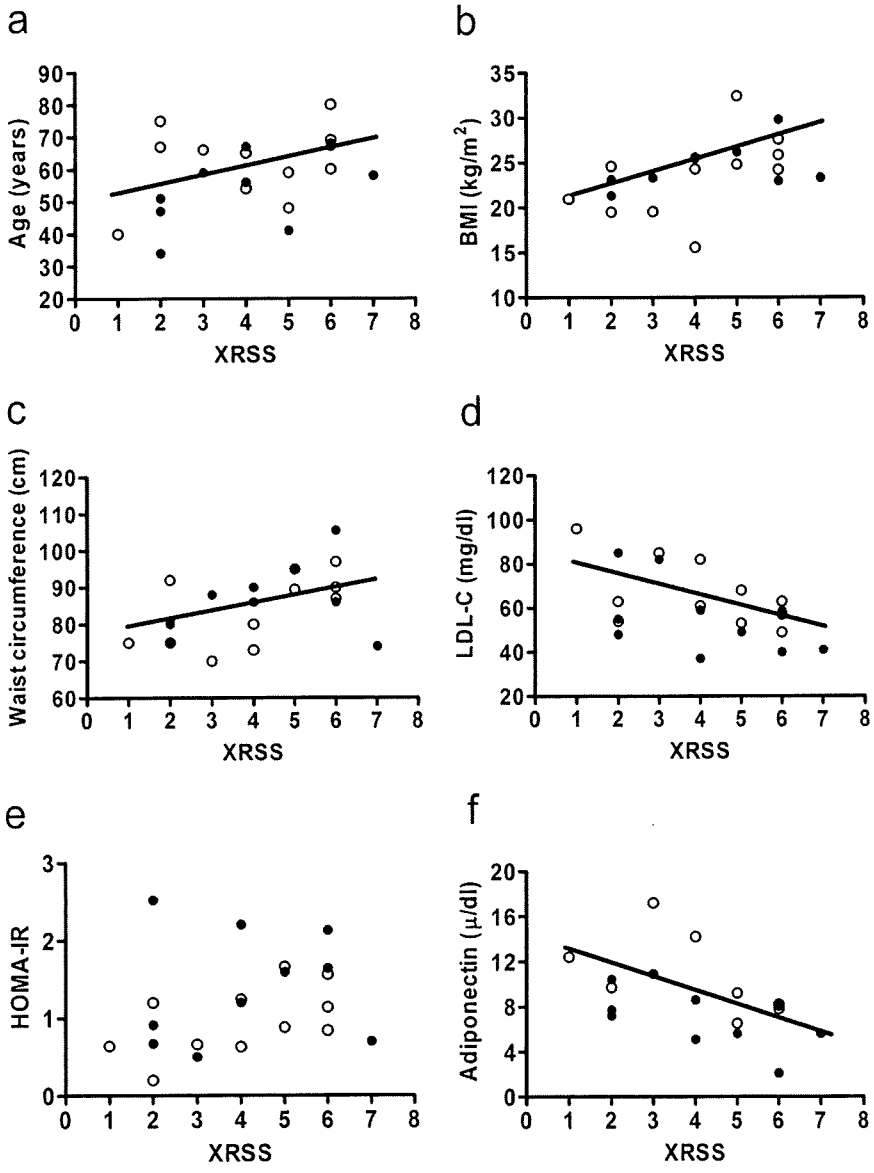

Figure 1 Regression analysis of XRSS with parameters of body measurement and metabolic markers. Relationship between XRSS and (a) age, (b) BMI, (c) waist circumference, (d) HDL-C, (e) HOMA-IR and (f) adiponectin. See Table 3 for abbreviations, *r*-values, and significance. ●, Male; ○, female.

correlated inversely with TG ($P = 0.036$), BMI ($P = 0.004$) and waist circumference ($P = 0.020$). Total ghrelin levels correlated with HOMA-IR ($P < 0.0001$), TG ($P = 0.010$), HDL-C ($P = 0.010$) and adiponectin ($P = 0.003$). Active ghrelin levels correlated with BMI ($P = 0.013$), HOMA-IR ($P < 0.001$), TG ($P = 0.037$) and adiponectin ($P = 0.012$).

Multiple linear regression analysis

Multiple linear regression analysis is shown in Table 4. Because many of the parameters correlated with each other (e.g. BMI and HOMA-IR, TG, HDL-C), multiple linear regression analysis was used to determine independent associations with XRSS. Serum adiponectin, BMI and TG levels were independently associated with XRSS ($P < 0.01$, <0.05, <0.05).

Discussion

The present study examined whether the findings from videoesophagography were related to GERD symptoms evaluated by QUEST. It also examined the relationships of XRSS as a measure of the severity of GER to body measurement, insulin resistance and lipid metabolism.

As we use a progressive pulsed method for decreasing X-ray exposure, videoesophagography is simple, non-invasive, reproducible, rapid and well tolerated by patients. It enables diagnosis of GER by detecting abnormal gastroesophageal barium reflux and hiatus hernia in the spinal LAO position after initial small-volume barium swallow. The findings correlated well with GERD symptoms. We previously evaluated the correlation between GERD symptoms and esophageal motility and clearance using videoesophagography after giving 20 mL barium in the spinal position.[11]

Table 4 Correlation between XRSS and metabolic markers (multiple regression analysis)

Variable	Standard coefficient	*P*-value
Age (years)	−0.190	0.052
BMI (kg/m^2)	0.146	0.018*
Waist (cm)	0.479	0.071
HOMA-IR (mg/dL)	−0.190	0.069
TG (mg/dL)	−0.371	0.012*
HDL-C (mg/dL)	−0.325	0.278
Adiponectin (μg/mL)	−0.905	0.008**
HbA_{1c} (%)	−0.599	0.027

*$P < 0.05$, **$P < 0.001$.

BMI, body mass index; HbA_{1c}, hemoglobin A_{1c}; HOMA-IR, homeostasis model assessment of insulin resistance; HDL-C, high-density lipoprotein–cholesterol; TG, triglyceride; XRSS, X-ray severity score for gastroesophageal reflux.

As several studies have shown that reflux is increased in patients in the right-side-down position, we chose a lying position with right side down to accelerate gastroesophageal reflux. The reason for this phenomenon may be related to increased transient esophageal sphincter relaxations (tLESR) in the right-side-down position, or possibly to the lower location of the gastric cardia than the pool of gastric juice in the right-side-down position.[12] Total reflux time, average acid clearance, and LES relaxations are significantly prolonged in patients lying on their right side compared with the left side down.[13] We believe that the barium X-ray finding of belching is associated with tLESR. The tLESR, a physiological venting system, exists to protect the stomach against damage caused by extreme dilatation. This relaxation differs in duration from swallow-induced LES relaxation. The rate of postprandial tLESR correlated with BMI and waist circumference using esophageal manometry and pH monitoring.[14] The reason for the increased rate of postprandial tLESR in obese subjects is unclear. Our findings are compatible with a previous study by Pandolfino *et al.* using high-resolution manometry that reported significant correlation between intragastric pressure and gastroesophageal pressure gradient with BMI and waist circumference.[15] There are some reports of esophageal motility disorder seen in GERD patients using esophageal manometry,[16] but no significant difference was shown in our preliminary examination using videoesophagography (data not shown). A reasonable algorithm for evaluating patients with GERD symptoms should include initial barium esophagography to evaluate the existence of GER and an anatomical lesion of the esophagus and gastric fundus, such as hiatus hernia. We could detect the majority of patients in whom a definitive diagnosis would significantly affect therapy and outcome. Therefore, esophageal manometry and 24-h esophageal pH test remain the gold standard for detecting esophageal motor disorder and GERD, despite being less well tolerated. It is suitable to regard videoesophagography as the initial screening test for GER in symptomatic patients.

In the present study, decreased levels of serum adiponectin were shown in patients with severe GER evaluated by videoesophagography and these levels correlated inversely with BMI, HOMA-IR and TG. Adiponectin is a plasma protein expressed in adipose tissue, the plasma levels of which are linked to insulin sensitivity.[6] In the present study, there was a possible link between adiponectin and the severity of GER. The notion of obesity as a cause of GERD is biologically plausible. Previous reports suggest that obesity is associated with increased intra-abdominal pressures,[17] impaired gastric emptying and decreased lower esophageal sphincter relaxation, thus leading to increased exposure to esophageal acid.[18] However, increased levels of adiponectin in the mesenteric adipose tissue of patients with Crohn's disease has recently been reported.[19] Expression of adiponectin receptors in esophageal or gastric smooth muscle has not been reported, nor has a relationship between adiponectin and the esophageal sphincter. Adiponectin may affect the regulation of esophagogastrointestinal motility and further investigation is required to examine the role of adiponectin in patients with GERD. Total and active ghrelin levels correlated inversely with insulin resistance in the present study, but only the level of active ghrelin correlated inversely with XRSS. Plasma ghrelin levels are significantly higher in patients with dysmotility-like functional dyspepsia diagnosed under Roma II.[20] The plasma concentration of active ghrelin changes more dynamically than that of total ghrelin and a similar tendency was observed in this study. As only 12 patients (six men and six women) had plasma ghrelin measured, we cannot assess the association between ghrelin and XRSS using multiple linear regression analysis due to the lack of data.

In conclusion, the present study suggests that videoesophagography is a useful procedure for initial evaluation of patients with GER symptoms. Functional GER is seen in obese patients with decreased levels of adiponectin and ghrelin. Based on the results of the present study, a large-scale clinical study examining videoesophagography and metabolic markers should be conducted.

Acknowledgments

We thank Mr Yujiro Nakamura for a great contribution to this study. This work was supported in part by a grant-in-aid for Young Scientists B from Japan Society for the Promotion of Science (No. 18790471 to E.I.), a grant-in-aid for Exploratory Research, JSPS (No. 19659057 to H.S.) in part from the Nateglinide Memorial Toyoshima Research and Education Fund.

References

1 Hampel H, Abraham NS, El-Serag HB. Obesity and the risk for gastroesophageal reflux disease and its complications. *Ann. Intern. Med.* 2005; **143**: 199–211.
2 Jacobson BC, Somers SC, Fuchs CS, Kelly CD, Camargo CA Jr. Body-mass index and symptoms of gastroesophageal reflux in women. *N. Engl. J. Med.* 2006; **354**: 2340–8.
3 El-Serag HB, Ergun GA, Pandolfino J, Fitzgerald S, Tran T, Kramer JR. Obesity increases esophageal acid exposure. *Gut* 2007; **56**: 749–55.
4 Aly YA. Digital radiography in the evaluation of oesophageal motility disorders. *Clin. Radiol.* 2000; **55**: 561–8.
5 Parkman HP, Maurer AH, Carlone DF, Miller DL, Krevsky B, Fisher RS. Optimal evaluation of patients with nonobstructive esophageal dysphagia. Manometry, scintigraphy, or videoesophagography? *Dig. Dis. Sci.* 1996; **41**: 1355–68.
6 Hotta K, Funahashi M, Arita Y *et al.* Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler. Thromb. Vasc. Biol.* 2000; **20**: 1595–9.

7 Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. *Diabetes* 2001; **50**: 707–9.
8 Mishima I, Adachi K, Arima N *et al.* Prevalence of endoscopically negative and positive gastroesophageal reflux disease in the Japanese. *Scand. J. Gastroenterol.* 2005; **40**: 1005–9.
9 Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. *Diabetes Care* 1997; **20**: 1087–92.
10 Masaoka T, Suzuki H, Hosoda H *et al.* Enhanced plasma ghrelin levels in rats with streptozotocin-induced diabetes. *FEBS Lett.* 2003; **541**: 64–8.
11 Iwasaki E, Suzuki H, Hibi T *et al.* Enhanced levels of insulin resistance in patients with gastro-esophageal reflux evaluated by flat-panel detector assisted videoesophagography (Abstract). *Am. J. Gastroenterol.* 2006; **101** (Suppl. 9): 132.
12 Meining A, Classen M. The role of diet and lifestyle measures in the pathogenesis and treatment of gastroesophageal reflux disease. *Am. J. Gastroenterol.* 2000; **95**: 2692–7.
13 van Herwaarden MA, Katzka DA, Smout AJ, Samsom M, Gideon M, Castell DO. Effect of different recumbent positions on postprandial gastroesophageal reflux in normal subjects. *Am. J. Gastroenterol.* 2000; **95**: 2731–6.
14 Wu JC, Mui LM, Cheung CM, Chan Y, Sung JJ. Obesity is associated with increased transient lower esophageal sphincter relaxation. *Gastroenterology* 2007; **132**: 883–9.
15 Pandolfino E, El-Serag HB, Zhang Q, Shah N, Ghosh SK, Kahrilas PJ. Obesity: a challenge to esophagogastric junction integrity. *Gastroenterology* 2006; **130**: 639–49.
16 Nguyen HN, Domingues GR, Lammert F. Technological insights: combined impedance manometry for esophageal motility testing-current results and further implications. *World J. Gastroenterol.* 2006; **12**: 6266–73.
17 Sugerman HJ, DeMaria EJ, Felton WL 3rd, Nakatsuka M, Sismanis A. Increased intra-abdominal pressure and cardiac filling pressures in obesity-associated pseudotumor cerebri. *Neurology* 1997; **49**: 507–11.
18 Barak N, Ehrenpreis ED, Harrison JR, Sitrin MD. Gastro-oesophageal reflux disease in obesity: pathophysiological and therapeutic considerations. *Obes. Rev.* 2002; **3**: 9–15.
19 Yamamoto K, Kiyohara T, Murayama Y *et al.* Production of adiponectin, an anti-inflammatory protein, in mesenteric adipose tissue in Crohn's disease. *Gut* 2005; **54**: 789–96.
20 Nishizawa T, Suzuki H, Hibi T *et al.* Enhanced plasma ghrelin levels in patients with functional dyspepsia. *Aliment. Pharmacol. Ther.* 2006; **24** (Suppl. 4): 104–10.

face, and mood disturbances such as apathy or euphoria in non-dominant hemisphere anterior lesions. In posterior watershed infarctions, hemi-anopsia is common. Watershed infarctions in the bilateral frontal lobes can cause severe dementia.[4]

PV is a chronic, myeloproliferatieve disorder, predominantly presenting in elderly patients with an incidence rate of 23.5 per 100,000 person-years.[5] Symptoms are due to hyperviscocity. A feared complication of PV is thrombosis (venous or arterial). Together with transformation into myelofibrosis, acute leukemia, or both, thrombosis is the main cause of death.[6] The median survival of treated patients exceeds 10 year.[7]

This case history illustrates that an unusual course of cognitive impairment necessitates in-depth clinical investigation.

