (80 wk old) experimentation (Fig. 3B). As expected, we observed a significant effect of age on body composition. In the saline-treated subgroup, adiposity increased, whereas

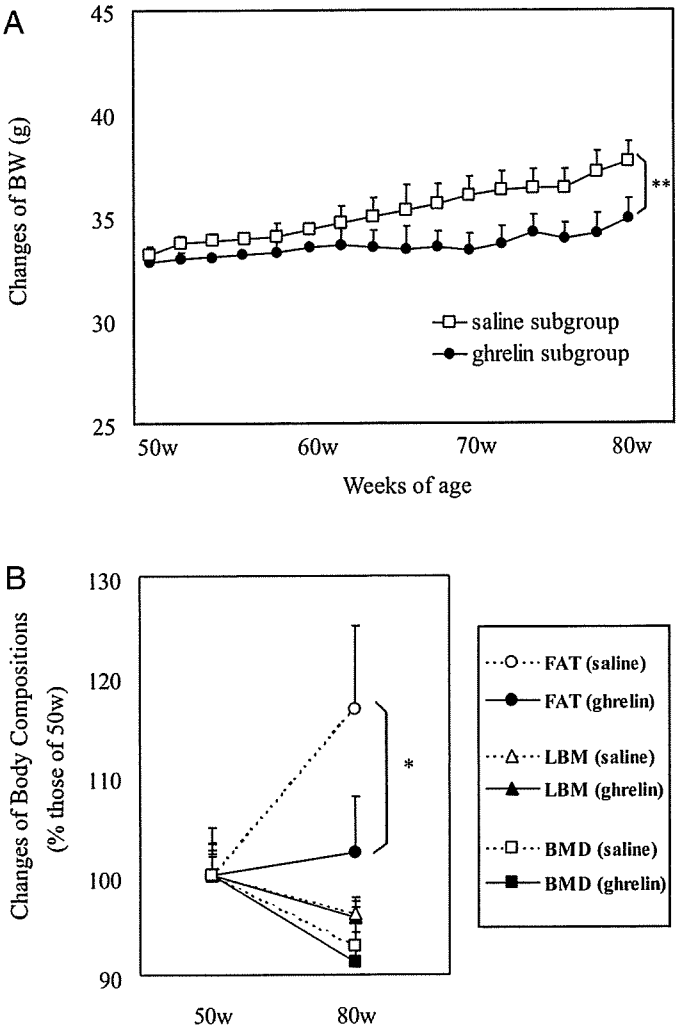

FIG. 3. Effect of long-term ghrelin administration on BW and body composition in aged mice. Fifty-week-old mice were injected with ghrelin (40 μg/kg twice weekly) for 30 wk (50–80 wk old). Data shown are the means ± SEM. Changes in BW (A) and body composition (B), as measured by CT (expressed as a percentage of the fat mass, LBM, or BMD of 50-wk-old animals), were measured in 80-wk-old mice. *, $P < 0.05$; **, $P < 0.005$ (*vs.* saline subgroup).

LBM and BMD decreased significantly with increasing age to 80 wk old (116.6 ± 8.4, 96.1 ± 1.6, and 92.9 ± 3.9% that seen for 50-wk-old animals, respectively) ($P < 0.01$). At the end of the experiment when animals were 80 wk old, LBM and BMD were similar for both the saline- and ghrelin-treated subgroups (saline *vs.* ghrelin subgroup, LBM was 96.1 ± 1.7 *vs.* 95.7 ± 2.1% of 50-wk-old mice, and BMD was 92.9 ± 3.9 *vs.* 91.2 ± 3.0% of 50-wk-old mice). Adiposity, however, decreased significantly in the ghrelin-treated subgroup (saline *vs.* ghrelin subgroup, 116.6 ± 8.4 *vs.* 102.2 ± 5.7% of 50-wk-old mice, $P < 0.05$).

