Endocrine Journal 2009, **56** (9), 1119-1128

NOTE

Ghrelin Increases Hunger and Food Intake in Patients with Restricting-type Anorexia Nervosa: A Pilot Study

MARI HOTTA*, **, RINA OHWADA**, TAKASHI AKAMIZU***, TAMOTSU SHIBASAKI#, KAZUE TAKANO** AND KENJI KANGAWA##

**Health Services Center, National Graduate Institute For Policy Studies, 106-8677 Tokyo, Japan*
*** Department of Medicine, Institute of Clinical Endocrinology, Tokyo Women's Medical University, School of Medicine, 162-8666 Tokyo, Japan*
****Ghrelin Research Project, Kyoto University School of Medicine, 606-8507 Kyoto, Japan*
 # Department of Physiology, Nippon Medical School, 113-8602 Tokyo, Japan
National Cardiovascular Center Research Institute, 565-8565 Osaka, Japan

Abstract. Ghrelin increases hunger sensation and food intake in various patients with appetite loss. Anorexia nervosa (AN) begins with psychological stress-induced anorexia and some patients cannot increase their food intake partly because of malnutrition-induced gastrointestinal dysfunction. The effects of ghrelin on appetite, food intake and nutritional parameters in anorexia nervosa (AN) patients were examined. Five female restricting-type AN patients (age: 14-35 y; body mass index: 10.2-14.6 kg/m^2) had persistently complained of gastrointestinal symptoms and failed to increase body weight. They were hospitalized for 26 days (6 days' pre-treatment, 14 days' ghrelin-treatment, and 6 days' post-treatment) and received an intravenous infusion of 3 μg/kg ghrelin twice a day. Ghrelin infusion improved epigastric discomfort or constipation in 4 patients, whose hunger scores evaluated by visual analogue scale questionnaires also increased significantly after ghrelin infusion. Daily energy intake during ghrelin infusion increased by 12-36 % compared with the pre-treatment period. Serum levels of total protein and triglyceride as nutritional parameters significantly increased after ghrelin treatment. There were no serious adverse effects including psychological symptoms. We found that ghrelin decreases gastrointestinal symptoms and increases hunger sensation and daily energy intake without serious adverse events in AN patients. Although the present study had major limitations of the lack of a randomized, placebo-controlled group, non-blindness of the investigators and the small number of patients recruited, it would contribute to further investigations for therapeutic potential of ghrelin in AN patients.

Key words: Ghrelin, Anorexia nervosa, Hunger, Food intake

(*Endocrine Journal* **56**: 1119-1128, 2009)

GHRELIN is mainly secreted by the stomach during starvation and it exerts a potent stimulatory effect on food intake and growth hormone (GH) secretion [1-3]. Endogenous ghrelin and its receptors are involved in the regulation of food intake, adiposity, and GH secretion [4]. Intravenous infusion of ghrelin is reported to increase food intake and body weight in healthy subjects [5-7] and to stimulate appetite and food intake in patients with congestive heart failure [8], chronic obstructive pulmonary disease [9], cancer [10], and functional dyspepsia [11].

Anorexia nervosa (AN) usually begins with psychological stress-induced anorexia and is characterized by fear of weight gain, starvation-induced abnormal behaviors, and a variety of biochemical and endocrinological abnormalities due to malnutrition. Chronic malnutrition induces both functional and organic changes in the gastrointestinal tract [12-14]. Most AN patients complain of chronic or recurrent upper abdominal discomfort and fullness, and chronic constipation. Laboratory examinations of the stomach reveal atrophy of the mucosa, alteration of peristalsis, and de-

Received Jun. 9, 2009; Accepted Aug. 28, 2009 as K09E-168
Released online in J-STAGE as advance publication Sep. 16, 2009

Correspondence to: Mari HOTTA, M.D., Ph.D., Health Services Center, National Graduate Institute For Policy Studies, 7-22-1 Roppongi, Minato-ku, Tokyo 106-8677 Japan.
E-mail: marihs@grips.ac.jp

Table 1. Clinical profile of AN patients in the present study

Case No	1	2	3	4	5
Age on entry (yrs)	27	31	25	35	14
Height (cm)	161	157	156	154	150
Weight before illness (kg) (BMI kg/m^2)	48 (18.5)	48 (19.5)	44.2 (18.2)	50 (21.1)	43 (19.1)
Age of onset (yrs)	16	24	17	20	13
Duration of illness (yrs)	12	6	8	15	1
The minimal weight (kg) (BMI kg/m^2)	29 (11.2)	30 (12.2)	32 (13.1)	23 (9.70)	27.4 (12.2)
Weight on entry (kg) (BMI kg/m^2)	37.9 (14.6)	32.5 (13.2)	35.0 (14.4)	24.2(10.2)	28.2 (12.5)
The increment of daily energy intake (%)	12	36	16	33	14
Weight on the end of study (kg) (BMI kg/m^2)	36.4 (14.0)	31.5 (12.8)	35.7 (14.7)	26.6 (11.2)	28.4 (12.6)
Weight on 6 months after discharge (kg) (BMI kg/m^2)	43 (16.6)	38.5 (15.6)	38.2 (15.7)	28 8 (12.1)	34.5 (15.3)

layed emptying time [15]. Even after becoming fully motivated to gain body weight, AN patients often cannot increase their food intake because of malnutrition-induced gastrointestinal dysfunction, and this delays recovery. Currently prescribed appetite-stimulating drugs such as metoclopramide, cyproheptadine, and sulpiride are not always effective, and any increase in appetite may be minor. Therefore, there is a pressing need for effective appetite-stimulating therapies for AN patients.

