III-like contractions was lower in the antrum of neuropeptide Y (NPY) Y2 receptor knockout mice than in wild-type mice (Tanaka et al., 2009). The manometric method for the measurement of gastroduodenal motility in conscious rats and mice reported by Fujino et al. (2003) and Tanaka et al. (2009) is described below.

2.2.1 Animal preparation (manometric method)

1. Rats weighing 200–250 g or mice weighing 20–25 g are anesthetized by the intraperitoneal injection of pentobarbital sodium (50 mg/kg body weight).
2. Two polyurethane tubes (3-Fr, 1 mm diameter for rats or ID 0.30 × OD 0.84 mm for mice) are used as a manometric catheter for the motility recordings.
3. One tube is inserted into the stomach through a small incision to the gastric body with the tip placed at the gastric antrum. The other is inserted through the duodenal wall, and the tip is placed 3 cm for rats or 7 mm for mice from the pylorus.
4. The tubes are fixed on the gastric wall and duodenal wall by purse-string sutures, which run subcutaneously to emerge at the top of the neck, and are then secured on the neck skin.
5. Animals are allowed to recover for 1 week before the experiments.

2.2.2 Measurement of gastroduodenal motility (Fig. 18.2A)

1. Mice are deprived of food but not water for 16 h before the experiment.
2. The manometric catheters from the stomach and duodenum are connected to the infusion swivel on a single-axis counter-weighted swivel mount to allow free movement and then connected to a pressure transducer.
3. The catheters are continuously infused with bubble-free saline or distilled water from an infusion pump at the rate of 1.5 ml/h for rats or 0.15 ml/h for mice.
4. The data are recorded and stored in a PowerLab.
5. The mice are placed in a black box (150 × 200 × 300 mm) with the top open.
6. Motor activity is analyzed as described in Section 2.1.2.

2.3. Measurement of gastroduodenal motility in conscious house musk shrews (*S. murinus*)

Ghrelin has a structural resemblance to motilin, and the ghrelin receptor exhibits a 50% identity with the motilin receptor (Asakawa et al., 2001). Ghrelin induces premature phase III contractions in the human stomach

(Tack et al., 2006). Motilin also induces phase III contractions through the cholinergic pathway in humans and dogs (Itoh et al., 1976; Luiking et al., 1998; Suzuki et al., 1998). Ghrelin and motilin are expected to have additive or synergistic effects on the induction of GI contraction. The motilin gene is inactivated in rodents, and mice and rats are therefore not suitable animals for the study of motilin–ghrelin interactions (He et al., 2010).

A recent report (Sakahara et al., 2010) has demonstrated that both physiological ghrelin and motilin are produced and stimulate gastric motility in the house musk shrew (laboratory name: suncus). Strain-gauge force transducers were implanted on the serosa of the gastric body and duodenum in the free-moving, conscious suncus. As a result, clear fasted contractions similar to those observed in humans (Vantrappen et al., 1977) and dogs (Szurszewski, 1969) were observed in the suncus stomach (gastric body). These coordinated contractions consist of three phases: phase I (a period of motor quiescence), phase II (a period of preceding irregular contractions), and phase III (a period of clustered potent contractions). These phases were clearly recognized at regular intervals (every 80–150 min). In addition, ghrelin and/or motilin stimulated suncus gastric motility (Sakahara et al., 2010). Suncus, a small laboratory animal, may be useful as an alternative to humans and dogs for studying the physiological relationships between ghrelin and motilin on GI motility.

3. THE GI MOTOR EFFECT OF GHRELIN MEDIATED BY THE GUT–BRAIN AXIS

3.1. The brain mechanism responsible for mediating GI motility

The fasted pattern in GI motility is disrupted and replaced by the fed pattern after feeding. Intracerebroventricular injection of NPY, a powerful orexigenic peptide in the brain, induces fasted motor activity in fed rats (Fujimiya et al., 2000). The frequency of phase III-like contractions was lower in the antrum of NPY-Y2 knockout mice than in wild-type mice (Tanaka et al., 2009). However, anorexigenic peptides such as the corticotrophin-releasing factor (CRF) (Bueno et al., 1986), urocortin (Kihara et al., 2001), and cholecystokinin (Rodriguez-Membrilla and Vergara, 1997) cause the disruption of fasted motor activity in animals. These findings may represent an integrated mechanism linking the feeding behavior and GI motor activity through the gut–brain axis.

The effects of intravenous injection of ghrelin were blocked by the immunoneutralization of NPY in the brain, suggesting that peripheral ghrelin induces fasted motor activity by activating the NPY neurons in the brain, probably through ghrelin receptors on vagal afferent neurons (Fujino et al., 2003). Various recent studies have demonstrated the brain mechanism responsible for mediating GI motility. Central and peripheral administration of des-acyl ghrelin has been shown to significantly decrease food intake in food-deprived mice and to decrease gastric emptying (Asakawa et al., 2005). Des-acyl ghrelin exerts inhibitory effects on antrum motility but not on duodenal motility in fasted animals (Chen et al., 2005). Obestatin exerts inhibitory effects on the motility of the antrum and duodenum in the fed state but not in the fasted state (Ataka et al., 2008). CRF receptors in the brain may mediate the actions of des-acyl ghrelin and obestatin. Central administration of nesfatin-1, which has been identified as a hypothalamic anorexigenic peptide, has been shown to decrease food intake and inhibit gastroduodenal motility in mice (Atsuchi et al., 2010). In the experiments that measure gastroduodenal motility, the peptide should be injected through a catheter to avoid the effect of handling stress. The methodology for catheter implantation in rats is described below.

3.1.1 Vessel catheter (Figs. 18.1A and 18.2A)

A vessel catheter (ID 0.36 × OD 0.84 mm, Eicom, Kyoto, Japan) is inserted into the right jugular vein in rats and also led out from the back of the neck. The catheter is filled with heparinized saline (100 units/ml) to avoid blood coagulation. The operation can be performed at the same time as the implantation of a strain-gauge force transducer.

3.1.2 Intracerebroventricular catheter (Figs. 18.1A and 18.2A)

1. The implantation of an intracerebroventricular catheter is performed 4 days before the implantation of a strain-gauge force transducer.
2. The anesthetized rats are placed in a stereotaxic apparatus and implanted with a guide cannula (25 gauge; Eicom, Kyoto, Japan), which reaches the right lateral ventricle.
3. The stereotaxic coordinates are 0.8 mm posterior to bregma, 1.4 mm right lateral to the midline, and 3.4 mm below the outer surface of the skull, when using a Kopf stereotaxic frame (Tujunga, CA, USA), with the incisor bar set at the horizontal plane passing through bregma and lambda.

4. The guide cannula is secured with dental cement anchored by two stainless steel screws that are fixed on the dorsal surface of the skull.
5. After surgery, a dummy cannula (Eicom) is inserted into each guide cannula and a screw cap (Eicom) is placed on the guide cannula to prevent blockade.
6. The correct placement of the intracerebroventricular catheter is verified by the administration of a dye (e.g., 0.05% cresyl violet) into the right lateral ventricle by the brain sections at the end of the experiments.