Gonnie M. Alkemade, MD
Department of Internal Medicine
Leiden University Medical Center
Leiden, the Netherlands

Jorien M. Willems, MD
Department of Gerontology and Geriatrics
Leiden University Medical Center
Leiden, the Netherlands

ACKNOWLEDGMENTS

We thank Professor R. G. J. Westendorp for thoroughly reading this manuscript. We also thank Dr. L. van der Wiel, neuropsychologist, Dr. W. Bollen, clinical neurologist, and Professor M. A. van Buchem, neuroradiologist, for their contributions.

Conflict of Interest: The editor in chief has reviewed the conflict of interest checklist provided by the authors and has determined that the authors have no financial or any other kind of personal conflicts with this letter.

Author Contributions: Both authors contributed equally to the preparation of this letter.

Sponsor's Role: None.

REFERENCES

1. Ringelstein EB, Stogbauer F. Border zone infarcts. In: Bogousslavsky J, Caplan L, eds. Stroke Syndromes. New York: Cambridge University Press, 2001, pp 564–582.
2. Di Pollina L, Juillerat-Van der Linden AC, Michel JP et al. Cognitive impairment in polycythemia vera: Partial reversibility upon lowering of the hematocrit. Eur Neurol 2000;44:57–59.
3. Bladin CF, Chambers BR. Clinical features, pathogenesis, and computer tomographic characteristics of internal watershed infarction. Stroke 1993;24:1925–1932.
4. Hashiguchi S, Mine H, Ide M et al. Watershed infarction associated with dementia and cerebral atrophy. Psychiatry Clin Neurosci 2000;54:163–168.
5. Ania BJ, Suman VJ, Sobell JL et al. Trends in the incidence of polycythemia vera among Olmsted County, Minnesota residents, 1935–1989. Am J Hematol 1994;47:89–93.
6. Rozman C, Giralt M, Fleiu E et al. Life expectancy of patients with chronic nonleukemic myeloproliferative disorders. Cancer 1991;67:2658–2663.
7. Berk PD, Goldberg JD, Donovan PB et al. Therapeutic recommendations in polycytemia vera based on Polycythemia Vera Study Group protocols. Semin Haematol 1986;23:132–143.

EFFECTS OF GHRELIN TREATMENT ON PATIENTS UNDERGOING TOTAL HIP REPLACEMENT FOR OSTEOARTHRITIS: DIFFERENT OUTCOMES FROM STUDIES IN PATIENTS WITH CARDIAC AND PULMONARY CACHEXIA

To the Editor: Elderly subjects, who exhibit low growth hormone (GH) secretion and low lean tissue reserves, are vulnerable to postoperative catabolism.[1] Osteoarthritis (OA) of the hip, a leading cause of disability in elderly people, often requires surgical treatment for the late-stage or severe forms. Ghrelin, which strongly stimulates secretion of GH, has been reported to reduce muscle wasting and improve functional capacity in elderly patients with congestive heart failure (CHF)[2] and chronic obstructive pulmonary disease (COPD).[3] In this study, we evaluated the effects of repeated administrations of ghrelin at the same dose and duration as in previous studies on physical performance and body composition in patients with OA undergoing elective total hip replacement (THR).

This Phase II study was randomized, double-blind, and placebo-controlled. Thirty-two patients (25 women, 7 men; aged 43–79, mean 60.4 ± 10.9) were assigned to two groups of 16 subjects each; the ghrelin group received intravenous injections of 2 μg/kg of ghrelin, and the placebo group received a vehicle alone. Subjects received twice-daily (1 hour before lunch and dinner) injections for 3 weeks beginning 1 week before surgery (Week 1, Days 1–7) and 2 weeks thereafter (Weeks 2–3, Days 9–22). Patients were hospitalized for 4 weeks postoperatively for rehabilitation (Weeks 2–6, Days 11–36). Human ghrelin and placebo were prepared as previously described.[4,5] As the primary endpoints, the alterations in muscle strength (hip abductors and knee extensors) and walking ability (Timed Up-and-Go Test)[6] due to ghrelin administration from Week 1 (before ghrelin administration) to Week 6 were assessed. Muscle strength of the hip flexors, hip extensors, and knee flexors; 10-m walking time; and handgrip strength were measured as secondary endpoints. Hip joint function was evaluated using the Japan Orthopaedic Association (JOA) hip score.[7] Mid-thigh muscle cross-sectional area (MMCA) and body composition were determined using magnetic resonance imaging and dual-energy X-ray absorptiometry, respectively.

There were no significant differences between the placebo and ghrelin groups in any baseline patient characteristics studied. Changes in muscle strength, walking ability, MMCA, and JOA hip score from pretreatment to 2 weeks after treatment did not differ significantly between the two groups. Ghrelin treatment significantly increased lean body mass ($P = .01$) and decreased fat mass ($P = .02$) (Figure 1A). None of the hormonal or metabolic parameters assessed, with the exception of serum insulin-like growth factor level at Week 2 (Day 8) (5.6 ± 28.5% vs 30.0 ± 34.6% (placebo vs ghrelin), $n = 16$, $P = .04$), changed significantly after ghrelin administration. GH

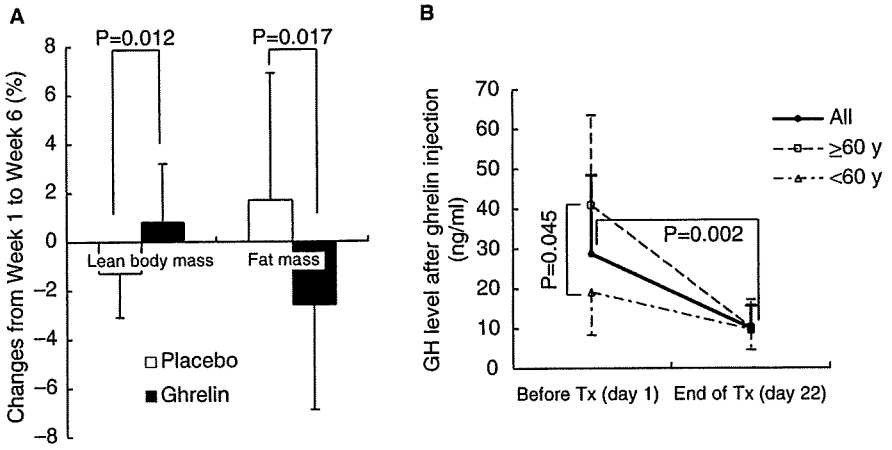

Figure 1. (A) Percentage change in lean body mass and fat mass from baseline (Week 1) to the posttreatment phase (Week 6) as measured using dual-energy X-ray absorptiometry. (B) Growth hormone (GH) responses after ghrelin injection; serum GH concentrations were measured 30 minutes after ghrelin injections on Days 1 and 22. Data are expressed as means ± standard deviations. Statistical analyses were performed using the Student *t*-test or the Wilcoxon rank-sum test when data were not normally distributed. A two-tailed *P*-value less than .05 was determined to be significant.

responses to ghrelin administration (30 minutes after morning injection) were significantly attenuated after 3 weeks of treatment (Day 1, 28.7 ± 19.7 (n = 16) vs Day 22, 10.0 ± 5.8 ng/mL (n = 15); $P = .002$) (Figure 1B). Although GH responses during the pretreatment phase were significantly greater in elderly subjects than in younger patients (41.0 ± 22.6 vs 19.2 ± 10.7 ng/mL; $P = .045$), GH responses on Day 22 had equilibrated to approximately 10 ng/mL in both groups (Figure 1B).

Although ghrelin treatment reduces muscle wasting and improves functional capacity in cachectic patients with CHF[2] or COPD,[3] no effect of ghrelin on mid-thigh muscle mass or functional performance was observed in this study. Several possibilities may explain these discrepancies. First, because patients with OA of the hip are often obese, not cachectic (body mass index 22.7 kg/m^2), their responses to ghrelin may differ from those of cachectic patients; GH responses to ghrelin may be lower in obese subjects.[8] Second, the timing of morning ghrelin injections in this study was 1 hour before lunch; GH responses, averaging 28.7 ± 19.7 ng/mL, appeared to be lower than those observed after early-morning injection.[2,4,9] Finally, the mean age of subjects who received ghrelin in the current study (62.5) was lower than those in the two previous studies (75 and 78).[2,3]

It was observed that GH responses to ghrelin were greater in elderly patients than younger subjects, contrary to a previous report.[9] GH responses were significantly attenuated after 3 weeks of administration, reflecting the homologous desensitization exerted by ghrelin on the receptor. Nonetheless, GH responses were preserved, up to approximately 10 ng/mL after 3 weeks of treatment.

Several revisions of the study protocol will be required to achieve optimal efficacy of ghrelin in the clinic. In particular, the duration of ghrelin administration may be critical to induce functional alterations in this patient population, because GH was administered for 18 weeks in the previous study.[10] Exploration of these factors will provide opportunities to obtain a better understanding of ghrelin actions and to determine whether ghrelin can be useful in the treatment of catabolism and aging-related disorders.

Takashi Akamizu, MD
Hiroshi Iwakura, MD
Hiroyuki Ariyasu, MD
Ghrelin Research Project
Translational Research Center
Kyoto University Hospital
Faculty of Medicine
Kyoto University
Kyoto, Japan

Toshinori Murayama, MD
Eriko Sumi, MD
Department of Clinical Innovative Medicine
Translational Research Center
Kyoto University Hospital
Faculty of Medicine
Kyoto University
Kyoto, Japan

Satoshi Teramukai, PhD
Department of Clinical Trial Design and Management
Translational Research Center
Kyoto University Hospital
Faculty of Medicine
Kyoto University
Kyoto, Japan

Koji Goto, MD
Eijiro Ohnishi, MD
Haruhiko Akiyama, MD
Keiichi Kawanabe, MD
Department of Orthopaedic Surgery
Kyoto University Hospital
Faculty of Medicine
Kyoto University
Kyoto, Japan

Manabu Nankaku, MS
Department of Physical Therapy
Kyoto University Hospital
Faculty of Medicine
Kyoto University
Kyoto, Japan

Noriaki Ichihashi, PhD
Tadao Tsuboyama, MD
Department of Physical Therapy
School of Health Science
Faculty of Medicine
Kyoto University
Kyoto, Japan

Ken Tamai, MD
Masako Kataoka, MD
Department of Diagnostic Imaging and Nuclear Medicine
Kyoto University Hospital
Faculty of Medicine
Kyoto University
Kyoto, Japan

Takashi Nakamura, MD
Department of Orthopedic Surgery
Kyoto University Hospital
Faculty of Medicine
Kyoto University
Kyoto, Japan

Kenji Kangawa, PhD
Ghrelin Research Project
Translational Research Center
Kyoto University Hospital
Faculty of Medicine
Kyoto University
Kyoto, Japan
National Cardiovascular Center Research Institute
Osaka, Japan

for the Osteoarthritis—Total Hip Replacement Clinical Study Team

ACKNOWLEDGMENTS

The Osteoarthritis—Total Hip Replacement Clinical Study Team consists of T. Irako, K. Miura, T. Yokota, I. Bando, H. Tada, A. Matsuyama, S. Kojima, T. Wada, Y. Wakatsuki, K. Togashi, A. Shimizu, M. Fukushima, and M. Yokode at Kyoto University Hospital. We would like to thank Dr. Hashida and Dr. Kobayashi for preparing the drugs used in this study.

Conflict of Interest: None of the authors have any financial or any other kind of personal conflicts with this manuscript. This study was partly supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan; the Ministry of Health, Labour, and Welfare of Japan; the Foundation for Growth Science; the Japan Health Foundation; and the Daiwa Securities Health Foundation.

Author Contributions: TA, HI, HA, HA, KK, TN, and KK: study concept and design, and drafting of manuscript. TM and ES: data acquisition and management. ST: statistical analyses. KG and EO: recruitment of participants. NI and TT: study concept and design. MN, KT, and MK: biological determinations and interpretation of the results. All authors contributed to and approved the final version of the manuscript.

Sponsors's Role: None.

REFERENCES

1. Corpas E, Harman SM, Blackman MR. Human growth hormone and human aging. Endocr Rev 1993;14:20–39.
2. Nagaya N, Moriya J, Yasumura Y et al. Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure. Circulation 2004;110:3674–3679.
3. Nagaya N, Itoh T, Murakami S et al. Treatment of cachexia with ghrelin in patients with COPD. Chest 2005;128:1187–1193.
4. Akamizu T, Takaya K, Irako T et al. Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. Eur J Endocrinol 2004;150:447–455.
5. Akamizu T, Iwakura H, Ariyasu H et al. Repeated administration of ghrelin to patients with functional dyspepsia: Its effects on food intake and appetite. Eur J Endocrinol 2008;158:491–498.
6. Podsiadlo D, Richardson S. The Timed "Up & Go": A test of basic functional mobility for frail elderly persons. J Am Geriatr Soc 1991;39:142–148.
7. Takeda H, Kamogawa J, Sakayama K et al. Evaluation of clinical prognosis and activities of daily living using functional independence measure in patients with hip fractures. J Orthop Sci 2006;11:584–591.
8. Alvarez-Castro P, Isidro ML, Garcia-Buela J et al. Marked GH secretion after ghrelin alone or combined with GH-releasing hormone (GHRH) in obese patients. Clin Endocrinol (Oxford) 2004;61:250–255.
9. Broglio F, Benso A, Castiglioni C et al. The endocrine response to ghrelin as a function of gender in humans in young and elderly subjects. J Clin Endocrinol Metab 2003;88:1537–1542.
10. Weissberger AJ, Anastasiadis AD, Sturgess I et al. Recombinant human growth hormone treatment in elderly patients undergoing elective total hip replacement. Clin Endocrinol (Oxford) 2003;58:99–107.