To develop a possibly new medical treatment for AN, we investigated the effects of ghrelin on appetite, energy intake, and nutritional parameters in restricting-type AN patients without binge eating/purging as a pilot study.

Subjects and Methods

Subjects

Subjects in the present study comprised 5 Japanese female amenorrheic AN patients aged 26 ± 8 yr (mean ±SD) (range, 14-35 yr) and mean body mass index (BMI) of 13.0 ±1.8 kg/m^2 (range, 10.2-14.6 kg/m^2) (Table 1). Patients met the Diagnostic and Statistical Manual IV (DSM IV) criteria for AN [16], in addition to those of the Survey Committee for Eating Disorders of the Japanese Ministry of Health, Labor and Welfare [17]. All patients had restricting AN, and had never reported binge eating, vomiting or laxative/diuretic abuse. All subjects were tested to be negative for *Helicobacter (H) pylori*. None of the patients had started medication prior to the trial. Four patients except for case 5 had complained of such as epigastric discomfort, abdominal fullness or pain after eating and constipation for several years and had been treated with intensive pshychotherapy as well as supervision of dieticians. All patients had been admitted to undertake hyperalimentation therapy but then lost weight again. They had been motivated to gain weight, but could not increase their food intake, in part because of gastrointestinal discomfort. The study protocol was approved by the institutional review board of Tokyo Women's Medical University. All patients provided written informed consent to participate in this study.

Methods

Study design

Due to ethical reasons, randomized controlled or blind methods were not applied for the present study. Subjects were hospitalized for 26 days (day -6 to day 20) in Tokyo Women's Medical University Hospital (Figure 1). Food intake and subjective hunger sensation were measured for 24 days (day -5 to day 19). The pre-treatment period was defined as the 5 days before ghrelin injection (day -5 to day -1). Subjects received an intravenous infusion of ghrelin (3 μg/kg body weight) for 5 min twice a day (before breakfast and dinner) for 14 days (day 1 to day 14) [11]. After ghrelin infusion, subjects were monitored for the clinical efficacy and safety of ghrelin for 5 days (day 15 to day 19) as a post-treatment period. Since ghrelin at doses of 1 and 5 μg/kg tended to increase appetite dose-dependently and repeated administration of ghrelin at a dose of 3μg/kg increase food intake without severe adverse effects [6, 11], we chose 3μg/kg of ghrelin in the present study.

Fig. 1. The timeline of the present study
Subjects were hospitalized for 26 days (day −6 to day 20) and subjective hunger sensation was measured for 24 days (day −5 to day 19). The pre-treatment period was defined as the 5 days before ghrelin injection (day −5 to day −1). Subjects received an intravenous infusion of ghrelin for 14 days (day 1 to day 14). After ghrelin infusion, subjects were monitored for the clinical efficacy and safety of ghrelin for 5 days (day 15 to day 19) as a post-treatment period. Blood and urine samples for biochemical and endocrinological parameters were taken in the morning after overnight fasting and psychological assessment was done on day −5, day 1, day 8, day 15, and day 19, respectively.

Ghrelin used in the present study

Human ghrelin was prepared as previously described [11]. Acylated peptide was dissolved in 3.75 % D-mannitol to yield a final concentration of 180 μg/mL. The solutions were filtered and stored at -20ºC 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.

Assessment of food intake and attitudes toward food

The primary endpoint of this study was energy intake. Patients were initially served with an amount of food equivalent to their meals at home before hospitalization plus an additional 200 Kcal. Each dish was weighed before and after eating. Energy intake was calculated by dieticians as total energy, carbohydrate, fat, and protein intakes. When subjects ate all of the food served and wanted more, they were allowed to eat self-prepared foods yielding approximately 200 Kcal such as fruit or other snacks. Their attitudes toward food were evaluated by a questionnaire incorporating visual analogue scales (VAS) rating hunger, satiety, prospective consumption, fullness, desire for some meat or fish, desire of something salty, desire of something sweet and desire of something fatty. During pre- and post-treatment, AN patients answered VAS questionnaire at before and after every meal. During ghrelin treatment, they did at 15 min before ghrelin infusion and breakfast or dinner, 15 min after ghrelin infusion before breakfast or dinner, and after those meals. It is demonstrated that food intake correlates with perceptions of hunger and fullness as assessed by VAS in healthy volunteers [18].