3.2. Ghrelin and GI disorders

Ghrelin and its receptor agonists possess strong prokinetic properties and therefore have the potential to serve in the treatment of diabetic, neurogenic, or idiopathic gastroparesis as well as for chemotherapy-associated dyspepsia; postoperative, septic, or postburn ileus; opiate-induced bowel dysfunction; and chronic idiopathic constipation (Sallam and Chen, 2010). Abnormalities in gastroduodenal motility are considered key players in the pathogenesis of upper-GI symptoms in certain disorders such as functional dyspepsia and gastroparesis (Suzuki et al., 2006). Zheng et al. (2009b) reported that acute restraint stress inhibits solid gastric emptying and abolishes gastric phase III-like contractions via central CRF in rats. During subsequent chronic stress, the impaired gastric phase III-like contractions were restored by an adaptation mechanism that involves the upregulation of ghrelin expression. Recent work has shown that the central serotonin (5-HT) 2c receptor pathway decreases the peripheral levels of ghrelin, resulting in a shift from fasted to fed-like motor activity. Intravenous administration of ghrelin was shown to replace fed with fasted motor activity in rats treated with fenfluramine, which stimulated 5-HT2cR signaling in the central nervous system. Rikkunshito is widely prescribed for patients exhibiting functional dyspepsia (Kusunoki et al., 2010; Suzuki et al., 2009). Oral administration of rikkunshito has been shown to reduce the incidence of anorexia and improve gastric emptying in animals through increased peripheral plasma ghrelin concentrations (Fujitsuka et al., 2009; Sadakane et al., 2011; Saegusa et al., 2011; Takeda et al., 2008; Yakabi et al., 2011), stimulated central ghrelin secretion (Yakabi et al., 2010), or increased hypothalamic ghrelin receptor activity (Takeda et al., 2010). Recent studies have demonstrated that oral administration of rikkunshito improves gastroduodenal dysmotility in a rat model of cancer anorexia–cachexia by the potentiation of ghrelin receptor signaling

(Fujitsuka et al., 2011). These findings suggest that stimulation of ghrelin signaling may be an attractive approach for the treatment of upper-GI motor dysfunction.

4. SUMMARY

Recent technical advances have permitted the measurement of GI motility in conscious small animals, including rats, mice, and house musk shrews (*S. murinus*). Transgenic and knockout mice are tools to investigate the pathogenesis of disease models. The suncus may be useful as an alternative to humans and dogs for studying the physiological relationships between ghrelin and motilin in the context of GI motility. Recent experiments on free-moving, conscious animal have demonstrated that ghrelin regulates physiological fasted motor activity in the antrum and duodenum. Intravenous injection of ghrelin increases the MI and the frequency of phase III-like contractions, both of which are mediated by hypothalamic NPY neuron activation though ghrelin receptors at the vagal afferent terminal.

Stress hormone and anorexigenic peptides cause the disruption of fasted motor activity through a brain–gut interaction, which is involved in the pathogenesis of upper-GI symptoms in disorders such as functional dyspepsia and gastroparesis. Ghrelin is a signal potentiator that promotes GI motility and could be a good therapeutic target for GI disorders.

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Editorial

A New Horizon of Herbal Medicines in Anorexia-Cachexia Syndrome

The role of complementary and alternative medicine (CAM) continues to evolve in the daily lifestyle and treatment regimens of patients such as cancer. More than half of the cancer patients have used some form of CAM treatment during their cancer therapy in USA, and the situation is similar in other nations such as Europe and Japan. CAM use may be inclusive of holistic spiritual practice and physical exercise, as well as vitamins and herbal medicines for enhanced tumoricidal activity or reduction in treatment-related adverse events. Herbal medicine has been practiced for a long time in China, Korea, Japan, and other countries to achieve its key goal of restoring the balance of energy in the body.

Many effective chemotherapeutic agents for cancer are burdened by toxicities that can reduce patient quality of life or hinder their effective use. Attempts to minimize the toxicity by using isolated compounds have been unsatisfactory. Herbal medicines, composed of multiple biologically active compounds, are widely used to help improve such conditions. Recent studies have shown that the herbal medicines such as rikkunshito improve nausea, appetite loss and cachexia associated with cancer or cancer chemotherapy which worsens QOL and life expectancy of the patients. The mechanism involves an enhancement of signaling by ghrelin [1, 2] which was discovered in 1999 as an appetite-stimulating peptide from the stomach. It has a rivaling action to leptin, an afferent signal from fat tissue which informs the brain the size of body adiposity [3]. Currently, ghrelin agonists and antagonists are being developed and tested for treatment of anorexia/cachexia and obesity, respectively.

Although herbal medicines have not been fully accepted by mainstream medicine because of the complex nature of the formulae, the stringent quality control of Japanese herbal medicine and reproducibility of preclinical findings, together with few adverse events, have made herbal medicines more and more attractive for the management on intractable diseases such as cancer. The multi-component herbal medicines capable of targeting multiple sites could be useful for future drug discovery. Mechanistic studies and identification of active compounds could lead to new discoveries in biological and biomedical sciences.

This review series cover the translational aspects of herbal medicine on cancer treatment, particularly for cancer anorexia-cachexia syndrome (Fig. **1**). A focus will be put on rikkunshito and its active components that are able to potentiate ghrelin signaling [4] and mitigate the anorexia-cachexia syndrome. The review would provide a new horizon of herbal medicine from scientific point of view and be a basis for further development of CAM for patients with cancer and other intractable diseases.