Blood glucose (*ad libitum*), serum insulin (*ad libitum*), triglyceride, total cholesterol, GH, and IGF-I levels did not differ between the saline- and ghrelin-treated subgroups. Blood glucose measured after an overnight fast was significantly lower in ghrelin-treated animals than in the saline subgroup ($P < 0.05$). Serum insulin levels after an overnight fast were undetectable in both groups (Table 1).

TABLE 1. Blood glucose (*ad libitum* or overnight fast), serum insulin (*ad libitum* or overnight fast), triglyceride, total cholesterol, GH, and IGF-I levels at the end of experiment 3 (80-wk-old mice)

	Saline-injected mice	Ghrelin-injected mice
Ad libitum		
Blood glucose (mg/dl)	128.6 ± 4.7	128.1 ± 8.4
Immunoreactive insulin (ng/ml)	2.9 ± 0.5	2.0 ± 0.3
Overnight fast		
Blood glucose (mg/dl)	89.4 ± 5.1	73.2 ± 3.4[a]
Immunoreactive insulin (ng/ml)	ND	ND
Triglyceride (mg/dl)	164.3 ± 11.3	177.4 ± 12.1
Total cholesterol (mg/dl)	110.6 ± 2.8	110.2 ± 3.8
GH (ng/ml)	8.0 ± 0.8	8.9 ± 0.8
IGF-I (ng/ml)	377.1 ± 9.4	372.7 ± 14.0

Data are the means ± SEM. ND, Not detected.
[a] $P < 0.05$ (*vs.* saline subgroup).

Discussion

Many studies have demonstrated that as people age, they eat less food (1, 2). Elderly people have an impaired ability to recover BW fully after physical and mental stresses, such as acute and chronic illnesses, surgery, or bereavement (1, 8). Animal models of aging also reflect the decline in FI observed in humans both spontaneously and in response to disturbances in feeding (43, 45, 46). A spontaneous decline in FI with aging was also observed in C57BL6 mice in this study. Wolden-Hanson and colleagues (41–43) reported that, in comparison with young animals, aging male Brown Norway rats fail to regulate BW in response to the metabolic stressor of a 72-h fast and fail to increase FI appropriately after the fast. In accordance with those results, we observed that aging male C57BL6 mice recover BW more slowly than younger mice in response to the stressor of a 72-h fast. Thus, C57BL6 mice can serve as an animal model for the anorexia of aging.

As shown in Fig. 2, ghrelin treatment increased both BW gain and FI in 75-wk-old mice after the physical stress of a 72-h fast in comparison with untreated age-matched controls. The time course of BW recovery in ghrelin-treated 75-wk-old mice resembled that of 10-wk-old animals. CT measurement of body composition revealed that the improvement in BW resulted from increased LBM, which might include changes in body water, in ghrelin-treated mice. Yukawa and colleagues (47) demonstrated that ghrelin treatment prevented BW loss after surgery without increasing visceral fat mass in aging animals. Recent publications suggested that endogenous ghrelin may play a role in obesity and exogenous ghrelin-induced weight gain due to increased fat mass (23, 48–50). Most of these studies were performed under healthy conditions. On the other hand, under unhealthy conditions, it is reported that ghrelin administration to the patient with functional dyspepsia and chronic heart failure increased FI and LBM without fat accumulation (51, 52). As is often the case with an inpatient, when decreased BW recovers to the baseline level after physical and mental stress, such as acute and chronic illness and surgery, a nonfat component, which is necessary to maintain a normal physical function, recovers first, and then fat mass accumulates. We therefore think that the result of experiment 2, where ghrelin administration hastened the recovery of BW after 72-h fasting without fat accumulation, were not inconsistent with the reported effects of ghrelin. Involuntary weight loss in which loss of muscle predominates may predispose the elderly to muscle weakness and protein energy malnutrition, leading to increased risk for extended hospitalization, mortality, and morbidity (53). Several drugs have been suggested for the treatment of the anorexia of aging, such as cyproheptadine (an antihistaminergic, antiserotonergic drug), GH, ornithine oxoglutarate, and anabolic steroids. None, however, have been established in the management of weight loss in elderly people (1). This study and previous findings support a possible role for ghrelin as a means to prevent weight loss after acute and chronic illness, surgery, or bereavement in elderly patients.