Measurement of biochemical and endocrinological parameters

Blood and urine samples for biochemical and endocrinological parameters were taken in the morning after overnight fasting longer than 10 h on day -5, day 1, day 8, day 15, and day 19. Blood samples for ghrelin assay were collected in tubes with 1 mg/mL EDTA-2Na and 500 U/mL aprotinin. They were immediately centrifuged at 4ºC, and plasma samples were then acidified with 1 normal HCl and stored at -80ºC until assay.

Immunoradiometric assays were utilized to measure levels of plasma GH (Eiken Chemical Co. , Tokyo, Japan) and serum IGF-I (Daiichi Pharmaceutical Co., Tokyo, Japan). Plasma insulin measurements were performed using an ELISA kit (Eiken Chemical Co., Tokyo, Japan). Plasma levels of intact and desoctanoyl ghrelin were measured using Active Ghrelin and Desacyl-Ghrelin ELISA kits (Mitsubishi Kagaku Iatron, Tokyo, Japan), respectively.

Psychological assessment

Depression and anxiety levels were evaluated using the Japanese versions of the self-rating depression scale (SDS) [19] and state-trait anxiety inventory (STAI) [20] on day -5, day 1, day 8, day 15 and day 19, respectively. Eating behaviors, weight, and body image concerns were also assessed by eating disorder inventory (EDI) [21] on the same time as described.

Statistics

Data are expressed as mean ± SE. Two-way analysis of variance (ANOVA) was used for energy and nutrient intakes and for biochemical and endocrinologic data. Appetite scores were analyzed by a Wilcoxon

signed rank test comparing the changes in VAS. Statistical analyses were performed using the computer statistical package SPSS (version 13.0.; SPSS Inc., Chicago, IL). Levels of significance were determined at $p<0.05$.

Results

Gastrointestinal symptoms and hunger sensation

After ghrelin injection, all patients except for case 2 reported that they had sensations of stomach activity or that their upper abdominal fullness disappeared. Borborygmi were frequently audible just after each ghrelin infusion in all patients. During ghrelin treatment, no patients reported constipation. As case 5 complained of loose stools, the dose of ghrelin was reduced to 1.5 μg/body weight from day 7 to day 14 and this improved her symptoms.

Hunger sensation evaluated by VAS was higher just after ghrelin infusion than that before ghrelin infusion in all patients except for case 2 (Figure 2). The stimulatory effects of ghrelin on hunger sensation disappeared after eating and did not last until next meal. Only in case 1, hunger scores before breakfast or dinner during ghrelin treatment were lower than those during both the pre- and post-treatment periods.

Food intake and body weight

The mean daily intakes of energy, carbohydrate, fat and protein are presented in Figure 3. The daily energy intake of the 5 patients during the pre-treatment period ranged from 825 to 1426 Kcal. During ghrelin infusion, all patients except for case 5 showed a statistically significant increase in daily energy intake. The mean increase in daily energy intake during ghrelin infusion was 20 ± 4 % when compared with the pre-treatment period. The mean food intake during ghrelin treatment in case 2, who did not report an increase in hunger sensation after ghrelin injection, significantly increased compared to that of pre-treatment. Analysis of nutrients revealed significant increases in daily intakes of carbohydrate (in 3 patients; cases 2, 3, and 4), fat (in 1 patient; case 4) and protein (in all patients). During the post-treatment period, daily energy, carbohydrate and protein intakes remained higher than those in the pre-treatment period in 3 patients (cases 2, 3, and 4). The daily fat intake during post-treatment period also remained higher than that in the pre-treatment period in 4 patients (cases 2, 3, 4, and 5). The increments of body weight in 5 patients were ranged from -1.5 to 2.4 kg during the ghrelin study (Table 1). Case 4 increased water and fat components evaluated by dual X-ray absorptiometry (data not shown).

Biochemical and endocrinological changes

Complete blood count did not change significantly during this study. Serum total protein and triglyceride levels significantly increased after ghrelin treatment (Table 2). Other nutritional markers including serum levels of transferrin and glucose showed a tendency to increase during and after ghrelin treatment, but this did not reach statistical significance. With the exception of case 4, in whom elevated transaminase levels due to malnutrition were improved by ghrelin treatment, liver function was stable over the study period.

Mean plasma levels of insulin and leptin did not increase significantly during ghrelin treatment. Although the elevated plasma level of GH decreased and the suppressed serum level of IGF-I improved during the study in case 4, other patients did not show a significant change in those parameters. Mean plasma levels of PRL and ACTH measured in the morning before ghrelin injection did not change significantly during ghrelin treatment.