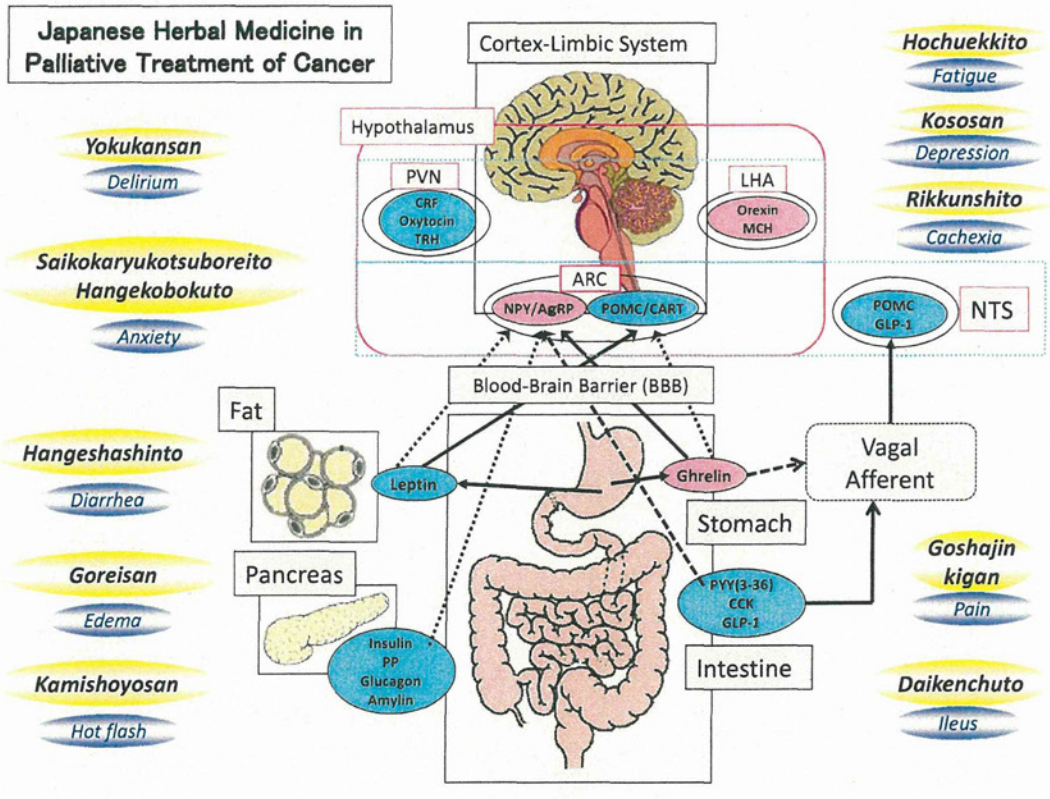

Fig. (1). Shown are the examples of Japanese herbal medicine in the palliative treatment of cancer. The quality control of herbal medicines is performed by 3 dimensional HPLC analysis that roughly estimates the main components of the crude drugs. The herbal medicines depicted are used to improve the cancer associated conditions such as anorexia-cachexia, depression, fatigue, anxiety, delirium, ileus, pain, edema, diarrhea and hot flash based on the scientific evidence in animal experiments and human studies. Brain-gut peptides are deeply involved in the actions of several herbal medicines: ghrelin in rikkunshito, adrenomedullin in daikenchuto, orexin in kososan, and CRF and opioid system in saikokaryukotuboreito and goshajinkigan. Solid lines show stimulation and dotted lines inhibition. See Perspectives in the last chapter of this special issue for details.

Abbreviations: Neuropeptide Y (NPY); pancreatic polypeptide (PP); melanin-concentrating hormone (MCH); agouti-related peptide (AgRP); corticotropin-releasing factor (CRF); glucagon-like peptide I (GLP-I); cocaine- and amphetamine-related transcript (CART); proopiomelanocortin (POMC); arcuate nucleus (ARC); paraventricular nucleus (PVN); cholecystokinin (CCK); lateral hypothalamic area (LHA); nucleus tractus solitarius (NTS); thyrotropin-releasing hormone (TRH)

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Ghrelin Treatment of Cachectic Patients with Chronic Obstructive Pulmonary Disease: A Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract

Background: Pulmonary cachexia is common in advanced chronic obstructive pulmonary disease (COPD), culminating in exercise intolerance and a poor prognosis. Ghrelin is a novel growth hormone (GH)-releasing peptide with GH-independent effects. The efficacy and safety of adding ghrelin to pulmonary rehabilitation (PR) in cachectic COPD patients were investigated.

Methodology/Principal Findings: In a multicenter, randomized, double-blind, placebo-controlled trial, 33 cachectic COPD patients were randomly assigned PR with intravenous ghrelin (2 μg/kg) or placebo twice daily for 3 weeks in hospital. The primary outcomes were changes in 6-min walk distance (6-MWD) and the St. George Respiratory Questionnaire (SGRQ) score. Secondary outcomes included changes in the Medical Research Council (MRC) scale, and respiratory muscle strength. At pre-treatment, serum GH levels were increased from baseline levels by a single dose of ghrelin (mean change, +46.5 ng/ml; between-group $p<0.0001$), the effect of which continued during the 3-week treatment. In the ghrelin group, the mean change from pre-treatment in 6-MWD was improved at Week 3 (+40 m, within-group $p=0.033$) and was maintained at Week 7 (+47 m, within-group $p=0.017$), although the difference between ghrelin and placebo was not significant. At Week 7, the mean changes in SGRQ symptoms (between-group $p=0.026$), in MRC (between-group $p=0.030$), and in maximal expiratory pressure (MEP; between-group $p=0.015$) were better in the ghrelin group than in the placebo group. Additionally, repeated-measures analysis of variance (ANOVA) indicated significant time course effects of ghrelin versus placebo in SGRQ symptoms ($p=0.049$) and MEP ($p=0.021$). Ghrelin treatment was well tolerated.

Conclusions/Significance: In cachectic COPD patients, with the safety profile, ghrelin administration provided improvements in symptoms and respiratory strength, despite the lack of a significant between-group difference in 6-MWD.

Trial Registration: UMIN Clinical Trial Registry C000000061

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Introduction

Pulmonary cachexia is common in the advanced stage of chronic obstructive pulmonary disease (COPD), and it is an independent risk factor for death in such patients [1,2]. Based on the notion that advanced COPD affects the whole body and causes wasting syndromes, many different therapeutic approaches have been attempted to improve this syndrome [1,3].

Pulmonary rehabilitation (PR) including exercise training is well accepted to improve exercise performance and quality of life in COPD patients [4], and it has been regarded as a nutritional adjunct therapy [5].

During the 1970s and 1980s, many gut peptides were identified [6]. Ghrelin, first discovered in 1999 as a novel growth hormone (GH)-releasing peptide isolated from the stomach, has been identified as an endogenous ligand for GH secretagogue receptor

[7]. Ghrelin also has a variety of GH-independent effects, such as causing a positive energy balance and weight gain by decreasing fat utilization [8], stimulating food intake [9], and inhibiting sympathetic nerve activity [10,11]. In addition, plasma ghrelin levels were elevated in cachectic COPD patients and were associated with the cachectic state and pulmonary function abnormalities, suggesting that endogenous ghrelin increased to compensate for the cachectic state and may provide important clues to improve the catabolic-anabolic imbalance in such patients[12]. In an open-label pilot study, we showed that ghrelin treatment increased walking distance in cachectic COPD patients [13]. Based on the above available evidence, a multicenter, randomized, double-blind, placebo-controlled study was conducted to test the hypothesis that the addition of ghrelin treatment to PR might benefit cachectic COPD patients. The objectives were to investigate the efficacy and safety of adding ghrelin to PR in cachectic COPD patients.

Methods

The protocol for this trial, supporting CONSORT checklist, and Supplementary Methods are available as supporting information; see Protocol S1, Checklist S1, and Supplementary Methods S1.