RARE DISEASES IN ELDERLY PERSONS

To the Editor: Primary biliary cirrhosis (PBC) is a chronic, progressive, cholestatic liver disease that usually affects middle-aged women, who are 95% of patients. The onset is usually between the ages of 30 and 65, but the disease has been reported in women as young as 15 and as old as 93. Estimates of prevalence range from 19 to 151 cases per million population, whereas estimates of incidence range from 3.9 to 15 cases per million population per year.[1–6] The incidence of PBC in a well-defined population (Rochester, MN) was detected to be 2.7 per 100,000 person-years, the age- and sex-adjusted prevalence per 100,000 persons was 65.4 for women and 12.1 for men.[7]

A case of PBC in an 86-year-old man with past medical history of ischemic cardiomyopathy, congestive heart failure, peripheral arterial disease, chronic obstructive pulmonary disease, previous cholecystectomy for gall stones, and chronic renal failure is described. The patient was hospitalized in January 2006 with a history of weight gain, fatigue, shortness of breath, and swollen legs. Echocardiography showed a dilated and hypokinetic left ventricle, ejection fraction of 37%, and moderate mitral valve regurgitation. At that time, blood examination of liver function showed a normal serum bilirubin concentration (0.5, normal range 0.2–1.3 mg/dL), slightly high serum aspartate aminotransferase (AST) level (56, normal range 5–48 UI/L), normal alanine aminotransferase (ALT) level

0013-7227/08/$15.00/0
Printed in U.S.A.

Endocrinology 149(10):5172–5176

doi: 10.1210/en.2008-0472

Early Ghrelin Treatment after Myocardial Infarction Prevents an Increase in Cardiac Sympathetic Tone and Reduces Mortality

Daryl O. Schwenke, Takeshi Tokudome, Ichiro Kishimoto, Takeshi Horio, Mikiyasu Shirai, Patricia A. Cragg, and Kenji Kangawa

Department of Physiology (D.O.S., P.A.C.), University of Otago, Dunedin 9054, New Zealand; Department of Biochemistry (T.T., I.K., T.H., K.K.), National Cardiovascular Center Research Institute, Osaka 565-8565, Japan; and Faculty of Health Sciences (M.S.), Hiroshima International University, Hiroshima City, Hiroshima Prefecture 730-0016, Japan

Acute myocardial infarction (MI) initiates an increase in cardiac sympathetic nerve activity (CSNA), which ultimately exacerbates chronic cardiac dysfunction. Ghrelin (Ghr), a GH-releasing peptide, is an effective treatment for improving cardiac function in chronic heart failure. Ghr also suppresses renal sympathetic nerve activity (SNA) and, therefore, may have important therapeutic benefits in the early stages of acute MI: by reducing CSNA. In this study we hypothesized that early Ghr administration may prevent an increase in CSNA in the acute phase after MI. CSNA was continuously recorded in urethane-anaesthetized rats before and for 5 h after acute MI (or sham). MI was induced by ligation of the left anterior descending coronary artery. Rats received an injection of either saline or Ghr (150 μg/kg, sc) 1 min, or 2 h, after the infarct. CSNA remained stable during the 5-h recording duration in sham rats. MI induced a maximal 110% increase in SNA, which was prevented in rats that received Ghr 1 min after infarct. When Ghr was injected 2 h after MI (SNA had increased by ~85%), SNA decreased to pre-MI activity. Importantly, early Ghr administration significantly reduced the high mortality rate associated with MI (61% mortality in untreated MI rats *cf.* ~23% in Ghr-treated MI rats). These results show that early Ghr treatment prevents the increase in CSNA after MI, which may contribute to the improved chances of survival. Whether these early beneficial effects of Ghr also have long-term benefits for improving cardiac function is an area that requires further investigation. (*Endocrinology* 149: 5172–5176, 2008)

MYOCARDIAL infarction (MI) is the most common cause of death in industrialized societies. In most instances, death occurs within the early stage, *i.e.* the first few hours, after MI. This high morbidity has been strongly associated with an adverse and sustained increase in cardiac sympathetic nerve activity (CSNA), which begins within the first hour of the initial infarction (1). Even for those that survive the immediate infarct, the adverse damage to cardiac tissue impairs the functional capacity of the heart, which is further exacerbated by the sustained increase in sympathetic tone, such that the long-term survival prognosis is bleak.

The peptide hormone ghrelin (Ghr), first discovered in 1999 (2), has improved cardiac function in patients suffering from end-stage chronic heart failure (3). Ghr is a GH-releasing peptide, and so the mechanism by which Ghr improves cardiac function has been linked, at least in part, to the anabolic properties of GH. To date, there is a paucity of studies describing the treatment of myocardial ischemia within the first few hours of onset. Indeed, it is this time period when autonomic modulation of cardiac function is enhanced, in which the opportunity to improve outcome by therapeutic intervention is so great.

Some studies have shown that Ghr is able to centrally suppress renal sympathetic nerve activity (SNA) (4, 5). More recently, we have also shown, using heart-rate spectral analyses, that Ghr treatment appears to attenuate cardiac sympathetic tone within the first week after MI (6). However, it is the initial increase in CSNA within the first hours after MI that significantly contributes to ventricular arrhythmia (1, 7) and, consequently, a high mortality (8). Therefore, in this study we hypothesized, and aimed to show, that the early administration of Ghr immediately after MI would be able to prevent, or at least attenuate, the early increase in CSNA, which could potentially improve early survival prognosis.

Materials and Methods

Animals

Experiments were conducted on 41 male Sprague Dawley rats (8 wk old; body weight ~280–340 g). All rats were on a 12-h light, 12-h dark cycle at 25 ± 1 C, and provided with food and water *ad libitum*. All experiments were approved and conducted in accordance with the guidelines stipulated by the Animal Ethics Committee of the University of Otago, New Zealand.

Anesthesia and surgical preparation

Rats were anesthetized with urethane (1.5 g/kg, ip). Adequate anesthesia was confirmed by elimination of the limb withdrawal reflex. Throughout the experiment, body temperature was maintained at 38 C using a rectal thermistor coupled with a thermostatically controlled heating pad. The trachea was cannulated, and the lungs were ventilated

First Published Online July 3, 2008

Abbreviations: ABP, Arterial blood pressure; CSNA, cardiac sympathetic nerve activity; Ghr, ghrelin; GHS-R, GH secretagogue receptor; HR, heart rate; LAD, left anterior descending; MABP, mean arterial blood pressure; MI, myocardial infarction; NTS, nucleus tractus solitarius; SNA, sympathetic nerve activity.

Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

with a Harvard rodent ventilator (model 680; Harvard Apparatus, Holliston, MA). The inspirate gas was enriched with O_2 (~50% O_2), and the ventilator settings were adjusted (tidal volume ~3.5 ml; breathing rate ~80/min) to maintain arterial P_{CO2} normocapnic. The femoral artery and vein were cannulated for measurement of systemic arterial blood pressure (ABP) and fluid administration (saline at 3 ml/h), respectively. The arterial line contained heparinized saline (50 U/ml).

Recording CSNA

The stellate ganglion was exposed through a left thoracotomy between the first and second rib. The cardiac sympathetic nerve was identified as a branch from the stellate ganglion, dissected free of surrounding connective tissue, sectioned, and the proximal section (containing efferent fibers) was placed on a pair of platinum recording electrodes. The signal was filtered (low cutoff 0.1 kHz; high cutoff 1 kHz;) and amplified, and subsequently passed through an amplitude discriminator (model WD-2, Dagan Corp., Minneapolis, MN) for counting nerve discharge frequency (impulse frequency).

Raw SNA, impulse frequency, and ABP were continuously sampled at 4 KHz, 200 Hz, and 400 Hz, respectively, using a PowerLab data-acquisition system (model 8/S; ADInstruments Pty Ltd., Bella Vista New South Wales, Australia). Heart rate (HR) was derived from the arterial systolic peaks. The raw nerve signal was rectified and integrated (1-sec resetting interval) online, and the integrated nerve signal was displayed in real time.

Experimental protocol

A 7.0-Prolene suture (Ethicon, Inc., Johnson & Johnson, Somerville, NJ) was loosely placed around the left anterior descending (LAD) coronary artery, which was located between the appendage of the left atrium and the base of the pulmonary artery. CSNA, and mean ABP (MABP) and HR were continuously recorded before occlusion of the LAD coronary artery, and for 5 consecutive hours after: 1) no manipulation (sham, n = 7); 2) LAD occlusion (MI, n = 13); and 3) MI with an immediate injection of Ghr (150 μg/kg, sc) (MI plus Ghr, n = 13). We also aimed to assess whether Ghr could reduce sympathetic tone after it had already increased after MI (to simulate those patients that receive delayed therapeutic treatment). Therefore, in this study we also tested a group of rats that received Ghr 2 h after MI (MI plus delayed Ghr, n = 8). Ghr was obtained from the Peptide Institute, Inc. (Osaka, Japan).

Measurement of infarct size

At the completion of each experiment, each rat was euthanized, and the heart was excised and sectioned into 2-mm horizontal slices down the vertical plane. The sections were then stained with 2,3,5-triphenyltetrazolium solution (Sigma-Aldrich Corp., St. Louis, MO) and subsequently fixed in 10% formalin for 20 min. Slices were mounted and photographed. Total infarct size was determined by measuring the area of the infarction for each slice, multiplying the area by the slice thickness, and summing the area of all slices. Infarct size was presented as a percentage of the total left ventricular wall.

Statistical analysis

All statistical analyses were conducted using StatView (version 5.01; SAS Institute Inc., Cary, NC). All results are presented as means ± SEM. Two-way ANOVA (repeated measures) was used to test significance for temporal changes in CSNA after LAD occlusion. One-way ANOVA (factorial) was used to test for differences among the groups of rats. Where statistical significance was reached, *post hoc* analyses were incorporated using the paired or unpaired *t* test with the Dunnet's correction for multiple comparisons. The Kaplan-Meier survival analysis was performed to compare survival curves between saline-treated and Ghr-treated rats after LAD occlusion. A *P* value less than or equal to 0.05 was predetermined as the level of significance for all statistical analyses.

Results

Survival

Eight out of 13 MI rats (61% mortality) died within 6 h after MI, compared with 23% of those MI plus Ghr rats (three out of 13 rats; $P = 0.039$; Fig. 1). Of the eight MI rats that died, three died after the fifth hour. Rats treated with Ghr 2 h after infarction had a mortality of 25% (two of eight rats); one death occurring after 5 h recording. Therefore, we were able to collect 5 h CSNA data from seven rats for each group.

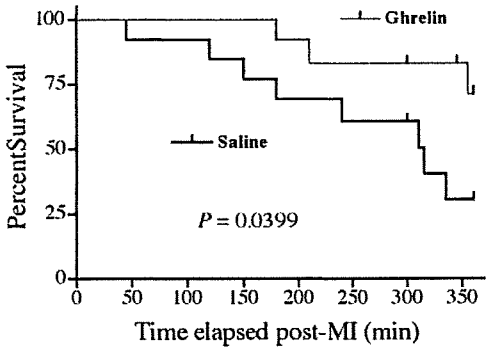

FIG. 1. Kaplan-Meier survival analysis showing a greater mortality rate in untreated MI rats (n = 13) compared with Ghr-treated rats (MI plus Ghr; n = 13) within 6 h after LAD occlusion ($P = 0.0399$).

Arrhythmias

Cardiac arrhythmias were evident within the first few minutes of LAD occlusion for all MI rats (treated with or without Ghr). As illustrated in Fig. 2A, arrhythmic episodes often consisted of numerous ectopic beats occurring in succession, followed by the return of a "normal" cardiac rhythm. In untreated MI rats, the arrhythmic episodes often persisted

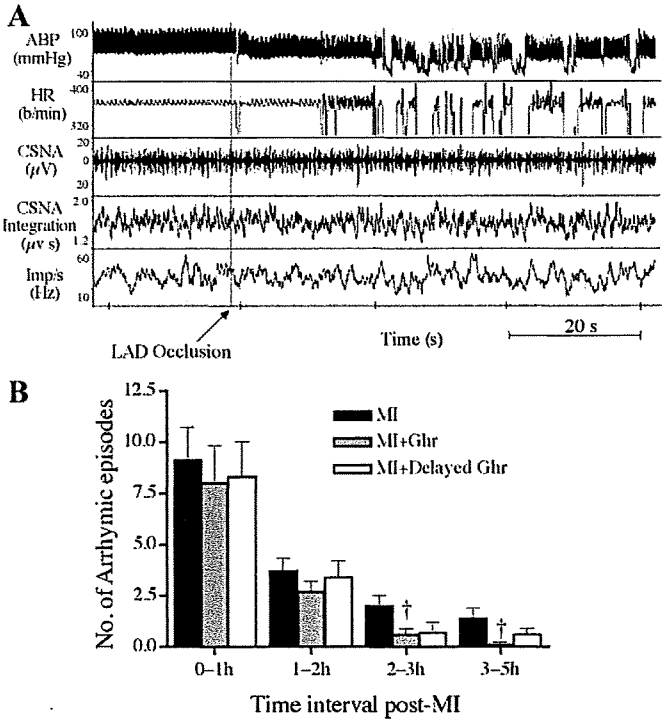

FIG. 2. "Chart" recording showing a severe example of arrhythmias immediately after LAD occlusion (this rat subsequently died of cardiac arrest ~2 h after MI) (A), and the incidence of arrhythmic episodes at each time interval period (*i.e.* 0–5 h) after MI (B). †, Significant difference between MI (n = 7) and MI plus Ghr rats (n = 6) ($P < 0.05$). Imp, Impulse frequency; LAD, left anterior descending artery.