We previously reported mean levels of plasma active and desacyl ghrelin in healthy young women as 29.9 ± 3.1 and 94.1 ± 7.5 pmol/L, respectively [22]. In the present study, the plasma levels of active ghrelin in AN patients ranged from 13 to 73 pmol/L (mean, 42) before ghrelin treatment and then did not show a significant change. Plasma levels of desacyl ghrelin in AN patients ranged from 80 to 731 pmol/L (mean, 280) before treatment, and then showed a tendency to decrease during ghrelin treatment.

Adverse effects

No serious adverse events occurred in all cases during ghrelin treatment. We did not detect any changes in vital signs or biochemical and endocrinologic data after ghrelin treatment. The only exceptions were loose stools in case 5 and an occasional warm sensation in the trunk or mild sweating in 2 subjects. No patients developed somnolence during ghrelin treat-

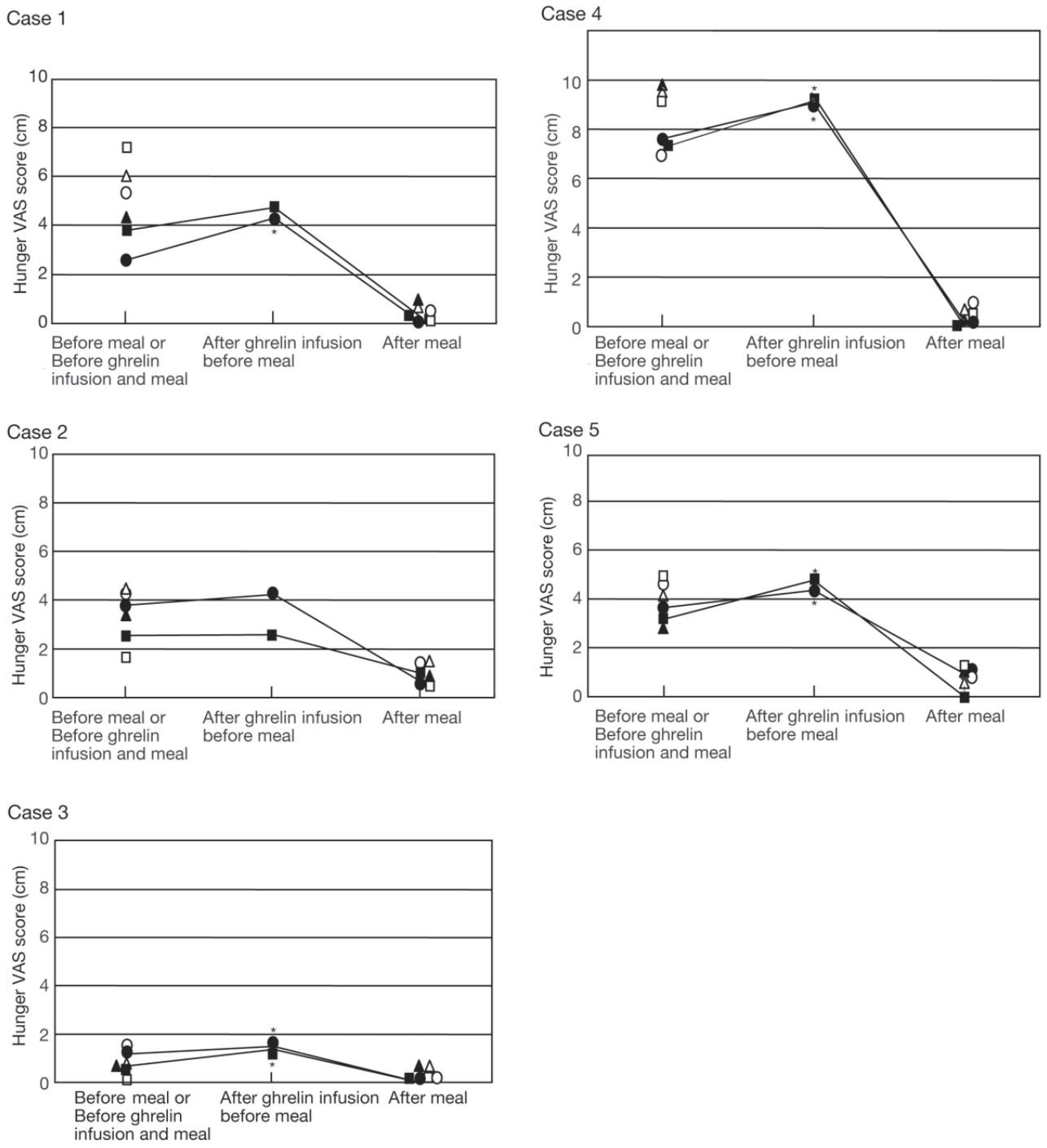

Fig. 2. Changes in hunger evaluated by VAS in AN patients

During pre- and post-treatment, AN patients answered VAS questionnaire at before and after every meal. During ghrelin treatment, they did at 15 min before ghrelin infusion, 15 min after ghrelin infusion before breakfast or dinner, and after those meals. Open circles (○), triangles (△) and squares (□) represent the mean of VAS hunger scores for breakfast, lunch, and dinner during pre and post-treatment periods, respectively. Closed circles (●), triangles (▲) and squares (■) represent the mean of VAS hunger scores for breakfast, lunch, and dinner during ghrelin treatment, respectively.