Study Design and Patients

The study was a 3-week, multicenter, randomized, double-blind, placebo-controlled trial of ghrelin administration during PR. The study was finally conducted at four clinical centers (National Cerebral and Cardiovascular Center, Miyazaki University School of Medicine, Nara Medical University, and National Hospital Organization Toneyama National Hospital) in Japan from September 2005 through May 2009, because Graduate School of Medicine, Osaka City University did not participate just before the start of the clinical trial. The study was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines and approved by the ethics committees of all participating study centers: The ethics committee of the National Cerebral and Cardiovascular Center (approval number, M17–13); The ethics committee of Miyazaki University School of Medicine (approval number, 218): The ethics committee of Nara Medical University (approval number, 05–012); and The ethics committee of the National Hospital Organization Toneyama National Hospital (approval number, 0311). All patients gave written

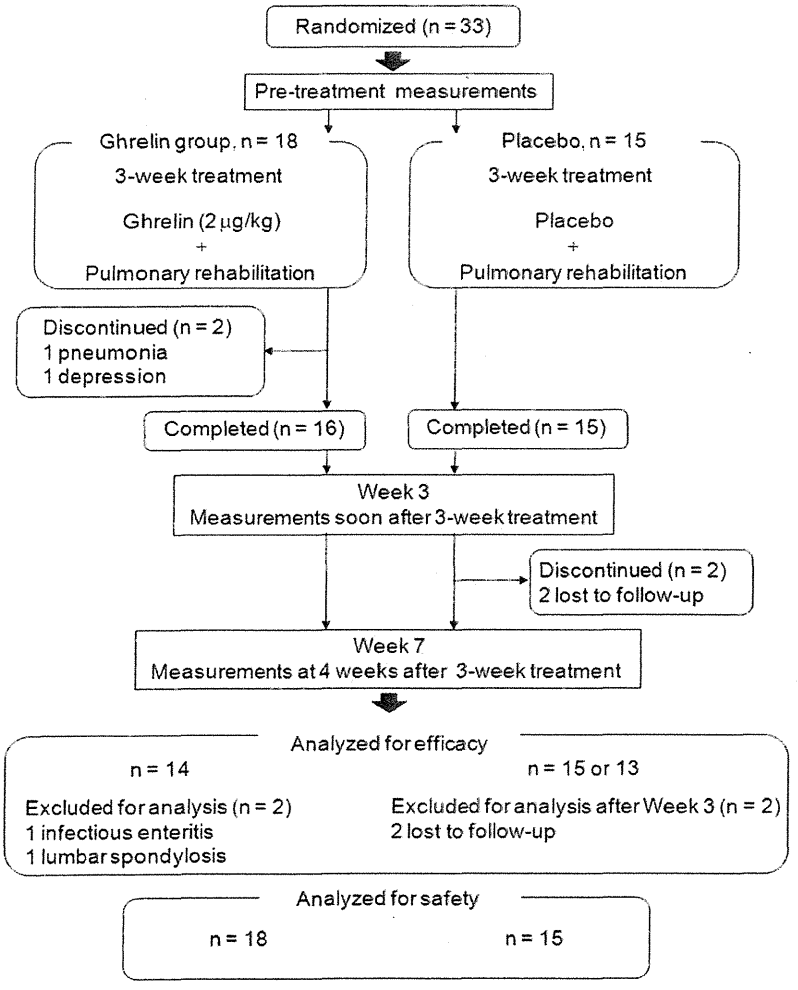

Figure 1. Trial profile.
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informed consent (in Japanese). The inclusion criteria were as follows: 1) severe to very severe COPD (forced expiratory volume in one second (FEV1)/forced vital capacity (FVC) of less than 70% and FEV1 percent predicted of less than 50%); 2) underweight (body mass index (BMI)<21 kg/m^2); 3) clinically stable and able to participate in PR; 4) between 20 and 85 years old; and 5) signed the agreement for participation in this study. Participants were excluded for any of the following: 1) malignant tumors; 2) active infection; 3) severe heart disease; 4) hepatic dysfunction (serum aspartate aminotransferase and alanine aminotransferase levels at least twice the upper limit of normal); 5) renal dysfunction (serum creatinine levels ≥2.0 mg/dl); 6) asthma; 7) definitely or possibly pregnant; 8) change in drug regimen within 4 weeks before participation in this study; or 9) judged to be unable to participate in this study by their physician. This study was registered with UMIN (University Hospital Medical Information Network in Japan: http://www.umin.ac.jp/ctr/), number C000000061.

Randomization and Interventions

Randomization was done in each center considered as a block. The randomization list was generated by a statistician from Hamamatsu University School of Medicine and maintained there until the study was finished and unblinded. Neither the physicians nor the patients were aware of the treatment assignments. Patients who met the eligibility criteria were enrolled and randomly assigned in a 1:1 ratio to receive PR with either ghrelin (2 μg/kg) or placebo twice a day for 3 weeks in hospital. The administration of ghrelin (2 μg/kg, ghrelin solution with 10 ml saline) or placebo was done intravenously over 30 minutes at a constant rate and repeated twice a day for 3 weeks. Patients were tested at pre-treatment, Week 3 after start of ghrelin or placebo administration with PR, and Week 7 after start of ghrelin or placebo administration with PR, i.e., 4 weeks after the completion of the combination treatment (Figure 1).

Preparation of Human Ghrelin

Human ghrelin obtained from the Peptide Institute Inc. was dissolved in distilled water with 3.75% D-mannitol and sterilized as described previously [13]. Ghrelin was stored in 2-ml volumes, each containing 120 μg ghrelin. The chemical nature and content of the human ghrelin in vials were rarefied as described previously [13]. All vials were stored frozen at −30°C until the time of preparation for administration.

Table 1. Patients' baseline characteristics. *

	Ghrelin, n = 14	Placebo, n = 15	p value
Age, years†	70.5 (6.2), 63–80	73.9 (6.0), 63–82	0.15
Sex, male/female‡	13/1	13/2	1.00
BMI, kg/m^2†	18.6 (2.1), 14.4–20.9	18.0 (2.1), 14.7–20.9	0.38
Cigarette smoking, pack years†	62.0 (30.9), 3.8–125	52.5 (28.8), 0.0–97.5	0.38
Pulmonary function†			
FEV1, L	0.78 (0.20), 0.54–1.21	0.77 (0.21), 0.47–1.21	0.90
%FEV1, % predicted	31.6 (8.1), 21.2–49.5	34.5 (9.1), 17.7–45.9	0.32
FEV1/FVC, %	38.0 (8.9), 24.6–50.5	38.8 (8.7), 25.4–52.9	0.74
VC, L	2.48 (0.37), 1.90–3.45	2.52 (0.50), 1.62–3.69	0.98
%VC, %	78.8 (9.3), 64.0–94.3	84.5 (12.6), 71.4–113.4	0.38
Exercise capacity on ICPET†			
Peak $\dot{V}o_2$, ml/kg/min	11.5 (3.3), 5.2–17.5	11.3 (3.5), 6.2–18.7	0.74
6-MWD, m†	328 (110), 148–619	315 (118), 85–498	0.84
SGRQ†			
Total score	58.2 (16.5), 36.3–84.4	50.2 (15.5), 21.3–77.3	0.23
Symptoms score	61.5 (22.5), 29.4–97.5	51.6 (19.8), 19.7–78.5	0.34
Activity score	72.5 (14.9), 41.7–92.5	65.9 (16.3), 35.3–92.5	0.34
Impacts score	46.7 (19.5), 20.0–84.4	39.2 (17.7), 9.4–69.7	0.53
Medications‡			
LAMA	9	6	0.27
SAMA	3	2	0.65
LABA	9	7	0.46
SABA	2	0	0.22
ICS	5	2	0.21
Methylxanthines	7	7	1.00

Data are presented as means (SD), and the minimum and maximum values unless otherwise stated. BMI = body mass index; FEV_1 = forced expiratory volume in one second; FVC = forced vital capacity; ICPET = incremental cardiopulmonary exercise testing; ICS = inhaled corticosteroids; LABA = long-acting β_2-agonist; LAMA = long-acting muscarinic antagonist; SABA = short-acting β_2-agonist; SAMA = short-acting muscarinic antagonist; VC = vital capacity.