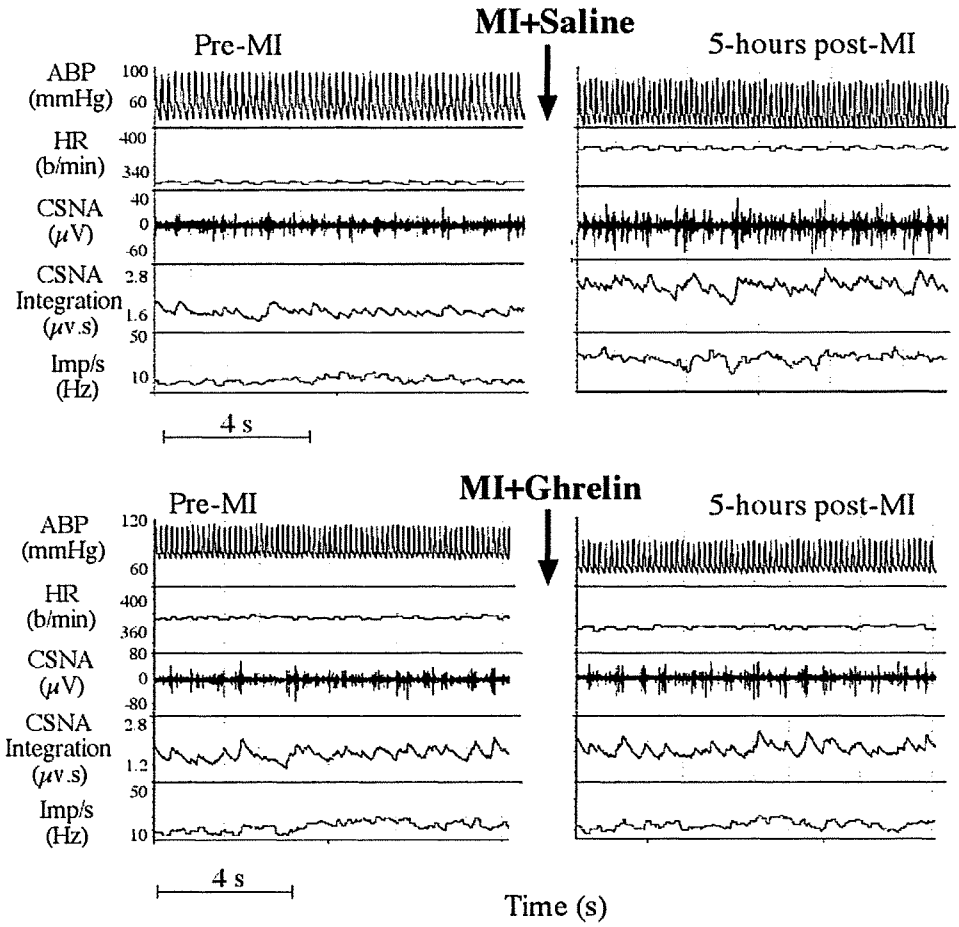

FIG. 3. Chart recordings showing "typical" changes in hemodynamic variables, and CSNA-integrated signal of the raw trace and burst frequency [impulse frequency (Imp) per second] in a MI rat (*top* trace) and MI plus Ghr rat (*bottom* trace) 5 h after MI.

for up to 5 h recording, although the incidence of episodes became less frequent with time (Fig. 2B), and there were less ectopic beats per episode. For all MI rats that died within 5 h after MI (n = 5), the cause of death was due to sudden cardiac arrest after an arrhythmic insult. Of the three rats that died after the fifth hour after MI, one died of sudden cardiac arrest after an arrhythmic insult, whereas critical hypotension (MABP ~30 mm Hg) after the fifth hour after MI preempted the death of the remaining two rats.

The incidence of arrhythmias within the first hour after MI for MI plus Ghr rats was similar to untreated MI rats, although in the ensuing hours, MI plus Ghr rats had fewer arrhythmic insults, significant by the second to third hour after MI (Fig. 2B). Indeed, in all but two of the MI plus Ghr rats, the incidence of arrhythmias had subsided from the third hour after MI. The persistence of arrhythmias in one MI plus Ghr rat ultimately resulted in cardiac arrest and death. The cause of death in the remaining two MI plus Ghr rats that died prematurely was linked to critical hypotension.

CSNA

Typical chart recordings of hemodynamic and CSNA data before and 5 h after either MI or MI plus Ghr are presented in Fig. 3. Baseline cardiovascular and CSNA data are presented in Table 1. In sham rats (*i.e.* control), CSNA remained stable for the 5-h recording period. MI rats elicited a significant increase in CSNA (110 ± 27%), which was completely prevented in those rats that also immediately received Ghr after MI (*i.e.* MI plus Ghr) (Fig. 4). Moreover, when Ghr was administered 2 h after MI (when CSNA had increased by 85 ± 23%), CSNA declined to pre-MI activity by the fifth hour of recording. MABP and HR did not significantly change in

TABLE 1. Baseline (before MI) CSNA (integrated signal and impulse frequency/sec), MABP, and HR of four groups of rats: control (n = 7); MI (n = 7); MI rats immediately treated with Ghr (150 μg/kg, iv) (MI plus Ghr, n = 7); and rats that received Ghr 2 h after the (MI MI plus delayed Ghr, n = 7)

	Control	MI	MI + Ghr	MI + delayed Ghr
Integrated CSNA (μV/sec)	2.88 ± 0.57	1.29 ± 0.47[a]	1.32 ± 0.23[a]	1.94 ± 0.52
CSNA (imp/sec)	29.7 ± 6.5	17.8 ± 5.2[a]	34.1 ± 1.9	26.5 ± 4.6
MABP (mm Hg)	82 ± 4	81 ± 4	88 ± 4	83 ± 3
HR (beats/min)	390 ± 14	386 ± 23	392 ± 23	397 ± 19

Data are presented as mean ± SEM. imp, Impulse frequency.
[a] Significantly different from control rat values ($P < 0.05$).

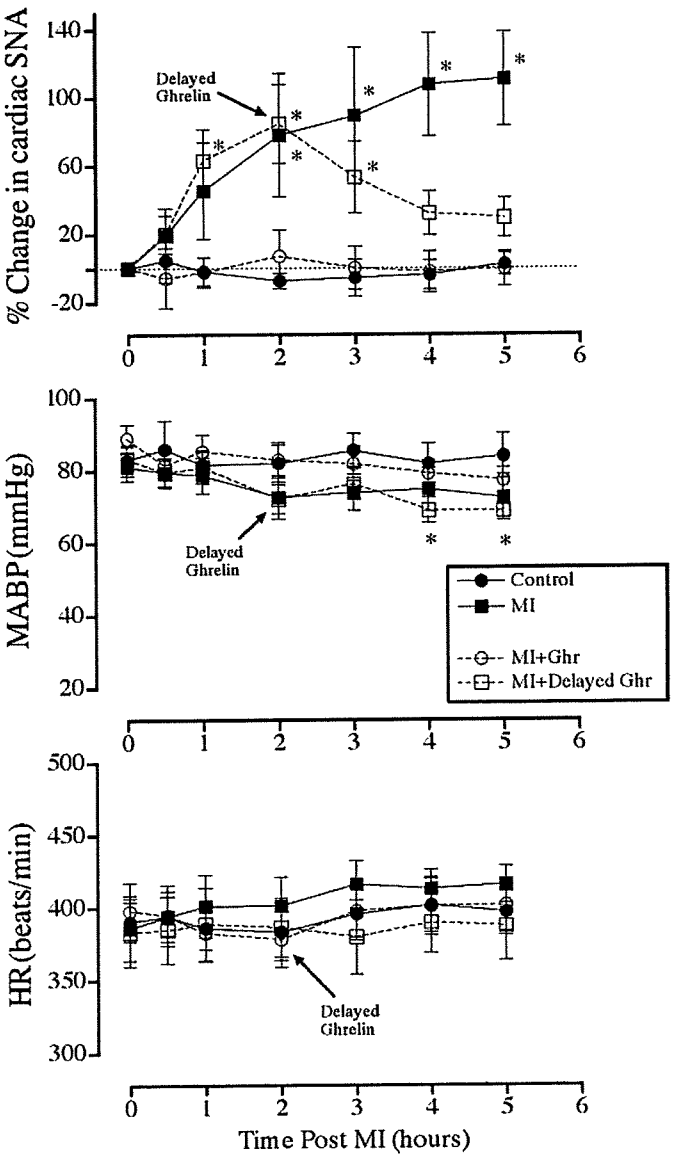

FIG. 4. Transient responses in CSNA (percent increase in CSNA of integrated area of the raw nerve signal), MABP (mm Hg), and HR (beats per minute) in sham rats (n = 7) and three groups of MI rats: untreated (MI; n = 7); Ghr treated immediately after MI (MI plus Ghr; n = 7); and Ghr treated 2 h after MI (MI plus delayed Ghr; n = 7). *, Significantly different from before MI (time "0") ($P < 0.05$).

sham or MI plus Ghr rats. MI rats experienced a mild 12% decrease in MABP (ΔMABP 9 ± 3 mm Hg) and a 15% increase in HR (ΔHR 55 ± 24 beats/min; not significant). The hypotension was more pronounced in MI plus delayed Ghr rats (ΔMABP 14 ± 3 mm Hg), although HR did not increase.

Infarct size

The size of the infarct of the left ventricular wall did not significantly differ among MI (40.0 ± 2.8%), MI plus Ghr (38.3 ± 1.6%), and MI plus delayed Ghr rats (42.4 ± 3.3%).

Discussion

The primary findings of this study highlight the important benefits of early Ghr intervention in preventing the adverse increase in CSNA after acute MI, as well as improving early survival prognosis. Although the therapeutic effects of Ghr have largely been attributed to the release of GH (3, 9), evidence is accumulating that supports a direct cardioprotective effect of Ghr through the central modulation of SNA (4–6).

In this study we reported that MI initiated an increase in CSNA, which is consistent with previous anesthetized animal models (10–12). More recently, Jardine *et al.* (1) described a transient increase in CSNA in conscious sheep: significant by the second hour after MI, which was sustained for at least 7 d. We also observed a rapid and sustained increase in CSNA, although, one limitation of this study is that we could only record CSNA for 5 h in an anesthetized, open-chest, rat model.

The mechanisms for the increase in CSNA after MI remain to be elucidated fully, although altered neural reflexes (*e.g.* from baroreceptors and chemoreceptors), increased levels of hormones (*e.g.* angiotensin II), and changes in central mechanisms that may amplify the responses to these inputs have been implicated (13). Although the increase in CSNA after MI appears to have immediate benefits, providing inotropic support to the heart to maintain cardiac output, this enhanced sympathetic tone is associated with an increased risk of ventricular arrhythmias (1), a leading cause for sudden heart failure and death (8). Indeed, we noted in our study that ventricular arrhythmias often preceded, or even instigated, cardiac failure and sudden death.

Early Ghr treatment after MI prevented the adverse increase in CSNA within the first 5 h of recording, and reduced the incidence of arrhythmias. Further research is required to reveal the exact mechanism(s) by which Ghr suppresses CSNA after MI, although studies have shown that the receptor for Ghr [GH secretagogue receptor (GHS-R)] is located in the main cardiovascular control centers in neurons of the nucleus tractus solitarius (NTS) (5), and that the central administration of Ghr directly attenuates renal SNA (4, 5).

We have also demonstrated the existence of GHS-R in the infarcted myocardium (6). Costaining with acetylcholine esterase suggests that the GHS-R is localized in the vagal nerve terminals in the heart, which send afferent projections to the NTS, so that Ghr may act to enhance vagal tone and thereby decrease SNA.

An enhanced vagal tone has also been reported to centrally augment baroreflex control of CSNA (14). Yet, our results imply that Ghr may have reduced baroreflex sensitivity, at least in MI plus delayed Ghr rats, because we observed that CSNA was not elevated above baseline (5 h after MI), despite a 17% decrease in MABP. Matsumura *et al.* (4) similarly reported that Ghr (iv) reduced MABP without changing renal SNA, but further showed that centrally administered Ghr did decrease SNA, HR, and MABP, and enhance baroreflex sensitivity. They reasoned that iv Ghr has a direct peripheral vasodilatory effect as well as a direct central sympathoinhibitory effect.

Therefore, in this study it is possible that peripheral Ghr administration prevented the increase in CSNA in the acute phase after MI, at least in part, by suppressing SNA directly at the level of the NTS (which would also prevent a baroreflex increase in SNA in response to the vasodilatory effects of Ghr), and indirectly through activation of cardiac vagal afferent nerves. Of course Ghr, which can cross the blood-brain barrier (15), has diverse effects both peripherally and within the central nervous system, and, thus, it is likely that Ghr

could modulate CSNA at sites other than the NTS. This is an area of research that warrants further investigation.

In this study, untreated MI rats had a survival rate of only 39%, compared with a 77% survival rate for MI rats treated with Ghr (MI plus Ghr). The observed difference between the two groups of MI rats is likely linked to the fact that Ghr prevented an increase in CSNA in treated rats. Indeed, it may be reasonable to suggest that this benefit of early Ghr treatment contributes, at least in part, to the improved survival prognosis of MI plus Ghr rats (survival of 77%), especially given that arrhythmia-related deaths were less prevalent in MI plus Ghr rats compared with MI rats.

In reality, it may not be possible for all MI patients to receive immediate treatment (*i.e.* within minutes), and because neurohumoral changes often precede the development of clinically recognizable symptoms of acute heart failure (13), treatment may be delayed by several hours. Yet, it is within this time interval that CSNA has already begun to increase (1). In this study we were able to demonstrate that a 2-h delayed treatment of Ghr was able to reduce the MI-induced increase in CSNA. Furthermore, the survival rate of these rats (75%) was improved compared with untreated rats.