Data are expressed as mean. * $p<0.05$ *vs.* before ghrelin infusion

The mean of hunger scores before breakfast or dinner evaluated by VAS significantly increased after ghrelin infusion in all cases except for case 2.

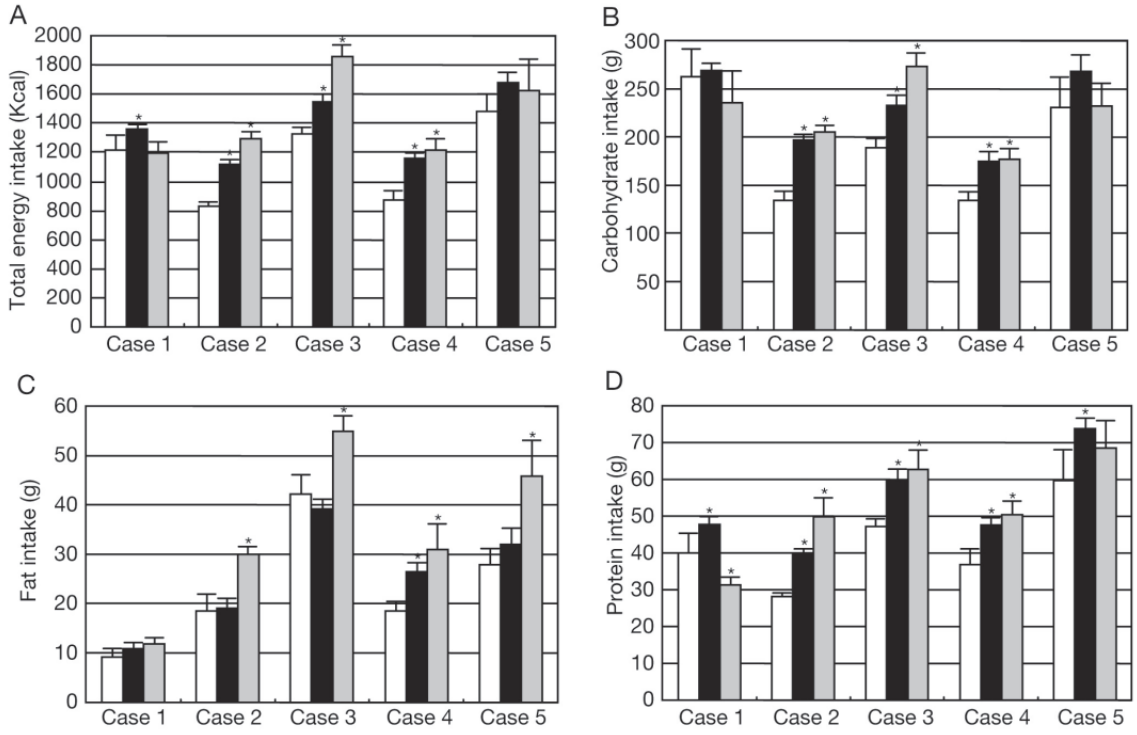

Fig. 3 Changes in the mean of total energy (panel A), carbohydrate (panel B), fat (panel C), and protein (panel D) intakes of AN patients.

Open (□), closed (■) and grey (■) bars represent the mean of intake during pre-treatment, ghrelin treatment, and post-treatment periods, respectively. Data are expressed as mean ± SE. * $p<0.05$ *vs.* pre-treatment period.

Across the 5 patients, mean increase in daily energy intake during ghrelin infusion was 12-36%. Energy intake in the post-treatment period remained higher than that in the pre-treatment period in 3 patients.

Table 2. Changes in biochemical and endocrinological data in AN patients during the present study

	Day 1	Day 8	Day 15	Day 19
White blood cell (/μL)	3200±230	2820±331	2660±388	3240±614
Hemoglobin (g/dL)	12.7±0.8	13.0±1.2	12.7±1.1	13.0±0.9
Platelet (x10^4/μL)	15.8±2.4	16.0±2.4	15.6±1.9	16.4±1.8
Total protein (g/dL)	6.5±0.4	6.9±0.1	6.8±0.4	7.1±0.3*
Transferrin (mg/dL)	179±22	195±21	196±15	208±10
Retinol binding protein (mg/dL)	3.0±0.3	3.0±0.4	3.1±0.3	3.1±0.2
Blood sugar (mg/dL)	75±5	79±2	81±2	81±2
AST(U/L)	86±58	32±7	27±2	31±3
ALT(U/L)	164±139	60±35	37±11	38±10
Cholinesterase (U/L)	220±29	220±30	214±28	216±25
Triglyceride (mg/dL)	47±10	80±15*	72±9*	83±10*
Total cholesterol (mg/dL)	179±23	187±23	170±24	182±18
Immunoreactive insulin (U/mL)	2.00±0.29	1.57±0.46	2.21±0.46	2.39±0.27
Leptin (ng/mL)	1.4±0.3	1.2±0.2	1.3±0.1	1.3±0.1
GH (ng/mL)	16.6±14.6	11.2±10.3	8.7±7.0	3.6±1.9
IGF-I (ng/mL)	115±37	116±28	128±32	123±35
PRL (ng/mL)	10.8±2.0	8.2±1.3	9.9±1.5	9.3±1.5
ACTH (pg/mL)	28.3±7.4	19.1±4.3	22.9±2.6	25.1±3.5
Active ghrelin (pmol/L)	42±19	45±12	54±15	46±9
Desacyl ghrelin (pmol/L)	280±115	198±26	206±37	198±37