As suggested by Toshinai *et al.* (46) and Sun *et al.* (54), sc administration of ghrelin at 40 μg/kg increased serum GH levels and did not affect daily FI in 50-wk-old mice. In addition, administration of ghrelin with a dose of 40 μg/kg twice weekly did not attenuate the GH response to ghrelin, whereas repeated administration of ghrelin markedly attenuated the GH response to injected ghrelin. These results strongly suggest that some intervals are needed to maintain the stimulating effect of ghrelin on GH secretion and support our regimen. The stimulating effect of ghrelin on GH secretion persisted at 80 wk of age (the end of experiment 3, data not shown).

In this study, long-term administration of ghrelin maintained low adiposity in aged mice without impairing glucose tolerance or lipid metabolism. Serum insulin levels and *ad libitum* and fasting blood glucose levels in ghrelin-treated mice tended to be lower than those seen in saline-treated mice. It is reported that peripheral ghrelin administration increased BW and cumulative FI and reduced insulin secretion (23, 55). This BW gain mainly results from increased fat mass and might be caused by decreased energy expenditure in addition to increased FI (50). Previous reports using mice showed that the effects of ghrelin on fat deposition were obtained by ghrelin administration at relatively high doses (50, 56). In this study, aged mice were administered low-dose ghrelin, which stimulates GH secretion without increase in cumulative FI. Discrepancy between previous reports and our result could be explained by differences in the doses and frequencies of ghrelin administration. Indeed, serum GH levels were increased by sc administration of ghrelin at a dose of 40 μg/kg, but daily and weekly FI were not increased in this study. Ghrelin-induced GH secretion may contribute to low adiposity. In contrast to our expectations, long-term administration of ghrelin did not increase either LBM or BMD. Clinical studies in elderly humans have indicated that MK-677 can reactivate the GH/IGF-I axis, increasing serum IGF-I levels, improving lean body composition (57), increasing BMD (40), and restoring lower-extremity function (44). In this study, serum IGF-I levels in ghrelin-treated mice were similar to those seen in saline-treated mice. The lack of change in IGF-I is likely why no increases in LBM or BMD were observed. Different results might have been obtained with ghrelin administration at higher doses and frequencies. In that case, however, fat mass would likely also have increased. Additional studies will be needed to investigate the

optimal dose and frequency of ghrelin administration to improve the age-related metabolic and physiological changes.

In conclusion, we demonstrated that repeated ghrelin administration increased FI and hastened the recovery of BW after a short-term physical stress. In addition, long-term administration of ghrelin maintained low adiposity in aged mice. Multiple approaches have attempted to improve such age-related metabolic and physiological changes. None of these, however, have yet achieved satisfactory results. Our results suggest that ghrelin may be a candidate therapeutic approach to combat such age-related metabolic and physiological changes.