Collectively, the results of this study appear to indicate that early Ghr treatment, at least within the first hours after MI, may improve early survival prognosis, providing clinicians with critical time for implementing supplementary therapeutic measures. Furthermore, this benefit of Ghr is likely associated with the prevention or attenuation of an enhanced cardiac sympathetic drive. Whether these early beneficial effects of Ghr also have long-term benefits for improving cardiac function, and ultimately long-term survival, is an important area that urgently requires further investigation.

Acknowledgments

Received April 3, 2008. Accepted June 23, 2008.

Address all correspondence and requests for reprints to: Ichiro Kishimoto, Department of Biochemistry, National Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan. E-mail: kishimot@ri.ncvc.go.jp.

This study was supported by the Department of Physiology, University of Otago, New Zealand, and, in part, by the research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan, the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation of Japan, and the Takeda Scientific Foundation.

Disclosure Summary: The authors have nothing to disclose. There are no conflicts of interest concerning the material in this study.

References

1. **Jardine DL, Charles CJ, Ashton RK, Bennett SI, Whitehead M, Frampton CM, Nicholls MG** 2005 Increased cardiac sympathetic nerve activity following acute myocardial infarction in a sheep model. J Physiol 565(Pt 1):325–333
2. **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
3. **Nagaya N, Uematsu M, Kojima M, Ikeda Y, Yoshihara F, Shimizu W, Hosoda H, Hirota Y, Ishida H, Mori H, Kangawa K** 2001 Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. Circulation 104:1430–1435
4. **Matsumura K, Tsuchihashi T, Fujii K, Abe I, Iida M** 2002 Central ghrelin modulates sympathetic activity in conscious rabbits. Hypertension 40:694–699
5. **Lin Y, Matsumura K, Fukuhara M, Kagiyama S, Fujii K, Iida M** 2004 Ghrelin acts at the nucleus of the solitary tract to decrease arterial pressure in rats. Hypertension 43:977–982
6. **Soeki T, Kishimoto I, Schwenke DO, Tokudome T, Horio T, Yoshida M, Hosoda H, Kangawa K** 2008 Ghrelin suppresses cardiac sympathetic activity and prevents early left ventricular remodeling in rats with myocardial infarction. Am J Physiol Heart Circ Physiol 294:H426–H432
7. **Keating MT, Sanguinetti MC** 2001 Molecular and cellular mechanisms of cardiac arrhythmias. Cell 104:569–580
8. **Kaye DM, Lefkovits. J, Jennings GL, Bergin P, Broughton A, Esler MD** 1995 Adverse consequences of high sympathetic nervous activity in the failing human heart. J Am Coll Cardiol 26:1257–1263
9. **Nagaya N, Kangawa K** 2003 Ghrelin improves left ventricular dysfunction and cardiac cachexia in heart failure. Curr Opin Pharmacol 3:146–151
10. **Brown AM, Malliani A** 1971 Spinal sympathetic reflexes initiated by coronary receptors. J Physiol 212:685–705
11. **Malliani A, Schwartz PJ, Zanchetti A** 1969 A sympathetic reflex elicited by experimental coronary occlusion. Am J Physiol 217:703–709
12. **Ninomiya I, Matsukawa K, Honda T, Nishiura N, Shirai M** 1986 Cardiac sympathetic nerve activity and heart rate during coronary occlusion in awake cats. Am J Physiol 251(3 Pt 2):H528–H537
13. **Watson AM, Hood SG, May CN** 2006 Mechanisms of sympathetic activation in heart failure. Clin Exp Pharmacol Physiol 33:1269–1274
14. **Liu JL, Kulakofsky J, Zucker IH** 2002 Exercise training enhances baroreflex control of heart rate by a vagal mechanism in rabbits with heart failure. J Appl Physiol 92:2403–2408
15. **Pulman KJ, Fry WM, Cottrell GT, Ferguson AV** 2006 The subfornical organ: a central target for circulating feeding signals. J Neurosci 26:2022–2030

Endocrinology **is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

Pulmonary Pharmacology & Therapeutics 21 (2008) 774–779

Contents lists available at ScienceDirect

Pulmonary Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/ypupt

Ghrelin treatment suppresses neutrophil-dominant inflammation in airways of patients with chronic respiratory infection

Tsuyoshi Kodama [a], Jun-Ichi Ashitani [a,*], Nobuhiro Matsumoto [a], Kenji Kangawa [b], Masamitsu Nakazato [a]

[a] Third Department of Internal Medicine, Miyazaki University School of Medicine, Kihara 5200, Miyazaki 889-1692, Japan
[b] National Cardiovascular Center Research Institute, Osaka, Japan

ARTICLE INFO

Article history:
Received 29 January 2008
Received in revised form
2 May 2008
Accepted 12 May 2008

Keywords:
Ghrelin
Respiratory tract infection
Neutrophil
Cachectic state
IGF-1
Translational research

ABSTRACT

Background: Persistent neutrophil influx into the airways is a characteristic of chronic respiratory infection and contributes to the deterioration of pulmonary function. Ghrelin is a novel growth hormone (GH)-releasing peptide with potential anti-inflammatory activities. The present study investigated whether or not ghrelin can reduce neutrophil-dominant inflammation in airways of patients with chronic respiratory infection.

Populations and methods: Synthesized ghrelin was administered intravenously for 3 weeks to 7 cachectic patients with chronic respiratory infection to confirm ghrelin's effects on airway inflammation and nutrition state. Neutrophils, neutrophil products and inflammatory cytokines in sputum were used as markers of airway inflammation. Changes in serum protein levels were also evaluated along with plasma catecholamine levels. Exercise tolerance was assessed by measuring 6-min walking distance before and after 3 weeks of ghrelin treatment.

Results: Three-week ghrelin administration decreased neutrophil density and inflammatory cytokine levels in sputum, reduced plasma norepinephrine level, and increased body weight, serum protein level, and 6-min walking distance.

Conclusions: Ghrelin administration suppressed airway inflammation by decreasing neutrophil accumulation in lungs and increased body weight. These findings may contribute to the development of supportive therapies for patients with refractory chronic respiratory infection.

1. Introduction

Chronic respiratory infection is characterized by neutrophil-dominant inflammation in the airways of patients with pulmonary diseases such as bronchiectasis, cystic fibrosis and diffuse panbronchiolitis (DPB), leading to an end-stage cachectic state when multidrug-resistant pathogens colonize and repeatedly cause infections [1–3]. Neutrophils in the lungs continue to try to sterilize the airways of exogenous pathogens, but host cells are injured by excess proteases such as elastase and myeloperoxidase released from neutrophils [4]. The cytotoxity of accumulated neutrophils to bronchial and alveolar epithelial cells leads to deterioration of pulmonary function [5–8], resulting in excess energy expenditure and weight loss in patients [9]. Persistent colonization of the lower airways with bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which are often resistant to multiple antibiotics, causes further neutrophil influx in the airways in patients with chronic respiratory infection [10]. Since damage by neutrophils provides the environment in which such bacteria readily proliferate, suppression of excess neutrophilic influx may represent a suitable therapeutic target in refractory chronic respiratory infections.

Ghrelin is a novel growth hormone (GH)-releasing peptide that was isolated from the stomach and has been identified as an endogenous ligand for the GH secretagogue receptor [11]. Ghrelin induces a positive energy balance and weight gain by stimulating food intake through GH-independent mechanisms. Clinical trials using synthesized ghrelin for patients with cachectic diseases such as chronic obstructive pulmonary disease, anorexia nervosa and chronic heart failure have shown efficacy in appetite stimulation [12–14]. Besides improving appetite, ghrelin reportedly acts as an anti-inflammatory factor that protects against endotoxin shock by inhibiting expression of proinflammatory cytokines by activated monocytes and endothelial cells [15]. In an animal model of colitis, ghrelin ameliorated clinical and histopathological severity, resulting in increased survival rates [16]. Ghrelin's anti-inflammatory functions may be advantageous for airway inflammation in addition to increasing appetite in cachectic patients with chronic respiratory infections. However, it remains unclear whether or not

* Corresponding author. Tel.: +81 985 85 2965.
E-mail address: jashi2@fc.miyazaki-u.ac.jp (J.-I. Ashitani).

1094-5539/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pupt.2008.05.001

ghrelin can decrease neutrophils in sputum or induce clinical benefits.

Based on ghrelin's potential for anti-inflammatory actions, we investigated its therapeutic effect on neutrophil-dominant inflammation in the airways of cachectic patients with chronic respiratory infection.

2. Methods

2.1. Patients

We recruited 7 cachectic patients (3 women, 4 men) between 62- and 80-year-olds with chronic respiratory infections. At enrolment, the following inclusion criteria were applied: (i) persistent productive cough with purulent sputum for >6 months and (ii) isolation of multidrug-resistant pathogens from sputum. The following exclusion criteria were adopted: (i) patients treated with steroids, immunosuppressants or antibiotics other than macrolides prescribed within 3 months prior to the study and (ii) patients with pneumonia, cancer or diabetes mellitus. Cachectic patients were defined as those with documented nonedematous and nonintentional weight loss of >7.5% of previous normal weight over a period of ⩾6 months and body mass index (BMI) <21 at entry. All patients provided written informed consent, and the Research Ethics Committee of Miyazaki University approved all study protocols in advance.

2.2. Preparation of human ghrelin

Synthetic human ghrelin (National Cardiovascular Center Research Institute, Osaka, Japan) was dissolved in water containing 4% D-mannitol. This solution was sterilized using a 0.22-μm Millex filter (Millipore, Bedford, MA). The sterilized ghrelin solution was stored in 2-mL vials, each containing 120 μg of ghrelin. All vials were stored at −30 °C until administration.

2.3. Study protocol

Human ghrelin (2 μg/kg, 20 mL solution) was intravenously administered to the patients for 30 min at a constant rate as shown in a previous report [12,17] before breakfast and dinner for 3 weeks continuously. The body weight, height and dietary intake of each patient were measured at baseline and after the 3-week treatment. Dual radiography absorptiometry, arterial blood gas analysis and blood sampling were also performed before and after ghrelin therapy.

2.4. Dietary intake

Food intake for 3 consecutive days was measured by staff nurses before ghrelin administration and during the last week of ghrelin therapy. Three-day food intake was assessed by a calorie count based on a 10-point scale (0 = no intake to 10 = full intake, 1800 kcal) and averaged.

3. Sputum processing

During the study, sputum samples were collected from patients to evaluate changes in volume as assessed by weight. For cell counts and classification, the first sputum resulting from deep cough after gargling of the mouth with water was collected after waking up in the morning as shown in a previous report [18]. The whole sputum was treated with 0.1% DTT phosphate-buffered solution at a maximum volume ratio of 1:1 [19]. The mixture was then vortexed, and rocked for 15 min at 37 °C. The clear cell suspension was filtered through a 40-μm nylon gauze (LMS Co., Tokyo, Japan) to remove debris and mucus. In this technique, samples should be processed as early as possible within 2 h. Total cell count was performed using a hemocytometer (Neubauer chamber). The percentage of viable cells was evaluated by trypan blue exclusion, in which dead cells were stained blue. The samples were centrifuged at 500g for 1 min to prepare a cell-free supernatant and a cell pellet. The supernatant was decanted, aliquoted and stored at −40 °C for assays. The pellet was resuspended in phosphate buffer solution to prepare cytospin slides for measuring total cell counts and neutrophil ratio. Densities of interleukin (IL)-8, tumor necrosis factor (TNF)-α, polymorphonuclear neutrophil (PMN) elastase and myeloperoxidase (MPO) were measured from the preserved supernatant. To correct for variable salivary contamination, the results of differential leukocyte counts were expressed as percentages of nucleated cells excluding squamous cells, as performed by an observer blinded to the subject's clinical characteristics. The quantity of sputum, the number of neutrophils, and the densities of IL-8, TNF-α, PMN elastase and MPO in sputum samples were assessed at baseline and during the last week of ghrelin therapy.

3.1. Body composition

Height was determined to the nearest 0.5 cm, with subjects standing barefoot. Weight was assessed using a beam scale to the nearest 0.1 kg, with subjects standing barefoot in light clothing. Dual radiography absorptiometry (EXP-5000; Lunar Corporation, Madison, WI) was performed to assess the lean body, fat and bone mineral mass of patients.

3.2. Blood sampling and assay

Blood sampling was performed at baseline and during the week after the end of ghrelin therapy to measure levels of total protein, albumin, glucose, total cholesterol, triglyceride, rapid turnover proteins and C-reactive protein (CRP). Blood samples were taken from an antecubital vein after 30-min bed rest in the morning following an overnight fast. Serum GH and insulin-like growth factor (IGF)-1 were measured by immunoradiometric assay (Ab Bead HGH Eiken; Eiken Chemical, Tokyo, Japan and Somatomedin CII Bayer; Bayer Medical, Tokyo, Japan). Plasma epinephrine and norepinephrine were measured by high-performance liquid chromatography (HCL8030; Tosoh, Tokyo, Japan). Commercially available enzyme-linked immunosorbent assay kits were used to determine levels of IL-8 (Central Laboratory of the Netherlands Red Cross, Amsterdam, Netherlands), TNF-α (Invitrogen Corp., Carlsbad CA), PMN elastase (Bender MedSystems GmbH, Vienna, Austria) and MPO (Assay Designs, Ann Arbor, MI) in sputum and soluble intercellular adhesion molecule (ICAM)-type 1 (Invitogen Corp., Carlsbad CA) in serum.

3.3. Six-minute walking test and alveolar-arterial oxygen gradient

The 6-min walking test was performed in all patients according to a standardized protocol [20]. Patients were instructed to walk at their own pace, but to cover as much ground as possible in 6 min. All patients tolerated the test without any adverse effects. Arterial blood gas analysis was performed in room air after bed rest for 30 min following the test. The alveolar-arterial oxygen gradient ($P[A\text{-}a]O_2$) was estimated using the following formula: $P(A\text{-}a)O_2 = 150 - PaO_2 - 1.25\,PaCO_2$

3.4. Monitoring adverse effects of ghrelin treatment

To monitor the adverse effects of the administration of synthesized ghrelin, vital signs including body temperature, blood pressure and heart rate were monitored by staff nurses 3 times a day. Chest radiography and bacterial species isolated from sputum were confirmed once a week to exclude exacerbation of respiratory infection.