Data are expressed as mean ± SE. *$p<0.05$ compared to day 1.

ment. In terms of psychological tests, SDS and STAI showed no significant change during the study, and EDI did not show any increased fear of weight gain in these patients (data not shown).

Clinical course after discharge

All patients gained weight after discharge, as shown in Table 1. In case 3, menstruation resumed 6 months after discharge.

Discussion

The present study showed that ghrelin infusion (3 μg/kg twice a day) can decrease gastrointestinal symptoms and enhance hunger sensation and daily energy intake without serious adverse events in restricting-type AN patients. The major limitations of the present study relate to the lack of a randomized, placebo-controlled group and non-blindness of the investigators and the small number of patients recruited. A non-treated group is not possible due to ethical reasons. Although non-ghrelin infused subjects who receive intense counseling and supervision of dietitian might be considered as a control group, all subjects in the present study had already received those treatments as well as total parenteral nutrition during the previous admission but failed to increase body weight due to gastrointestinal symptoms. Since the daily energy intake of post-treatment period was still higher than that of pre-treatment period, we could not exclude a placebo effect of ghrelin. However, we insist that 4 patients who failed in gaining body weight for long periods but they could increase their food intake during and after ghrelin infusion. It is speculated as the patients told us that ghrelin triggered an improvement in gastrointestinal function, which ameliorated the fear of gastrointestinal discomfort after eating in these patients.

Ghrelin seems to improve gastrointestinal motility in AN patients in the present study. It is notable that borborygmi occurred immediately after ghrelin infusion and that abdominal fullness or constipation disappeared in all patients. Ghrelin plays a role in the regulation of gastrointestinal motility and acid secretion in rats [23-25] and increases the gastric emptying rate in normal-weight humans [26]. Although we did not investigate gastric emptying rate in AN patients after ghrelin injection, ghrelin improved epigastric discomfort. This was probably mediated partly through increased gastric peristalsis as shown in other diseases with gastrointestinal dysfunction [27-30].

Ghrelin infusion increased hunger scores evaluated by VAS questionnaires of AN patients in the present study. Although AN patients often report not to feel hunger or satiety sensation, hunger scores was higher just after ghrelin infusion than that before ghrelin infusion in 4 patients. Since the sensation of hunger is usually correlated with gastric emptying in humans [31], enhanced hunger sensation in AN patients may be caused in part by ghrelin-induced gastric motility. However, the stimulatory effects of ghrelin on hunger score did not last until the next meal. We considered that the short-term effect of ghrelin on hunger sensation is related to its rapid degradation. The plasma concentration of ghrelin reaches the peak at 15 min after injection and rapidly decreases [6]. Hunger scores before breakfast or dinner during ghrelin treatment were lower than those during both the pre- and post-treatment periods in case 1. It is likely that abdominal fullness induced by the increased amount of food eaten in the foregoing meal during ghrelin treatment probably disturbed the hunger sensation on the next meal.

In previous reports, continuous or repeated ghrelin infusion increased hunger sensation and food intake in healthy volunteers and various patients with appetite loss. Ghrelin infusion at a dose of 5 pmol/kg/min for 270 min increased food intake by 28 % in healthy young Caucasian volunteers [5] and by 31 % in middle-aged and elderly cancer patients [10]. Ghrelin infusion (2 μg/kg twice a day) for 3 weeks increased food intake and body weight by 0.8 kg in elderly patients with congestive heart failure [9], and by 1 kg in elderly patients with chronic obstructive pulmonary disease [8]. Moreover, in patients with functional dyspepsia, ghrelin infusion (3 μg/kg twice a day) for 2 weeks increased hunger sensation and food intake by 29 % without significant weight gain [11]. Since 1 kg weight gain requires 7000-8000 Kcal, the increase in energy intake achieved for 14 days in this study was not enough to lead to any considerable weight gain. Although case 4 gained 2.4 kg and showed remarkable improvement in nutritional parameters and malnutrition-related liver dysfunction, we believe that water retention during the refeeding period contributed to this weight gain [32]. A decrease in body weight of 2 patients (cases 1 and 2) during ghrelin study might be attributable to a decrease in malnutrition-induced fluid

retention or improvement in bowel movements.