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References

1. **Morley JE** 2001 Decreased food intake with aging. J Gerontol A Biol Sci Med Sci 56(Spec No 2):81–88
2. **Chapman IM** 2004 Endocrinology of anorexia of ageing. Best Pract Res Clin Endocrinol Metab 18:437–452
3. **Wurtman JJ, Lieberman H, Tsay R, Nader T, Chew B** 1988 Calorie and nutrient intakes of elderly and young subjects measured under identical conditions. J Gerontol 43:B174–B180
4. **Rolls BJ, Dimeo KA, Shide DJ** 1995 Age-related impairments in the regulation of food intake. Am J Clin Nutr 62:923–931
5. **Janicke B, Coper H** 1991 Effects of age and drugs on food and fluid intake. Growth Dev Aging 55:139–150
6. **Di Francesco V, Zamboni M, Dioli A Zoico E, Mazzali G, Omizzolo F, Bissoli L, Solerte SB, Benini L, Bosello O** 2005 Delayed postprandial gastric emptying and impaired gallbladder contraction together with elevated cholecystokinin and peptide YY serum levels sustain satiety and inhibit hunger in healthy elderly persons. J Gerontol A Biol Sci Med Sci 60:1581–1585
7. **Clarkston WK, Pantano MM, Morley JE, Horowitz M, Littlefield JM, Burton FR** 1997 Evidence for the anorexia of aging: gastrointestinal transit and hunger in healthy elderly vs. young adults. Am J Physiol 272:R243–R248
8. **Fischer J, Johnson MA** 1990 Low body weight and weight loss in the aged. J Am Diet Assoc 90:1697–1706
9. **Morley JE** 1987 Neuropeptide regulation of appetite and weight. Endocr Rev 8:256–287
10. **Kalra SP, Dube MG, Sahu A, Phelps CP, Kalra PS** 1991 Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. Proc Natl Acad Sci USA 88:10931–10935
11. **Schwartz MW** 1998 Orexins and appetite: the big picture of energy homeostasis gets a little bigger. Nat Med 4:385–386
12. **Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S** 2001 A role for ghrelin in the central regulation of feeding. Nature 409:194–198
13. **Glowa JR, Barrett JE, Russell J, Gold PW** 1992 Effects of corticotropin releasing hormone on appetitive behaviors. Peptides 13:609–621
14. **Lieverse RJ, Jansen JB, Masclee AA, Lamers CB** Satiety effects of a physiological dose of cholecystokinin in humans. Gut 36:176–179
15. **Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K** 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656–660
16. **Date Y, Murakami N, Kojima M, Kuroiwa T, Matsukura S, Kangawa K, Nakazato M** 2000 Central effects of a novel acylated peptide, ghrelin, on growth hormone release in rats. Biochem Biophys Res Commun 275:477–480
17. **Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, Suda M, Koh T, Natsui K, Toyooka S, Shirakami G, Usui T, Shimatsu A, Doi K, Hosoda H, Kojima M, Kangawa K, Nakao K** 2001 Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab 86:4753–4758
18. **Lu S, Guan JL, Wang QP, Uehara K, Yamada S, Goto N, Date Y, Nakazato M, Kojima M, Kangawa K, Shioda S** 2001 Immunocytochemical observation of ghrelin-containing neurons in the rat arcuate nucleus. Neurosci Lett 321: 157–160
19. **Cowley MA, Smith RG, Diano S, Tschop M, Pronchuk N, Grove KL, Strasburger CJ, Bidlingmaier M, Esterman M, Heiman ML, Garcia-Segura LM, Nillni EA, Mendez P, Low MJ, Sotonyi P, Friedman JM, Liu H, Pinto S, Colmers WF, Cone RD, Horvath TL** 2003 The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. Neuron 37:649–661
20. **Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, Mori K, Komatsu Y, Usui T, Shimatsu A, Ogawa Y, Hosoda K, Akamizu T, Kojima M, Kangawa K, Nakao K** 2000 Ghrelin strongly stimulates growth hormone release in humans. J Clin Endocrinol Metab 85:4908–4911
21. **Iwakura H, Akamizu T, Ariyasu H, Irako T, Hosoda K, Nakao K, Kangawa K** 2007 Effects of ghrelin administration on decreased growth hormone status in obese animals. Am J Physiol Endocrinol Metab 293:E819–E825