3.5. Statistical analysis

Data are expressed as mean ± standard deviation (SD). Changes in parameters during ghrelin treatment were analyzed using the Wilcoxon signed rank test. Values of $p<0.05$ denoted the presence of a statistically significant difference.

4. Results

4.1. Patient characteristics

The median subject age was 72 years (range, 62–80 years). The underlying pathology was bronchiectasis in 5 patients and chronic obstructive pulmonary disease in 2 patients. All patients had been treated for chronic respiratory infections for 5–8 years before entry. Five patients were nonsmokers and the other 2 were ex-smokers. All subjects had been administered ⩾3 kinds of antibiotics more than 3 months before study entry. The potential pathogenic microorganisms isolated from sputum samples taken from the 7 subjects at study entry were as follows: multidrug-resistant *P. aeruginosa* in 6 patients; methicillin-resistant *S. aureus* in 3 patients; and *Haemophilus influenzae* in 1 patient. More than 2 pathogens were present in the sputa of 3 of 7 subjects.

Table 1
Changes in food intake and body composition

	Before treatment	After treatment	
Food intake	7.1±2.2	8.9±1.9	$p<0.005$
Body weight (kg)	39.4±9.7	41.8±9.9	$p<0.005$
Body height (m)	1.58±0.09	1.58±0.09	NS
BMI	15.8±2.6	16.8±2.7	$p<0.005$
Lean body mass (kg)	32.4±7.1	34.5±11.0	NS
Fat mass (kg)	5.4±3.2	5.7±3.5	NS
Bone mineral mass (kg)	1.7±0.5	1.6±0.5	NS
Body fat rate (%)	13.2±6.0	13.3±6.3	NS

4.2. Effect of Ghrelin on food intake and body composition

Semiquantitative analysis demonstrated that treatment with ghrelin increased food intake in patients with chronic respiratory infections. After the 3-week administration, increases were seen in body weight (39.4±9.7 to 41.8±9.9 kg; $p<0.005$) and BMI (15.8±2.6 to 16.8±2.7; $p<0.005$) (Table 1).

4.3. Effect of Ghrelin on neutrophils and inflammatory cytokines in sputum and inflammatory markers in blood of patients with chronic respiratory infections

Administration of synthesized ghrelin for 3 weeks decreased neutrophils in sputum and volume in all subjects. Neutrophil rates decreased except for 1 case in which the total neutrophil count was decreased (Fig. 1). Levels of IL-8, TNF-α and MPO in sputum were lower after ghrelin therapy than before (Fig. 2). PMN elastase levels in sputum tended to be lower after ghrelin therapy than at entry, although no significant difference was identified. Ghrelin significantly decreased serum CRP (1.67±1.20 to 1.10±0.87 mg/dL; $p<0.01$) and soluble ICAM-1 levels (521±230 to 371±287 g/dL; $p<0.05$) (Table 2).

4.4. Effect of Ghrelin on nutrition status

Significant increases were seen in the levels of serum total protein (6.9±0.6 to 7.4±0.8 g/dL; $p<0.02$), albumin (3.7±0.3 to 3.9±0.4 g/dL; $p<0.05$) and rapid turnover proteins such as prealbumin (16.9±8.5 to 18.9±9.6 mg/dL; $p<0.05$), transferrin (208±36 to 238±47 mg/dL; $p<0.01$) and retinol-binding protein (1.67±0.64 to 1.96±0.81 mg/dL; $p<0.05$) (Table 3). Ghrelin therapy did not alter fasting glucose, total cholesterol or triglyceride levels.

4.5. Effect of Ghrelin on catecholamine and hormone levels

Plasma norepinephrine levels in patients with chronic respiratory infections were above normal, as determined based on pooled data from 7 age-matched healthy subjects (721±380 to 165±9 pg/mL; $p<0.05$). Ghrelin therapy decreased plasma

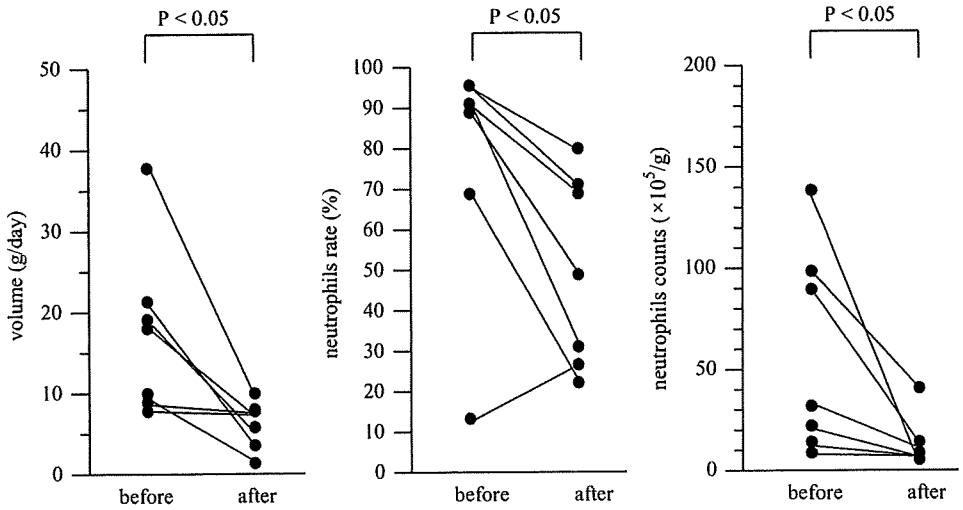

Fig. 1. Changes in sputum volume (A), neutrophil rate (B) and neutrophil count (C) in sputum before and after ghrelin therapy.

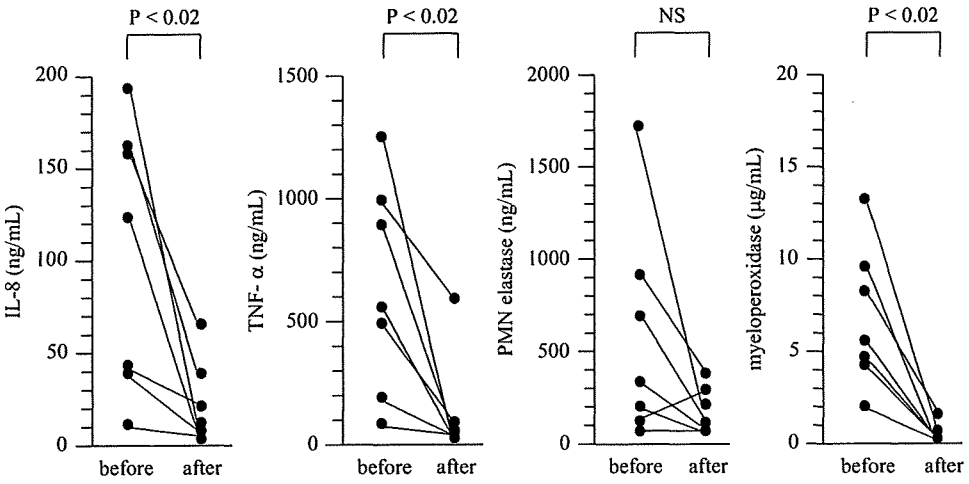

Fig. 2. Changes in levels of IL-8 (A), TNF-α (B), polymorphonuclear (PMN) elastase (C) and myeloperoxidase (D) in sputum before and after ghrelin therapy.

Table 2
Changes in inflammatory markers in blood

	Before treatment	After treatment	
CRP (mg/dL)	1.67±1.20	1.10±0.87	$p<0.01$
WBC (μL)	6686±1170	6386±1139	NS
Neutrophil (μL)	4083±1292	3758±796	NS
Lymphocyte (μL)	1919±518	1820±616	NS
SICAM-1 (ng/mL)	521±230	371±287	$p<0.05$

CRP = C-reactive protein, WBC = white blood cell count, sICAM-1 = soluble intercellular adhesion molecule-type 1.

Table 3
Changes in nutrition parameters

	Before treatment	After treatment	
Total protein (g/dL)	6.9±0.6	7.4±0.8	$p<0.02$
Albumin (g/dL)	3.7±0.3	3.9±0.4	$p<0.05$
Prealbumin (mg/dL)	16.9±8.5	18.9±9.6	$p<0.05$
Transferrin (mg/dL)	208±36	238±47	$p<0.01$
Retinol-binding protein (mg/dL)	1.67±0.64	1.96±0.81	$p<0.05$
Fasting glucose (mg/dL)	91±4	89±5	NS
Total-cholesterol (mg/dL)	164±20	173±27	NS
Triglyceride (mg/dL)	91±26	99±28	NS

Table 4
Changes in hormone and catecholamine levels

	Before treatment	After treatment	
GH (ng/mL)	0.8±0.5	2.0±1.5	NS
IGF-1 (ng/mL)	103±55	117±63	$p<0.05$
Leptin (ng/mL)	2.22±2.14	2.05±1.78	NS
Catecholamine			
Adrenaline (pg/mL)	53±27	38±12	NS
Noradrenaline (pg/mL)	721±380	487±197	$p<0.05$
Dopamine (pg/mL)	15±7	16±8	NS

GH = growth hormone.

Table 5
Changes in excise tolerance and oxygen capacity

	Before treatment	After treatment	
6 min-walk distance (m)	294±124	348±101	$p<0.05$
PaO_2 (Torr)	69.8±9.0	73.5±8.3	NS
$PaCO_2$ (Torr)	44.3±3.0	45.0±3.0	NS
PH	7.43±0.01	7.42±0.02	NS
$P(A\text{-}a)O_2$	24.8±5.3	20.2±7.5	$p<0.05$

$P(A\text{-}a)O_2$ = alveolar-arterial gradient in oxygen.

norepinephrine levels (721±380 to 487±197 pg/mL; $p<0.05$) (Table 4). Ghrelin increased serum IGF-1 levels (103±55 to 117±63 pg/mL; $p<0.05$). GH levels tended to be higher after ghrelin therapy than at entry, although no significant difference was identified.

4.6. Effect of Ghrelin on exercise tolerance and oxygen capacity

Treatment with ghrelin significantly prolonged 6-min walking distance (294±124 to 348±101 m; $p<0.05$) (Table 5) and decreased $P(A\text{-}a)O_2$ (24.8±5.3 to 20.2±7,5; $p<0.05$). Ghrelin therapy did not alter blood pH levels.

4.7. Adverse effects of ghrelin administration

Previous clinical trials with synthesized ghrelin caused subjects to feel warm and sleepy [14], although no such symptoms were reported in our studies. Bacterial species isolated from sputum did not change except for 1 patient who showed clearance of *S. aureus* from sputum after ghrelin therapy. During the study, there were no cases of pneumonia or exacerbation of infections requiring further use of antibiotics. No adverse effects such as diarrhea, eruption or liver dysfunction were seen in any subjects.

5. Discussion

The present study showed the capacity of ghrelin in suppressing neutrophil accumulation into the airways of patients with chronic respiratory infections. Levels of IL-8, TNF-α and MPO in addition to neutrophil density in sputum decreased in response to ghrelin administration. IL-8 is a neutrophil chemoattractant secreted from cultured bronchial epithelial cells following stimulation by TNF-α. TNF-α has been shown to play an important

role in neutrophil recruitment into the airways [21]. Neutrophils activate other inflammatory cells and epithelial cells to induce the release of proinflammatory cytokines [22]. Neutrophils and inflammatory cytokines are involved in lung damage while acting closely with each other when neutrophil influx is persistent. Neutrophil products such as PMN elastase and MPO induced mucus hypersecretion, impairment of ciliary function and damage to epithelial tissues, leading to protein lavage into airways through the epithelium [23–26]. Ghrelin therapy decreased sputum volume, which may be induced partly by a decrease in neutrophil products in the airways. Ghrelin reduced the levels of IL-8, TNF-α and neutrophil products as well as neutrophil density in sputum, which confirmed that the clinical application of ghrelin was beneficial to the treatment of neutrophilic airway inflammation.

Bacterial profiles in sputum showed no significant changes after ghrelin therapy, although quantitative sputum culture was not performed. DPB, a chronic lower respiratory tract infection, has become treatable with the advent of low-dose, long-term macrolide treatment [27]. Macrolide therapy has little impact on the bacterial flora in sputum, but is effective for DPB even in patients already colonized by *P. aeruginosa*, suggesting the possibility that decreases in sputum neutrophils and volume may improve clinical conditions without the clearance of pathogens from the airways in chronic respiratory infections.

Previous studies have suggested the following potential mechanisms for the effects of ghrelin on the suppression of neutrophil accumulation in the airways. First, detection of ghrelin and its receptor in immune cells indicates that the peptide plays a role in immune function. Second, ghrelin decreased TNF-α-induced adhesion molecule expression on vascular endothelial cells *in vitro* [15]. In our study, ghrelin therapy decreased the serum levels of soluble ICAM-1, the adhesion molecule on vascular endothelial cells for neutrophils, thus supporting the results of a previous *in vitro* study [15]. Finally, ghrelin suppresses the biosynthesis of TNF-α in *T*-cells and mononuclear cells [28]. Taken together, these findings suggest that the effects of ghrelin shown in the present study may exert therapeutic benefits through the control of immune cells and adhesion molecule expression for neutrophils.

Ghrelin has been shown to increase appetite and body weight in cachectic patients. Chronic infections induce weight loss, which may be followed by a decreased exercise tolerance. Malnutrition, a risk factor for pneumonia, is difficult to alleviate in cachectic patients with chronic respiratory diseases [29–31]. Ghrelin administration increased levels of serum protein including short turnover proteins, suggesting ghrelin induces an anabolic state in patients. Pulmonary cachexia is accompanied by increased levels of circulating inflammatory cytokines, which contribute to muscle atrophy [32]. Our study suggests that ghrelin's anabolic effect and anti-inflammatory action may contribute to the improvement of 6-min walking distance. Ghrelin therapy did not increase serum GH levels in the samples collected in early morning before breakfast. Single administration of ghrelin has rapidly increased serum GH levels, which returned to basal levels within 3 h [12]. As the blood samplings were done before ghrelin administration, we might not be able to confirm an increase in serum GH level.