*The groups shown represent only patients analyzed for efficacy. Medications are not mutually exclusive, and data are presented separately.

†Analyzed using a Wilcoxon rank sum test.

‡Analyzed using a Fisher's exact test.

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Pulmonary Rehabilitation

Exercise training, which was included in the PR program, was conducted in three sets daily, every weekday for 3 weeks (i.e. 15 days) at high-intensity targets. Additional details are described online in Supplementary Methods S1.

Outcome Measure

Efficacy: The primary outcomes were changes in 6-min walk distance (6-MWD) and the score evaluated using the St. George Respiratory Questionnaire (SGRQ) [14]. Secondary outcomes were changes in the health-related QoL (HRQoL) score using the Short-Form 36 questionnaire (SF 36 v2™ Health Survey, Japanese version) [15,16,17] and the Medical Research Council (MRC) dyspnea scale [18], peak oxygen uptake ($\dot{V}o_2$), food intake, FEV1/FVC, vital capacity (VC), respiratory muscle strength, and plasma norepinephrine levels in the resting condition.

Safety: All randomized patients who received at least one dose of the study treatments (ghrelin group, n = 18; placebo group, n = 15) were included in the safety analyses using intention-to-treat analysis. Blood tests were done up to Week 7. All serious adverse events were monitored throughout the study period.

6-min Walk Test

The 6-MWD was measured as described previously [13].

Cardiopulmonary Exercise Testing (CPET)

While breathing room air with a mask, symptom-limited CPET was conducted on an electrically braked cycle ergometer using an incremental protocol (continuous ramp rate of 5 W/min). Expired gas data were measured breath-by-breath and collected as 30-s averages at rest and during exercise. The CPET was done until subject exhaustion.

Food Intake

Food intake was assessed as described previously [13].

Respiratory and Peripheral Muscle Strength

The maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) were measured as described previously [13]. Peripheral muscle strength was measured by the maximal voluntary handgrip maneuver as described previously [13].

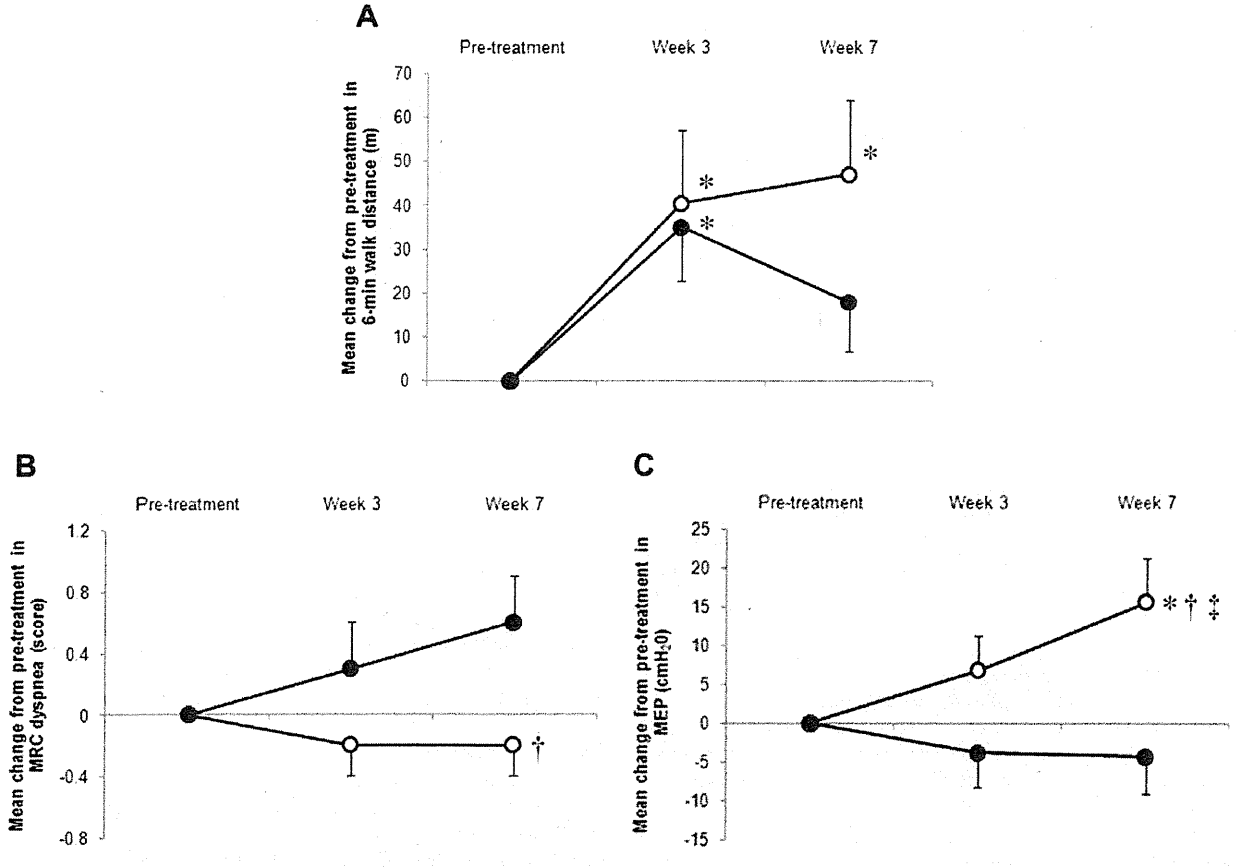

Figure 2. Change from pre-treatment in 6-min walk distance (6-MWD), Medical Research Council (MRC) score, and maximal expiratory pressure (MEP) over time. Open circles, ghrelin; closed circles, placebo. Data are presented as mean differences±SE. * $p<0.05$: change between pre- and post-treatment (within-group difference). † $p<0.05$: change between pre-treatment and post-treatment (between ghrelin and placebo group difference). ‡ $p<0.05$: time course effect of ghrelin versus placebo by repeated-measures ANOVA. A) In both groups, 6-MWD increases significantly to a similar level from pre-treatment at Week 3. Prolonged effects can be seen in the ghrelin group at Week 7, though the improvement in 6-MWD declined in the placebo group. B) Though the MRC score became progressively worse in the placebo group, the maintained effects in the MRC score can be seen in the ghrelin group at Week 7. C) Repeated-measures ANOVA indicated significant time course effects of ghrelin versus placebo in MEP ($F(2, 51) = 4.17$, $p = 0.021$).
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Table 2. Changes in pre-treatment exercise capacity, pulmonary function and other parameters during pulmonary rehabilitation with ghrelin or placebo.