There were two reports about the effects of ghrelin on appetite in AN patients. In one study, 5 pmol/kg/min ghrelin infusion for 300 min had little effect on appetite in severely emaciated as well as weight-recovered AN patients [33]. However, appetite was evaluated by VAS alone because the severely emaciated AN patients refused to eat in the study. Since it is well known that recognition of hunger and satiety in AN patients is generally impaired, appetite cannot be always analyzed correctly by VAS alone. Although 1 μg/kg ghrelin bolus infusion made AN patients feel hunger sensation in another study, their food intakes were not evaluated [34]. We therefore believe that studies aiming to investigate ghrelin as an appetite-stimulating substance should recruit only AN patients who are fully motivated to gain weight by psycho-educational therapy.

Adverse effects such as abdominal discomfort, diarrhea, transient flushing, truncal perspiration, and somnolence have been reported after ghrelin injection [6]. Two patients in the present study occasionally reported a warm sensation in the trunk and mild sweating. Since case 5 experienced mild abdominal pain and several episodes of loose stools per day, we reduced the dose of ghrelin to 1.5 μg/kg, which improved these symptoms. No other serious physical or biochemical deteriorations occurred. Moreover, malnutrition-related liver dysfunction and endocrinologic abnormalities in case 4 were improved after ghrelin treatment. Interestingly, ghrelin infusion increased somnolence in the study [33], however, none of the present 5 subjects reported increased sleepiness. We did not observe increased fear concerning weight gain, abnormal behavior, or unstable mental status owing to an increase in appetite during ghrelin treatment, and psychological tests did not demonstrate any significant change in mental state. The present patients who motivated to gain body weight felt happy to be able to eat after ghrelin infusion, and they were pleased to be free from uncomfortable gastrointestinal symptoms after this ghrelin study. It is notable that all patients gained weight after discharge.

In conclusion, we found that ghrelin decreases gastrointestinal symptoms and increases hunger sensation and daily energy intake without serious adverse events in AN patients. A double-blinded, randomized, and placebo-controlled study is indispensable for developing ghrelin as an effective appetite-stimulating therapy for AN patients. The present study would contribute to investigations for therapeutic potential of ghrelin in AN patients.

Acknowledgments

We would like to thank Dr. Chiori Shibasaki for the analysis of nutritional intake, and SRL (Tachikawa, Tokyo, Japan) for technical assistance with the ELISA assay for ghrelin. This study was supported by a fund from the Ministry of Health, Labor and Welfare of Japan. There is no conflict of interest that would prejudice the impartiality of the research.

References

1. 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.
2. 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.
3. 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.
4. Shuto Y, Shibasaki T, Otagiri A, Kuriyama H, Ohata H, Tamura H, Kamegai J, Sugihara H, Oikawa S, Wakabayashi I (2002) Hypothalamic growth hormone secretagogue receptor regulates growth hormone secretion, feeding, and adiposity. *J Clin Invest*. 109: 1429-1436.
5. 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: 5992-5995.
6. 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 (2004) Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. *Eur J Endocrinol* 150: 447-455.