22. **Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, Hayashi T, Inoue G, Hosoda K, Kojima M, Kangawa K, Nakao K** 2001 Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes 50:227–232
23. **Wren AM, Small CJ, Abbott CR, Dhillo WS, Seal le IJ, Cohen MA, Batterham RL, Taheri S, Stanley SA, Ghatei MA, Bloom SR** 2001 Ghrelin causes hyperphagia and obesity in rats. Diabetes 50:2540–2547
24. **Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS** 2001 A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50:1714–1719
25. **Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M** 2001 Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterology 120:337–345
26. **Ariyasu H, Takaya K, Hosoda H, Iwakura H, Ebihara K, Mori K, Ogawa Y, Hosoda K, Akamizu T, Kojima M, Kangawa K, Nakao K** 2002 Delayed short-term secretory regulation of ghrelin in obese animals: evidenced by a specific RIA for the active form of ghrelin. Endocrinology 143:3341–3350
27. **Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML** 2001 Circulating ghrelin levels are decreased in human obesity. Diabetes 50: 707–709
28. **Rigamonti AE, Pincelli AI, Corra B, Viarengo R, Bonomo SM, Galimberti D, Scacchi M, Scarpini E, Cavagnini F, Müller EE** 2002 Plasma ghrelin concentrations in elderly subjects: comparison with anorexic and obese patients. J Endocrinol 175:R1–R5
29. **Sturm K, MacIntosh CG, Parker BA, Wishart J, Horowitz M, Chapman IM** 2003 Appetite, food intake, and plasma concentrations of cholecystokinin, ghrelin, and other gastrointestinal hormones in undernourished older women and well-nourished young and older women. J Clin Endocrinol Metab 88: 3747–3755
30. **Akamizu T, Murayama T, Teramukai S, Miura K, Bando I, Irako T, Iwakura H, Ariyasu H, Hosoda H, Tada H, Matsuyama A, Kojima S, Wada T, Wakatsuki Y, Matsubayashi K, Kawakita T, Shimizu A, Fukushima M, Yokode M, Kangawa K** 2006 Plasma ghrelin levels in healthy elderly volunteers: the levels of acylated ghrelin in elderly females correlate positively with serum IGF-I levels and bowel movement frequency and negatively with systolic blood pressure. J Endocrinol 188:333–344
31. **Broglio F, Benso A, Castiglioni C, Gottero C, Prodam F, Destefanis S, Gauna C, van der Lely AJ, Deghenghi R, Bo M, Arvat E, Ghigo E** 2003 The endocrine response to ghrelin as a function of gender in humans in young and elderly subjects. J Clin Endocrinol Metab 88:1537–1542
32. **Smith RG, Jiang H, Sun Y** 2005 Developments in ghrelin biology and potential clinical relevance. Trends Endocrinol Metab 16:436–442
33. **Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, Garry PJ, Lindeman RD** 1998 Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol 147:755–763
34. **Morley JE** 2001 Anorexia, body composition, and ageing. Curr Opin Clin Nutr Metab Care 4:9–13
35. **Anawalt BD, Merriam GR** 2001 Neuroendocrine aging in men. Andropause and somatopause. Endocrinol Metab Clin North Am 30:647–669
36. **Lamberts SW, van den Beld AW, van der Lely AJ** 1997 The endocrinology of aging. Science 278:419–424
37. **Roubenoff, R** 2001 Origins and clinical relevance of sarcopenia. Can J Applied Physiol 26:78–89
38. **Kamel HK, Maas D, Duthie Jr EH** 2002 Role of hormones in the pathogenesis and management of sarcopenia. Drugs Aging 19:865–877
39. **Chapman IM, Bach MA, Van Cauter E, Farmer M, Krupa D, Taylor AM, Schilling LM, Cole KY, Skiles EH, Pezzoli SS, Hartman ML, Veldhuis JD, Gormley GJ, Thorner MO** 1996 Stimulation of the growth hormone (GH)-insulin-like growth factor I axis by daily oral administration of a GH secretogogue (MK-677) in healthy elderly subjects. J Clin Endocrinol Metab 81: 4249–4257