	At Week 3			At Week 7		
	Ghrelin, n = 14	Placebo, n = 15	Treatment effect (95% CI; p value)	Ghrelin, n = 14	Placebo, n = 13	Treatment effect (95% CI; p value)
Exercise capacity						
6-MWD, m	40 (17)*	35 (12)*	5 (−37 to 48; 0.81)	47 (17)*	18 (11)	29 (−15 to 73; 0.19)
Peak$\dot{V}O_2$, ml/min/kg	1.2 (0.4)*	0.5 (0.3)	0.7 (−0.4 to 1.8; 0.21)	ND	ND	ND
Peak $\dot{V}O_2$/HR, ml/beats	0.5 (0.2)*	−0.4 (0.5)	0.9 (−0.2 to 2.0; 0.11)	ND	ND	ND
PFT						
FEV1/FVC, %	−1.1 (1.0)	−2.7 (0.9)*	1.6 (−1.2 to 4.3; 0.26)	−1.7 (1.2)	−1.2 (1.1)	−0.5 (−3.8 to 2.8; 0.77)
VC, L	0.14 (0.07)	0.11 (0.07)	0.03 (−0.16 to 0.23; 0.74)	0.09 (0.11)	−0.10 (0.07)	0.19 (−0.09 to 0.47; 0.17)
Others						
MIP, cmH_20	−8.2 (4.9)	−9.8 (3.2)**	1.6 (−10.1 to 13.4; 0.78)	−8.4 (5.6)	−4.3 (2.6)	−4.1 (−17.7 to 9.5; 0.52)
MEP, cmH_20	6.8 (4.4)	−3.8 (4.5)	10.7 (−2.2 to 23.5; 0.099)	15.6 (5.7)*	−4.3 (4.8)	19.9 (4.1 to 35.6; 0.015)
Food intake, kcal/day	122 (93)	−17 (86)	139 (−122 to 399; 0.28)	ND	ND	ND
MRC, score	−0.2 (0.2)	0.3 (0.3)	−0.4 (−1.2 to 0.3; 0.22)	−0.2 (0.2)	0.6 (0.3)	−0.7 (−1.4 to −0.1; 0.030)
Plasma NE, ng/ml	−0.063 (0.061)	−0.066 (0.067)	0.004 (−0.183 to 0.190; 0.97)	ND	ND	ND
IL-6 NE, pg/ml	1.52 (1.33)	0.08 (0.21)	1.44 (−1.35 to 4.22; 0.31)	ND	ND	ND
TNF-α, pg/ml	0.29 (0.15)	0.08 (0.06)	0.21 (−0.12 to 0.54; 0.21)	ND	ND	ND
Mean BP, mmHg	−13 (3)**	−3 (4)	−10 (−20 to 1; 0.061)	−2 (3)	4 (4)	−6 (−17 to 4; 0.20)
Body weight, kg	0.1 (0.3)	0.4 (0.3)	−0.3 (−1.2 to 0.7; 0.58)	0.8 (0.4)	0.4 (0.4)	0.4 (−0.7 to 1.4; 0.49)
Total lean mass, kg	0.2 (0.5)	0.5 (0.3)	−0.2 (−1.5 to 1.1; 0.73)	ND	ND	ND
Grip strength, kg	0.3 (0.9)	−0.0 (0.5)	0.3 (−1.7 to 2.3; 0.76)	1.1 (0.9)	2.5 (1.1)*	−1.5 (−4.4 to 1.4; 0.31)

Data are means (SE), or mean effect (95% CI; p value) unless otherwise indicated. BP = blood pressure; FEV_1 = forced expiratory volume in one second; FVC = forced vital capacity; IL = interleukin; MEP = maximal expiratory pressure; MIP = maximal inspiratory pressure; MRC = medical research council; ND = not done; NE = norepinephrine; PFT = pulmonary function test; VC = vital capacity.
*$p<0.05$,
**$p<0.01$: change between pre-treatment and post-treatment within-group difference.
doi:10.1371/journal.pone.0035708.t002

Dual-Energy X-ray Absorptiometry (DEXA)

All participating centers measured dual energy x-ray absorptiometry (DEXA) to assess the total body composition, including lean body mass. The measurements were performed with the subject lying in a supine position. As a general rule, a single expert from each center analyzed the scans from the corresponding center.

Blood Samples and Analyses

Serum GH, serum insulin-like growth factor (IGF)-1, serum tumor necrosis factor α (TNF-α), serum interleukin-6 (IL-6), and plasma norepinephrine were measured as described previously [13]. Additional details are described online in Supplementary Methods S1.

Sample Size

The study's target accrual was 60 in the original protocol at the time of study design (see supporting information; Protocol S1). When 31 of the 33 randomized patients completed this study, we re-performed the power and sample size calculation, and confirmed that the number of patients that had completed the study exceeded the number necessary for the re-calculated sample size of 18. As a result, this trial ended prematurely. Because i) it is difficult to prolong hospitalization considering the current status of health care insurance in Japan, and ii) what constituted a clinically important change in 6-MWD after ghrelin treatment with PR was not known before the study ended; the sample size calculation was re-performed on the estimated effect of only ghrelin treatment for improving 6-MWD, which was based on information from the pilot study [13]. The resultant total sample size of 18 was finally used to provide the power (80%) to detect a mean difference of 60 m in 6-MWD with an estimated SD of 40 m using a two-sided alpha of 0.05, though the study's target accrual stated in the original protocol was 60.

Statistical Analysis

All data are expressed as means±SD or SE unless otherwise indicated. Comparisons of baseline characteristics between the two groups were made by Fisher's exact tests and Wilcoxon rank sum tests. Effects were examined once or twice; that is i) at Week 3 soon after 3-week treatment or ii) at Week 3 and Week 7 (i.e., 4 weeks after the completion of 3-week treatment). The results at Week 3 and Week 7, respectively, were compared with the pre-treatment within each group, and between the two groups using paired *t*-tests and unpaired *t*-tests, respectively. To assess the time course efficacy of ghrelin versus placebo, post-treatment data up to Week 7 were also assessed using a repeated-measures analysis of variance