The present study showed that ghrelin treatment was beneficial for the improvement of alveolar-arterial oxygen gradient in patients with chronic respiratory infection. Airway inflammation with secretion induces microatelectasis and edema, which are associated with ventilation–perfusion inequality and a decrease in diffusion capacity, respectively. Ghrelin may improve gas change by decreasing sputum and suppressing airway inflammation in the patients. The limitations of this trial relate to the small sample size and a lack of a placebo group. To determine the effects of ghrelin on the suppression of airway inflammation, double-blinded randomized trials with a large study population are required.

Ghrelin acts directly on the central nervous system to decrease sympathetic nervous activity [33]. Plasma norepinephrine levels, which were higher than those in normal subjects at study entry, decreased after ghrelin therapy. As increases in norepinephrine induce lipolysis in adipose tissue [34] and a cachectic state [35], a decrease in norepinephrine levels may attenuate the exaggerated energy expenditure in patients with chronic respiratory infections.

In conclusion, our preliminary trial suggests that repeated ghrelin administration decreases sputum neutrophils and volume, and increases body weight and serum protein levels. Administration of ghrelin may represent a new therapeutic approach to refractory chronic respiratory infections.

Acknowledgments

We would like to thank Ms. S. Tajiri for her excellent technical assistance. This study was supported in part by the Ministry of Education, Culture, Sports, Science and Technology of Japan; by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO) of Japan and by the Takeda Scientific Foundation.

References

[1] Murray TS, Egan M, Kazmierczak BI. Pseudomonas aeruginosa chronic colonization in cystic fibrosis patients. Curr Opin Pediatr 2007;19:83–8.
[2] Ichikawa Y, Ninomiya H, Koga H, Tanaka M, Kinoshita M, Tokunaga N, et al. Erythromycin reduces neutrophils and neutrophil-derived elastolytic-like activity in the lower respiratory tract of bronchiolitis patients. Am Rev Respir Dis 1992;146:196–203.
[3] Kadota J, Sakito O, Kohno S, Sawa H, Mukae H, Oda H, et al. A mechanism of erythromycin treatment in patients with diffuse panbronchiolitis. Am Rev Respir Dis 1993;147:153–9.
[4] Gadek JE. Adverse effects of neutrophils on the lung. Am J Med 1992;92:27S–31S.
[5] Konstan MW, Berger M. Current understanding of the inflammatory process in cystic fibrosis: onset and etiology. Pediatr Pulmonol 1997;24:137–42.
[6] Stockley RA. Proteolytic enzymes, their inhibitors and lung diseases. Clin Sci 1983;64:119–26.
[7] Deneuville E, Perrot-Minot C, Pennaforte F, Roussey M, Zahm JM, Clavel C, et al. Revisited physicochemical and transport properties of respiratory mucus in genotyped cystic fibrosis patients. Am J Respir Crit Care Med 1997;156:166–72.
[8] Martin TR, Raghu G, Maunder RJ, Springmeyer SC. The effects of chronic bronchitis and chronic air-flow obstruction on lung cell populations recovered by bronchoalveolar lavage. Am Rev Respir Dis 1985;132:254–60.
[9] Sridhar MK, Carter R, Lean ME, Banham SW. Resting energy expenditure and nutritional state of patients with increased oxygen cost of breathing due to emphysema, scoliosis and thoracoplasty. Thorax 1994;49:781–5.
[10] Hill AT, Campbell EJ, Hill SL, Bayley DL, Stockley RA. Association between airway bacterial load and markers of airway inflammation in patients with stable chronic bronchitis. Am J Med 2000;109:288–95.
[11] Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999;402:656–60.
[12] Nagaya N, Itoh T, Murakami S, Oya H, Uematsu M, Miyatake K, et al. Treatment of cachexia with ghrelin in patients with COPD. Chest 2005;128:1187–93.
[13] Broglio F, Gianotti L, Destefanis S, Fassino S, Abbate Daga G, Mondelli V, et al. The endocrine response to acute ghrelin administration is blunted in patients with anorexia nervosa, a ghrelin hypersecretory state. Clin Endocrinol 2004;60:592–9.
[14] Nagaya N, Uematsu M, Kojima M, Ikeda Y, Yoshihara F, Shimizu W, et al. Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. Circulation 2001;104:1430–5.
[15] Li WG, Gavrila D, Liu X, Wang L, Gunnlaugsson S, Stoll LL, et al. Ghrelin inhibits proinflammatory responses and nuclear factor-kappa B activation in human endothelial cells. Circulation 2004;109:2221–6.
[16] Gonzalez-Rey E, Chorny A, Delgado M. Therapeutic action of ghrelin in a mouse model of colitis. Gastroenterology 2006;130:1707–20.

[17] Nagaya N, Miyatake K, Uematsu M, Oya H, Shimizu W, Hosoda H, et al. Hemodynamic, renal, and hormonal effects of ghrelin infusion in patients with chronic heart failure. J Clin Endocrinol Metab 2001;86:5854–9.
[18] Burton GG, Hodgkin JE, Ward JJ. Specimen types and collection. In: Respiratory care. A guide to clinical practice. 4th ed. New York: Lippincott; 1997. p. 1061.
[19] Saraiva-Romanholo BM, Barnabé V, Carvalho AL, Martins MA, Saldiva PH, Nunes Mdo P. Comparison of three methods for differential cell count in induced sputum. Chest 2003;124:1060–6.
[20] Woo MA, Moser DK, Stevenson LW, Stevenson WG. Six-minute walk test and heart rate variability: lack of association in advanced stages of heart failure. Am J Crit Care 1997;6:348–54.
[21] Zheng L, Shum H, Tipoe GL, Leung R, Lam WK, Ooi GC, et al. Macrophages, neutrophils and tumor necrosis factor-α expression in bronchiectatic airways in vivo. Respir Med 2001;95:792–8.
[22] Taggart CC, Greene CM, Carroll TP, O'Neill SJ, McElvaney NG. Elastolytic proteases. Inflammatory resolution and dysregulation in chronic infective lung disease. Am J Respir Crit Care Med 2005;171:1070–6.
[23] Amitani R, Wilson R, Rutman A, Read R, Ward C, Burnett D, et al. Effects of human neutrophil elastase and *Pseudomonas aeruginosa* proteinases on human respiratory epithelium. Am J Respir Cell Mol Biol 1991;4:26–32.
[24] Sommerhoff CP, Nadel JA, Basbaum CB, Caughey GH. Neutrophil elastase and cathepsin G stimulate secretion from cultured bovine airway gland serous cells. J Clin Invest 1990;85:682–9.
[25] Smallman LA, Hill SL, Stockley RA. Reduction of ciliary beat frequency in vitro by sputum from patients with bronchiectasis: a serine proteinase effect. Thorax 1984;39:663–7.
[26] Gorin AB, Stewart PA. Differential permeability of endothelial and epithelial barriers to albumin flux. J Appl Physiol 1979;47:1315–24.
[27] Kudoh S, Azuma A, Yamamoto M, Izumi T, Ando M. Improvement of survival in patients with diffuse panbronchiolitis treated with low-dose erythromycin. Am J Respir Crit Care Med 1998;157:1829–32.
[28] Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, et al. Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T-cells. J Clin Invest 2004;114:57–66.
[29] Chailleux E, Laaban JP, Veale D. Prognostic value of nutritional depletion in patients with COPD treated by long-term oxygen therapy: data from the ANTADIR observatory. Chest 2003;123:1460–6.
[30] Harkness GA, Bentley DW, Roghmann KJ. Risk factors for nosocomial pneumonia in the elderly. Am J Med 1990;89:457–63.
[31] Alp E, Güven M, Yildiz O, Aygen B, Voss A, Doganay M. Incidence, risk factors and mortality of nosocomial pneumonia in intensive care units: a prospective study. Ann Clin Microbiol Antimicrob 2004;3:17.
[32] Langen RC, Schols AM, Kelders MC, van der Velden JL, Wouters EF. Muscle wasting and impaired muscle regeneration in a murine model of chronic pulmonary inflammation. Am J Respir Cell Mol Biol 2006;35:689–96.
[33] Matsumura K, Tsuchihashi T, Fujii K, Abe I, Iida M. Central ghrelin modulates sympathetic activity in conscious rabbits. Hypertension 2002;40:694–9.
[34] Lönnqvist F, Nyberg B, Wahrenberg H, Arner P. Catecholamine-induced lipolysis in adipose tissue of the elderly. J Clin Invest 1990;85:1614–21.
[35] Anker SD, Chua TP, Ponikowski P, Harrington D, Swan JW, Kox WJ, et al. Hormonal changes and catabolic/anabolic imbalance in chronic heart failure and their importance for cardiac cachexia. Circulation 1997;96:526–34.

Progress in Neuro-Psychopharmacology & Biological Psychiatry 32 (2008) 1527–1532

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnpbp

Decreased levels of ghrelin, cortisol, and fasting blood sugar, but not *n*-octanoylated ghrelin, in Japanese schizophrenic inpatients treated with olanzapine

Kazuhide Tanaka [a,b], Shigeru Morinobu [a,*], Mai Ichimura [b,c], Akihiro Asakawa [d], Akio Inui [d], Hiroshi Hosoda [e], Kenji Kangawa [e], Shigeto Yamawaki [a]

[a] Department of Psychiatry and Neurosciences, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8551, Japan
[b] Shigasato Hospital, 1-18-41 Shigasato, Otsu, 520-0006, Japan
[c] Department of Psychiatry, Shiga University of Medical Science, Setatsukinowa-cho, Otsu, 520-2192, Japan
[d] Department of Behavioral Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima, 890-8520, Japan
[e] Department of Biochemistry, National Cardiovascular Center Research Institute, 5-7-1 Fujishirodai, Suita, 565-8565, Japan

ARTICLE INFO

Article history:
Received 3 February 2008
Received in revised form 26 April 2008
Accepted 15 May 2008
Available online 22 May 2008

Keywords:
Body weight
Ghrelin
Hospitalized patient
Leptin
Olanzapine
Schizophrenia

ABSTRACT

The mechanism by which chronic administration of olanzapine induces a marked weight gain in patients with schizophrenia remains unknown. We examined the influence of long-term treatment with olanzapine on plasma levels of hormones regulating food intake and energy homeostasis in schizophrenia. In this study, olanzapine was administered to 28 Japanese inpatients for 16 weeks after switching from typical antipsychotic drugs or risperidone. At endpoint, no significant changes in body weight or body mass index were found. There was a significant decrease in the plasma levels of ghrelin without any accompanying change in active, *n*-octanoylated ghrelin. Serum levels of leptin tended to be increased and a significant reduction in plasma cortisol levels was found. In addition, the levels of fasting blood sugar as well as free fatty acid were significantly decreased. Furthermore, we did not confirm any marked weight gain induced by chronic administration of olanzapine as previously reported. The reason for this discrepancy may be due to differences in subjects and treatment settings. Based on these findings, it is unlikely that the decrease in plasma ghrelin levels by chronic administration of olanzapine affects weight gain. Further studies examining the effect of chronic olanzapine administration on weight and energy homeostasis in inpatients are required.

1. Introduction

Numerous studies have demonstrated that patients with schizophrenia have greater liability to chronic physical disease induced by weight gain than the general population (Casey et al., 2004; Coodin, 2001; Fontaine et al., 2001; Harris and Barraclough, 1998; Hennekens et al., 2005; Marder et al., 2004; Newcomer 2007; Newman and Bland, 1991; Paton et al., 2004). Previously, long-term administration of chlorpromazine, a typical first-generation antipsychotic drug, was reported to lead to body weight gain and obesity in a group of individuals with schizophrenia (Ganguli, 1999; Sletten et al., 1967). Furthermore, studies have shown that newly developed second-generation antipsychotic drugs, such as clozapine and olanzapine, commonly prescribed in the treatment of schizophrenia, are more likely to induce excessive body weight gain than first-generation antipsychotic drugs (Covell et al., 2004; Lambert et al., 2005; Liberman et al., 2005). A meta-analysis by Allison et al. (1999) indicated that the mean body weight gain following a 10-week administration of olanzapine was approximately 4.15 kg.

In addition, patients with schizophrenia treated with second-generation antipsychotic drugs, are at a higher risk than the general population for developing type 2 diabetes mellitus and hyperlipidemia (McIntyre et al., 2001; Newcomer et al., 2002; Saari et al., 2004; Sernyak et al., 2002). Diabetes mellitus, coronary heart disease, and hypertension, induced by body weight gain associated with antipsychotic drugs, are thought to contribute to a shorter life expectancy compared with that in the general population (Marder et al., 2004; Newman and Bland, 1991). Taking these findings together, the increase in the risk for type II diabetes mellitus and hyperlipidemia could be due to excessive body weight gain in response to long-term administration.

Recent advances in neuroendocrinology have revealed that various central and peripheral peptides are involved in the regulation of body weight (Horvath, 2005; Park and Bloom, 2005). In particular, ghrelin,

Abbreviations: BMI, body mass index; FBS, fasting blood sugar; FFA, free fatty acid; GH, growth hormone; HDL, high-density lipoprotein; HOMA-IR, homeostasis model of insulin resistance; LDL, low-density lipoprotein; PANSS, the positive and negative syndrome scale.

* Corresponding author. Department of Psychiatry and Neurosciences, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8551, Japan. Tel.: +81 82 257 5205; fax: +81 82 257 5209.
E-mail address: smoriob@hiroshima-u.ac.jp (S. Morinobu).