7. Druce MR, Wren AM, Park AJ, Milton JE, Patterson M, Frost G, Ghatei MA, Small C, Bloom SR (2005) Ghrelin increases food intake in obese as well as lean subjects. *Int J Obes (Lond)* 29: 1130-1136.
8. 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.
9. Nagaya N, Itoh T, Murakami S, Oya H, Uematsu M, Miyatake K, Kangawa K. (2005) Treatment of cachexia with ghrelin in patients with COPD. *Chest* 128: 1187-1193.
10. Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, Frost GS, Ghatei MA, Coombes RC, Bloom SR (2004) Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. *J Clin Endocrinol Metab* 89: 2832-2836.
11. Akamizu T, Iwakura H, Ariyasu H, Hosoda H, Murayama T, Yokode M, Teramukai S, Seno H, Chiba T, Noma S, Nakai Y, Fukunaga M, Nakai Y, Kangawa K, FD Clinical Study Team (2008) Repeated administration of ghrelin to patients with functional dyspepsia: its effects on food intake and appetite. *Eur J Endocrinol* 158: 481-498.
12. Haller E (1992) Eating disorders. A review and update. *West J Med* 157: 658-662.
13. Crisp AH (1985) Gastrointestinal disturbance in anorexia nervosa. *Postgrad Med J* 61: 3-5.
14. Abell TL, Malagelada JR, Lucas AR, Brown ML, Camilleri M, Go VL, Azpiroz F, Callaway CW, Kao PC, Zinsmeister AR (1987) Gastric electromechanical and neurohormonal function in anorexia nervosa. *Gastroenterology* 93: 958-965.
15. Domstad PA, Shih WJ, Humphries L, Deland FH, Digenis GA (1987) Radionuclide gastric emptying studies in patients with anorexia nervosa. *J Nucl Med* 28: 816-819.
16. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Washington D.C. American Psychiatric Association 1992.
17. Hotta M, Fukuda I, Sato K, Hizuka N, Shibasaki T, Takano K (2000) The relationship between bone turnover and body weight, serum insulin-like growth factor (IGF) I, and serum IGF-binding protein levels in patients with anorexia nervosa. *J Clin Endocrinol Metab* 85: 200-206.
18. Parker BA, Sturm K, MacIntosh CG, Feinle C, Horowitz M, Chapman IM (2004) Relation between food intake and visual analogue scale ratings of appetite and other sensations in healthy older and young subjects. *Eur J Clin Nutr* 58: 212-218.
19. Zung W W (1965) A self-rating depression scale. *Arch Gen Psychiatry* 12: 63-70.
20. Iwata N, Mishima N, Shimizu T, Mizoue T, Fukuhara M, Hidano T, Spielberger C (1998) The Japanese adaptation of the STAI Form Y in Japanese working adults - the presence or absence of anxiety. *Indust Heal* 36: 8-13.
21. Garner DM, Garfinkel PE (1979) The eating attitude test: an index of symptoms of anorexia nervosa. *Psychol Med* 9: 273-279.
22. Hotta M, Ohwada R, Katakami H, Shibasaki T, Hizuka N, Takano K (2004) Plasma levels of intact and degraded ghrelin and their responses to glucose infusion in anorexia nervosa *J Clin Endocrinol Metab* 89: 5707-5712.
23. Masuda Y, Tanaka T, Inomata N, Ohnuma N, Tanaka S, Itoh Z, Hosoda H, Kojima M, Kangawa K (2000) Ghrelin stimulates gastric acid secretion and motility in rats. *Biochem Biophys Res Commun* 276: 905-908.
24. Edholm T, Levin F, Hellstrom PM & Schmidt PT (2004) Ghrelin stimulates motility in the small intestine of rats through intrinsic cholinergic neurons. *Regul Pept* 121: 25-30.
25. Levin F, Edholm T, Ehrström M, Wallin B, Schmidt PT, Kirchgessner AM, Hilsted LM, Hellström PM, Näslund E (2005) Effect of peripherally administrated ghrelin on gastric emptying and acid secretion in the rat. *Regul Pept* 131: 59-65.
26. Levin F, Edholm T, Schmidt PT, Grybäck P, Jacobsson H, Degerblad M, Höybye C, Holst JJ, Rehfeld JF, Hellström PM, Näslund E (2006) Ghrelin stimulates gastric emptying and hunger in normal-weight humans. *J Clin Endocrinol Metab* 91: 3296-3302.
27. Murray CD, Martin NM, Patterson M, Taylor SA, Ghatei MA, Kamm MA, Johnston C, Bloom SR, Emmanuel AV (2005) Ghrelin enhances gastric emptying in diabetic gastroparesis: a double-blind, placebo-controlled, cross-over study. *Gut* 54: 1693-1698.
28. Binn M, Albert C, Gougeon A, Maerki H, Coulie B, Lemoyne M, Rabasa Lhoret R, Tomasetto C, Poitras P (2006) Ghrelin gastrokinetic action in patients with neurogenic gastroparesis. *Peptides* 27: 1603-1606.
29. Tack J, Depoortere I, Bisschops R, Verbeke K, Janssens J, Peeters T (2005) Influence of ghrelin on gastric emptying and meal-related symptoms in idiopathic gastroparesis. *Gut* 55: 327-333.
30. Strasser F, Lutz TA, Maeder MT, Thuerlimann B, Bueche D, Tschöp M, Kaufmann K, Holst B, Brändle M, von Moos R, Demmer R, Cerny T (2008) Safety, tolerability and pharmacokinetics of intravenous ghre-

lin for cancer-related anorexia/cachexia: a randomised, placebo-controlled, double-blind, double-crossover study. *Br J Cancer* 98: 300-308.
31. Sepple CP, Read NW (1989) Gastrointestinal correlates of the development of hunger in man. *Appetite* 13: 183-191.
32. Yücel B, Ozbey N, Polat A, Yager J (2005) Weight fluctuations during early refeeding period in anorexia nervosa: case reports. *Int J Eat Disord* 37: 175-177.
33. Miljic D, Pekic S, Djurovic M, Doknic M, Milic N, Casanueva FF, Ghatei M, Popovic V (2006) Ghrelin has partial or no effect on appetite, growth hormone, prolactin, and cortisol release in patients with anorexia nervosa. *J Clin Endocrinol Metab* 91: 1491-1495.
34. Broglio F, Gianotti L, Destefanis S, Fassino S, Abbate Daga G, Mondelli V, Lanfranco F, Gottero C, Gauna C, Hofland L, Van der Lely AJ, Ghigo E (2004) The endocrine response to acute ghrelin administration is blunted in patients with anorexia nervosa, a ghrelin hypersecretory state. *Clin Endocrinol (Oxf)* 60: 592-599.