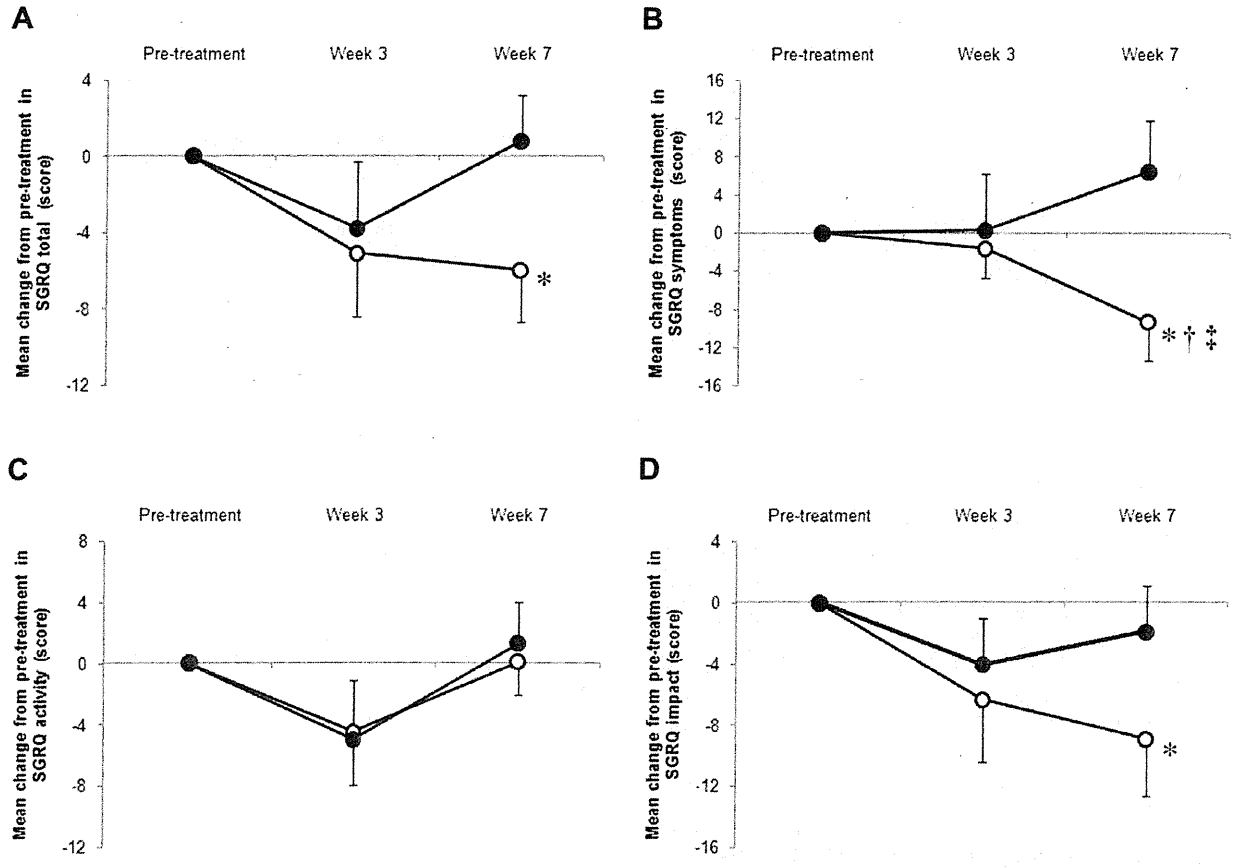

Figure 3. Change from pre-treatment in St. George Respiratory Questionnaire (SGRQ) scores over time. Open circles, ghrelin; closed circles, placebo. Data are presented as mean differences±SE. * $p<0.05$: change between pre- and post-treatment (within-group difference). † $p<0.05$: change between pre-treatment and post-treatment (between ghrelin and placebo group difference). ‡ $p<0.05$: time course effect of ghrelin versus placebo by repeated-measures ANOVA. At Week 3, marked improvements in SGRQ scores are not seen in both groups. However, SGRQ scores, especially SGRQ symptom scores, are significantly improved in the ghrelin group at Week 7. B) Repeated-measures ANOVA indicated significant time course effects of ghrelin versus placebo in SGRQ symptoms ($F(2, 51)=3.19$, $p=0.049$).
doi:10.1371/journal.pone.0035708.g003

(ANOVA). A p value<0.05 was considered significant (SAS 9.1.3, SAS Institute Inc., Cary, NC, USA).

Results

Of the 33 randomized patients, 31completed the 3-week study; 2 patients in the ghrelin group discontinued study medications due to pneumonia and depression, respectively. Of the 31 patients who completed the randomized 3-week study, in the ghrelin group, one patient had infective enteritis after 3 weeks of medications, and one had low back pain due to lumbar spondylosis before and throughout the 3 weeks of medications. Two patients in the placebo group were lost to follow-up after the Week 3 measurements. Therefore, 29 patients (ghrelin, n = 14; placebo, n = 15) were included in the study analyses to ensure adequate efficacy evaluation using pre-protocol analysis. The mean BMI in the enrolled patients (n = 29) was very low (mean±SD, 18.3±2.1 kg/m^2). The treatment groups were generally well-matched with regard to demographics and baseline characteristics (Table 1).

Somatotropic Function

At pre-treatment, compared with placebo, a single administration of ghrelin markedly increased serum GH levels from baseline (mean change±SE: ghrelin group 46.4±6.2 ng/ml at the mean peak time (35 min) versus the placebo group 1.1±0.5 ng/ml at the mean peak time (55 min); between group $p<0.0001$), the effect of which was maintained at Week 3 (mean change±SE: ghrelin group 15.8±2.1 ng/ml at the mean peak time (30 min) versus the placebo group 0.4±0.2 ng/ml at the mean peak time (65 min); between group $p<0.0001$). Three-week ghrelin-PR combination treatment tended to increase serum IGF-1 levels (mean change±SE: 12±6 ng/ml, within-group $p=0.093$).

Exercise Tolerance and Gas Exchange Measurements

At both Week 3 and Week 7, there were no significant differences between the ghrelin and placebo groups in 6-MWD. In each group, at Week 3, a similar significant increase from pre-treatment in 6-MWD was observed (mean difference: ghrelin group +40 m, within group $p=0.033$ versus placebo group +35 m, within group $p=0.013$). The effect remained at Week 7 in the ghrelin group, whereas in the placebo group, the

Table 3. Changes in pre-treatment scores of health-related quality of life during pulmonary rehabilitation with ghrelin or placebo

	At Week 3			At Week 7		
	Ghrelin, n = 14	Placebo, n = 15	Treatment effect (95% CI; p value)	Ghrelin, n = 14	Placebo, n = 13	Treatment effect (95% CI; p value)
SGRQ						
Total	−5.0 (3.2)	−3.9 (3.5)	−1.1 (−10.9 to 8.7; 0.83)	−6.0 (2.7)*	0.8 (2.4)	−6.8 (−14.4 to 0.7; 0.072)
Symptoms	−1.7 (3.0)	0.3 (5.9)	−1.9 (−16.2 to 12.3; 0.77)	−9.4 (4.0)*	6.4 (5.4)	−15.8 (−29.5 to −2.1; 0.026)
Activity	−4.5 (3.5)	−5.0 (3.9)	0.4 (−10.5 to 11.4; 0.94)	0.1 (2.2)	1.3 (2.7)	−1.2 (−8.3 to 5.9; 0.73)
Impacts	−6.3 (4.1)	−4.1 (3.1)	−2.2 (−12.6 to 8.2; 0.67)	−8.9 (3.7)*	−1.9 (3.0)	−7.0 (−16.9 to 2.9; 0.16)
SF-36						
Physical functioning	4.6 (6.1)	0.3 (3.9)	4.3 (−10.0 to 18.5; 0.55)	3.1 (4.7)	−6.9 (4.9)	10.0 (−3.9 to 23.9; 0.15)
Role physical	−8.3 (6.9)	−4.6 (5.4)	−3.7 (−21.6 to 14.1; 0.67)	−12.0 (4.1)*	−22.6 (7.3)**	10.6 (−6.8 to 27.9; 0.22)
Bodily pain	−6.8 (5.3)	8.4 (6.4)	−15.2 (−33.0 to 2.6; 0.090)	−7.6 (6.5)	−3.8 (6.8)	−3.8 (−23.2 to 15.7; 0.69)
General health	−0.6 (4.5)	2.9 (5.2)	−3.5 (−17.9 to 11.0; 0.63)	0.5 (3.4)	5.8 (5.4)	−5.3 (−18.5 to 7.9; 0.41)
Vitality	5.7 (5.5)	7.8 (4.4)	−2.0 (−16.3 to 12.3; 0.77)	3.4 (4.8)	−2.9 (3.4)	6.2 (−5.9 to 18.4; 0.30)
Social functioning	−3.1 (9.5)	3.3 (7.2)	−6.5 (−30.5 to 17.6; 0.59)	−12.5 (8.1)	−2.9 (6.0)	−9.6 (−30.5 to 11.3; 0.35)
Role emotional	−13.9 (5.2)*	−9.5 (9.2)	−4.4 (−27.7 to 18.8; 0.68)	−19.9 (6.6)*	−16.0 (10.4)	−3.9 (−29.3 to 21.5; 0.76)
Mental health	0.4 (6.0)	3.7 (4.2)	−3.3 (−18.0 to 11.5; 0.65)	3.5 (3.3)	−8.2 (4.6)	11.7 (0.0 to 23.4; 0.050)