40. **Murphy MG, Weiss S, McClung M, Schnitzer T, Cerchio K, Connor J, Krupa D, Gertz BJ; MK-677/Alendronate Study Group** 2001 Effect of alendronate and MK-677 (a growth hormone secretagogue), individually and in combination, on markers of bone turnover and bone mineral density in postmenopausal osteoporotic women. J Clin Endocrinol Metab 86:1116–1125
41. **Wolden-Hanson T, Marck BT, Matsumoto AM** 2002 Troglitazone treatment of aging Brown Norway rats improves food intake and weight gain after fasting without increasing hypothalamic NPY gene expression. Exp Gerontol 37:679–691
42. **Wolden-Hanson T, Marck BT, Matsumoto AM** 2004 Blunted hypothalamic neuropeptide gene expression in response to fasting, but preservation of feeding responses to AgRP in aging male Brown Norway rats. Am J Physiol Regul Integr Comp Physiol 287:R138–R146
43. **Wolden-Hanson T** 2006 Mechanisms of the anorexia of aging in the Brown Norway rat. Physiol Behav 88:267–276
44. **Bach MA, Rockwood K, Zetterberg C, Thamsborg G, Hébert R, Devogelaer JP, Christiansen JS, Rizzoli R, Ochsner JL, Beisaw N, Gluck O, Yu L, Schwab T, Farrington J, Taylor AM, Ng J, Fuh V; MK 0677 Hip Fracture Study Group** 2004 The effects of MK-0677, an oral growth hormone secretagogue, in patients with hip fracture. J Am Geriatr Soc 52:516–523
45. **Gruenewald DA, Marck BT, Matsumoto AM** 1996 Fasting-induced increases in food intake and neuropeptide Y gene expression are attenuated in aging male brown Norway rats. Endocrinology 137:4460–4467
46. **Toshinai K, Mondal MS, Shimbara T, Yamaguchi H, Date Y, Kangawa K, Nakazato M** 2007 Ghrelin stimulates growth hormone secretion and food intake in aged rats. Mech Ageing Dev 128:182–186
47. **Yukawa M, Weigle DS, Davis CD, Marck BT, Wolden-Hanson T** 2008 Peripheral ghrelin treatment stabilizes body weights of senescent male Brown Norway rats at baseline and after surgery. Am J Physiol Regul Integr Comp Physiol 294:R1453–R1460
48. **Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR** 2001 Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 86:5992
49. **Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG, Ghatei MA, Bloom SR** 2000 The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 141:4325–4328
50. **Tschöp M, Smiley DL, Heiman ML** 2000 Ghrelin induces adiposity in rodents. Nature 407:908–913
51. **Nagaya N, Moriya J, Yasumura Y, Uematsu M, Ono F, Shimizu W, Ueno K, Kitakaze M, Miyatake K, Kangawa K** 2004 Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure. Circulation 110:3674–3679
52. **Akamizu T, Iwakura H, Ariyasu H, Hosoda H, Murayama T, Yokode M, Teramukai S, Seno H, Chiba T, Noma S, Nakai Y, Fukunaga M, Kangawa K; FD Clinical Study Team** 2008 Repeated administration of ghrelin to patients with functional dyspepsia. Eur J Endocrinol 158:491–498
53. **Waters DL, Baumgartner RN, Garry PJ** 2000 Sarcopenia: current perspectives. J Nutr Health Aging 4:133–139
54. **Sun Y, Garcia JM, Smith RG** 2007 Ghrelin and growth hormone secretagogue receptor expression in mice during aging. Endocrinology 148:1323–1329
55. **Broglio F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, van der, Lely AJ, Deghenghi R, Ghigo E** 2001 Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. J Clin Endocrinol Metab 86:5083–5086
56. **Asakawa A, Inui A, Kaga T, Katsuura G, Fujimiya M, Fujino MA, Kasuga M** 2003 Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. Gut 52:947–952
57. **Svensson J, Lonn L, Jansson JO, Murphy G, Wyss D, Krupa D, Cerchio K, Polvino W, Gertz B, Boseaus I, Sjöström L, Bengtsson BA** 1998 Two-month treatment of obese subjects with the oral growth hormone (GH) secretagogue MK-677 increases GH secretion, fat-free mass, and energy expenditure. J Clin Endocrinol Metab 83:362–369

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