0278-5846/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2008.05.013

an endogenous ligand for growth hormone secretagogue receptor that was originally isolated from human and rat stomach, potently stimulated appetite, food intake, and body weight gain (Inui, 2001; Kojima et al., 1999). Since ghrelin is an orexigenic peptide (Klok et al., 2007), the peripheral increase in ghrelin levels correlates with hunger scores and induces food intake in healthy humans (Schmid et al., 2005; Tassone et al., 2003; Wren et al., 2001). Although it is plausible that an increase in plasma ghrelin levels by long-term administration of second-generation antipsychotic drugs, such as clozapine, and olanzapine, may be closely involved in excessive body weight gain in patients with schizophrenia, there have only been a few preliminary studies examining whether chronic treatment with olanzapine affects ghrelin blood levels in patients with schizophrenia (Hosojima et al., 2006; Murashita et al., 2004; Palik et al., 2005; Togo et al., 2004).

Although the majority of studies demonstrating excessive weight gain by long-term administration of olanzapine focus on outpatients, there are few studies examining the influence of olanzapine in hospitalized patients with schizophrenia, solely (Barak et al., 2004; Gattaz et al., 2004; Lambert et al., 2003). Furthermore, as compared to outpatients, blood sampling time as well as drug compliance is more accurate in inpatients. Overall, the individual differences in daily calorie intake and exercise in inpatients are smaller than those in outpatients. In this context, we examined the influence of long-term treatment with olanzapine on the body weight and plasma levels of hormones regulating food intake and energy homeostasis in Japanese inpatients with schizophrenia to elucidate mechanisms by which olanzapine induces body weight gain.

In addition, it is possible that other hormones regulating the homeostasis of energy metabolism, such as insulin (Woods et al., 1996), leptin (Campfield et al., 1996), growth hormone and cortisol, are also associated with excessive body weight gain induced by chronic administration of antipsychotics. For example, since leptin is an anorexigenic peptide, leptin induces weight loss by suppression of food intake in healthy humans (Klok et al., 2007). Although the effect of leptin on energy homeostasis is opposite to that of ghrelin, the relationship between leptin and ghrelin is still controversial (Ikezaki et al., 2002; Monti et al., 2006; Tschop et al., 2001; Yildiz et al., 2004). The result of the recent study examining the influence of recombinant leptin on ghrelin concentrations in plasma in healthy humans suggests that leptin has no effect on circulating ghrelin concentrations (Chan et al., 2004). Glucocorticoids also have a major effect on food intake and body weight (Dallman et al., 2004). Healthy humans with a high cortisol response to experimental stress conditions eat more calories comprising sweet and fatty foods than those with a low cortisol response (Epel et al., 2001). There is a significant correlation between the basal plasma levels of leptin and cortisol in healthy humans (Elimam et al., 1998; Newcomer et al., 1998). We, therefore, measured blood glucose, hemoglobin A_{1C}, triglycerides, total cholesterol, insulin, cortisol, GH, leptin, and ghrelin while monitoring body mass index (BMI) and body weight during a 16-week administration of olanzapine.

2. Methods

2.1. Subjects

Twenty-eight Japanese patients (18 males, 10 females) with schizophrenia who were hospitalized in the Shigasato Mental Hospital participated in this study. All patients were diagnosed with schizophrenia according to the DSM-IV criteria (Table 1). None of the patients had diabetes mellitus, or any other physical illness that could affect glucose–insulin homeostasis. The mean age of the patients was 59.5 ± 10.0 years (mean ± S.D.), and the duration of illness was 30.3 ± 11.2 years (mean ± S.D.). No patient with a family history of type 2 diabetes mellitus was involved in this study. This study was approved by the Ethics Committee of the Shigasato Mental Hospital, and written informed consent was obtained from patients or their legal guardians.

2.2. Treatment

At the start of the study, all patients were receiving either typical antipsychotics (10), risperidone (5), or both (13). At the time of switching, the doses of the ongoing antipsychotic drug(s) were tapered; all antipsychotic drugs were discontinued over a 2-week period (Fig. 1). At the beginning of this tapering period, 10 mg of olanzapine along with a half dose of the ongoing antipsychotic drug(s) was administered for 1 week. Following this, 20 mg of olanzapine was administered with a quarter dose of the ongoing antipsychotic(s) for 1 week. Finally, 2 weeks after beginning the tapering process, all ongoing antipsychotic drugs were withdrawn and patients received only 20 mg of olanzapine (Fig. 1). Concomitant medication for these patients included hypnotics (79% patients), anxiolytics (43% patients), mood stabilizers (18%), and antidepressants (0%). These were not changed during this study.

Based on a nutritional assessment of these patients at the Shigasato Hospital, the average of total energy in daily nutrient intake was 1800–2300 kcal/day during the study period. The average intake of carbohydrate, protein, and fat was 57, 15, and 20% of total energy, respectively.

2.3. Assessment of blood samples and clinical symptoms

The protocol for blood sampling and laboratory tests in this study is shown in Fig. 1. After overnight fasting, all blood samples were collected at 7:00 AM, prior to breakfast and medication. Fasting blood sugar (FBS) levels, serum levels of insulin, growth hormone (GH), cortisol, total cholesterol, triglyceride, free fatty acid (FFA), and leptin, and plasma levels of ghrelin were measured before beginning the switching process (baseline), and 8 and 16 weeks (endpoint) after beginning of switching. Body weight and BMI were recorded at the same times.

Blood glucose was measured using the hexokinase-glucose 6-phosphate dehydrogenase glucose oxidase method with a Quick Auto GLU-HK kit (Shino-test Corporation, Tokyo, Japan). Serum insulin levels were measured by a radioimmunoassay (RIA), using a commercial RIA kit (Insulin-Riabead kit, YAMASA Corporation, Tokyo, Japan). A homeostasis model was used to assess insulin resistance (HOMA-IR) by the following formula: fasting serum insulin level × fasting plasma glucose level/22.5. GH serum concentration was determined by immunoradiometric assay, using a Daiichi GH kit (TFB Inc., Tokyo, Japan). Cortisol serum concentration was measured by electro-chemiluminescence immunoassay (ECLusys Cortisol, Roche Diagnostics, Basel, Switzerland). The serum concentration of leptin was analyzed by immunoradiometric assay, using a Human Leptin RIA kit (Linco Research, Inc., St. Louis, MO.). Ghrelin plasma level was determined by RIA using two polyclonal rabbit antibodies raised against the N-terminal [1–11] and C-terminal [13–28] according to the method of Kojima et al. (1999). While active, *n*-octanoylated ghrelin

Table 1
Characteristics of schizophrenic inpatients at the baseline (before switching)

Patients characteristics	
Age, years	59.5 ± 10.0
Sex M/F, *N*	18/10
Duration of illness, years	30.3 ± 11.2
Schizophrenia type, *N*	
Paranoid	13
Disorganized	2
Undifferentiated	6
Residual	7
Concomitant therapy, %	
Sedative hypnotics	79
Anxiolytics	43
Mood stabilizer	18
Antidepressant	0

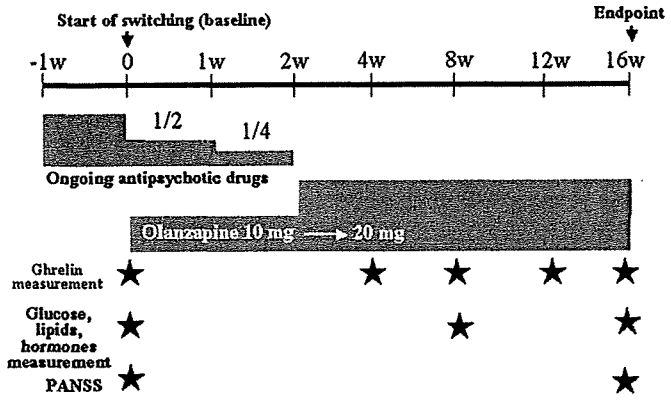

Fig. 1. Treatment and examination schedule.

was measured by RIA using anti-rat ghrelin [1–11] antiserum specific for *n*-octanoylated ghrelin, the level of total ghrelin (des-acyl and *n*-octanoylated ghrelin) was assessed by RIA using anti-rat ghrelin [13–28] (Ariyasu et al., 2001; Hosoda et al., 2000; Kojima et al., 1999).

Changes in clinical symptoms in response to switching were assessed using the positive and negative syndrome scale (PANSS). The clinical symptoms were assessed at baseline (before switching) and at endpoint (16 weeks after switching).

2.4. Statistical analysis

The Wilcoxon test was used in the statistical analyses. Differences were considered significant at $P<0.05$ for total, positive, and negative PANSS scores. Differences were considered significant at $P<0.025$ for body weight, BMI, FBS, HbA1c, cholesterol, triglycerides, FFA, insulin, HOMA, GH, cortisol, and leptin. Differences were considered significant at $P<0.0125$ for gherine. The strength of the linear relationship between the two parameters was calculated by Spearman rank correlation, and differences were considered significant at $P<0.05$.

3. Results

Based on the assessment by PANNS, the severity of psychiatric symptoms at Week 16 (endpoint) was significantly lower than that at baseline (Table 2). Body weight, BMI, FBS, HbA_1c, total cholesterol, triglycerides, FFA, insulin, HOMA, GH, cortisol, and leptin at baseline, Week 8, and Week 16 are shown in Tables 3 and 4.

3.1. Body weight and BMI

Whereas body weight gain (mean 2.3 kg; range 1–7 kg) occurred in 15 (53%) of 28 patients during olanzapine treatment, no significant change in body weight was found at Week 8, or at endpoint (Week 16) (Table 3). Similarly, there was no significant change in BMI from baseline to Week 8, or endpoint. In this study, 5 (18%) of 28 patients received combination treatment with olanzapine and valproic acid. No significant change in body weight or BMI was found at Week 8, or at endpoint in these 5 patients.

Table 2
Changes in psychiatric symptoms in a 16-week olanzapine treatment in patients with schizophrenia

	Baseline	Endpoint
PANSS		
Total	77.6±16.4	67.9±18.2*
Positive	17.6±6.1	14.8±6.0*
Negative	22.5±7.4	20.0±73*

Values are presented as mean±S.D.
*$P<0.05$ compared to baseline (before switching) by Wilcoxon test.

Table 3
Changes in weight, glucose, glucose metabolism and lipid in a 16-week olanzapine treatment in patients with schizophrenia

	Baseline	8 week	16 week
Body weight (kg)	58.4±12.7	58.5±12.5	58.1±13.0
BMI (kg/m^2)	22.2±3.7	22.3±3.6	22.2±3.8
FBS (mg/dl)	87.5±9.9	82.5±11.7*	80.7±10.9*
Insulin (μU/ml)	6.62±2.52	7.32±5.05	6.66±7.68
HOMA-IR	1.43±0.53	1.51±1.08	1.78±2.55
Hb A_{1c} (%)	4.89±0.37	4.83±0.37	4.89±0.43
FFA (mEq/l)	0.488±0.346	0.299±0.197*	0.306±0.148*
Total cholesterol (mg/dl)	178±36	169±28	182±32
HDL cholesterol (mg/dl)	56.5±18.9	50.7±15.5	54.5±16.6
LDL cholesterol (mg/dl)	102±24	97±23	104±27
Triglycerides (mg/dl)	106±51	111±61	116±68

Values are presented as mean±S.D.
*$P<0.025$ compared to baseline by Wilcoxon test.
BMI, body mass index; FBS, fasting blood glucose; HOMA-IR, homeostasis model of assessment of insulin release; Hb, hemoglobin; FFA, free fatty acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

3.2. Blood glucose, glucose metabolism, and lipid levels

The mean±SD levels of FBS at baseline, Week 8, and endpoint were 87.5±9.93 mg/dl, 82.5±11.7 mg/dl, and 80.7±10.9 mg/dl, respectively (Table 3). The mean level of FBS was significantly decreased from baseline at Week 8 ($P=0.0074$) as well as at endpoint ($P=0.0039$) (Table 3). On the other hand, no significant change in the mean value of HbA1c was found between baseline and Week 8, or between baseline and endpoint (Table 3). Also there was no significant change in mean level of serum insulin from baseline to Week 8, or to endpoint. The mean value of HOMA-IR at baseline did not differ from that of Week 8 or endpoint.

There was no significant change in the mean level of total cholesterol from baseline to Week 8, or to endpoint. Also no significant change was seen in the mean value of HDL- or LDL-cholesterol from baseline to Week 8, or to endpoint. The mean level of serum triglyceride at baseline did not differ from that of Week 8 or endpoint. However, the mean FFA level significantly decreased from baseline to Week 8 ($P=0.0158$) as well as to endpoint ($P=0.0111$) (Table 3).

3.3. Hormones and peptides

Mean±SD of total ghrelin levels were 227.7±114.3 fmol/ml, 217.7±98.3 fmol/ml, 193.5±108.6 fmol/ml, 208.2±104.2 fmol/ml, and 199.0±102.3 fmol/ml at baseline, Week 4, 8, 12, and 16 (endpoint), respectively (Fig. 2). The mean total ghrelin level was significantly decreased from baseline at Week 8 ($P<0.0001$), at Week 12 ($P=0.0075$), and at endpoint ($P=0.0010$). In contrast, there was no significant change in mean active ghrelin from baseline to Week 4, 8, 12, or endpoint (Fig. 2). There was no significant correlation between ghrelin level and BMI ($P=0.114$) or FBS ($P=0.630$) at endpoint. Whereas, at both baseline (M: 174.3±14.0 fmol/ml, F: 330.8±34.9 fmol/ml, $P=0.003$) and endpoint (M: 154.8±8.5 fmol/ml, F: 278.4±42.5 fmol/ml, $P=0.0054$), the total ghrelin level in females was significantly higher than that in male

Table 4
Changes in leptin, GH and cortisol in a 16-week olanzapine treatment in patients with schizophrenia

	Baseline	8 week	16 week
Leptin (ng/ml)	5.71±5.76	7.74±11.7	8.64±13.9
GH (ng/ml)	1.50±2.29	1.20±1.84	1.75±2.63
Cortisol (μg/dl)	20.0±6.8	15.3±5.2*	15.6±3.4*

Values are presented as mean±S.D.
*$P<0.025$ compared to baseline by Wilcoxon test. GH, growth hormone.