Data are means (SE), or mean effect (95% CI; p value) unless otherwise indicated. SGRQ = St. George Respiratory Questionnaire; SF 36 = short-Form 36.
*p<0.05,
**p<0.01: change between pre-treatment and post-treatment within-group difference.
doi:10.1371/journal.pone.0035708.t003

improvement in 6-MWD was reduced at Week 7 (mean difference: ghrelin group within group +47 m, p = 0.017 versus placebo group +18 m, within group p = 0.14) (Table 2 and Figure 2A). To assess the time course efficacy of ghrelin versus placebo in 6-MWD, a repeated-measures ANOVA was performed. There was no significant time course effect of ghrelin versus placebo in 6-MWD ($F(2, 51) = 1.10$, p = 0.34).

Table 4. Adverse events.

Event	Ghrelin, n = 18	Placebo, n = 15
Patients with at least 1 adverse event	12 (67)	5 (33)
Adverse events not considered study therapy-related		
Pneumonia	1 (6)	0 (0)
Depression	1 (6)	0 (0)
Infective enteritis	1 (6)	0 (0)
Lung cancer*	1 (6)	0 (0)
Hypercalcemia	0 (0)	1 (7)
Adverse events considered study therapy-related		
Stomach rumbling	3 (17)	2 (13)
Feeling of being warm	4 (22)	0 (0)
Feeling of hunger	2 (11)	2 (13)
Thirst	2 (11)	0 (0)
Slight liver dysfunction	1 (6)	0 (0)
Hypercholesterolemia	1 (6)	0 (0)
Hypoproteinemia	1 (6)	2 (13)

Values are presented as n (% of group). * One patient developed lung cancer 2 years and 9 months after study treatment.
doi:10.1371/journal.pone.0035708.t004

In the ghrelin group, the peak $\dot{V}o_2$ and $\dot{V}o_2$/HR were significantly increased by 1.2 ml/kg/min and 0.5 ml/beats, respectively, from pre-treatment (within-group p = 0.021, p = 0.019, respectively) (Table 2). However, there was no significant difference between the two groups in the peak $\dot{V}o_2$ and $\dot{V}o_2$/HR. In the ghrelin group, the ventilatory equivalents for oxygen ($\dot{V}E/\dot{V}o_2$) was relatively improved by −3.9 from pre-treatment (within group p = 0.060).

HRQoL and MRC Measures

In both groups, there was no significant difference in each SGRQ score and MRC score between pre-treatment and at Week 3. At Week 7, there was a significant treatment effect between the two groups in SGRQ symptoms (between-group: p = 0.026, Table 3 and Figure 3B), and in the MRC score (between-group p = 0.030, Table 2 and Figure 2B). At Week 7, in the ghrelin group, SGRQ total was decreased by 6.0 from pre-treatment (within-group p = 0.046, between-group p = 0.072) (Table 3 and Figure 3A). Furthermore, there was a significant time course effect of ghrelin versus placebo in SGRQ symptoms (repeated-measures ANOVA, $F(2, 51) = 3.19$, p = 0.049, Figure 3B).

Body Weight and Food Intake

In the ghrelin group, at Week 1, the relative increase in body weight was+0.42 kg (within group p = 0.092), which was reduced by Week 3 and followed by a re-increase at Week 7 (+0.8 kg, within group: p = 0.054). However there was no significant difference in body weight between the groups at each Week (Table 2). No affect on whole lean body mass from ghrelin was seen at Week 3 (Table 2). No significant increase from baseline in food intake was observed at Week 3 in both groups (Table 2).

Respiratory and Peripheral Muscle Strength

In the ghrelin group, at Week 3, the post-treatment increase in respiratory muscle strength, as indicated by MEP and MIP, was not significantly different from that in the placebo group, but at Week 7, the mean increase from pre-treatment in MEP (+15.6 cmH_2O) was significantly different from that in the placebo group (between group $p = 0.015$) (Table 2). Furthermore, there was a significant time course effect of ghrelin versus placebo in MEP (repeated-measures ANOVA, $F(2, 51) = 4.17$, $p = 0.021$, Figure 2C).

At Week 3 and Week 7, there was no significant treatment effect between the two groups in grip strength (Table 2).

Pulmonary Function, Plasma Norepinephrine, and Other Hormone Levels

Ghrelin treatment did not significantly change any parameters of the pulmonary function tests, serum TNF-α, serum IL-6, or plasma norepinephrine at rest (Table 2).

Safety

Throughout this trial, 67% of patients in the ghrelin group and 33% of patients in the placebo group reported 12 and 5 adverse events, respectively, but there was no significant difference between the groups (Table 4). In the ghrelin group, alanine aminotransferase increased to 41 IU/L in one patient (6%), and total cholesterol increased to 270 mg/dl in one patient (6%); both increases disappeared at Week 7. Two patients randomized to ghrelin discontinued as a result of adverse events: one because of bacterial pneumonia, and one because of depression, both of which were not considered related to ghrelin treatment. One patient randomized to ghrelin developed lung cancer 2 years and 9 months after the end of ghrelin administration, but this was judged by the efficacy and safety committee as not causally related to ghrelin treatment, considering the period of disease development and the incidence rate of lung cancer [19].

Discussion

The present study is the first multicenter, randomized, double-blind, placebo-controlled study to assess the effect and safety of repeated ghrelin administration to very severe cachectic patients with COPD. The main results of this study can be summarized as follows. In the ghrelin group, single administration of ghrelin was accompanied by a significant increase in serum GH levels during 3-week treatment, and there was no significant difference in 6-MWD between ghrelin and placebo at Week 3 and at Week 7. With ghrelin, symptomatic improvements in SGRQ symptoms and MRC score were not obtained at Week 3, but significant differences between ghrelin and placebo were seen at Week 7. In the ghrelin group, no significant within-group improvement from pre-treatment was seen in respiratory muscle strength, as indicated by MEP and MIP, at Week 3, but there was a significant difference in MEP between ghrelin and placebo at Week 7. Repeated-measures ANOVA showed significant time course effects of ghrelin versus placebo in SGRQ symptoms and MEP. Finally, ghrelin treatment was well tolerated.

Ghrelin treatment may have beneficial, continuing effects after treatment on HRQoL and MRC measures in this population. Though this study was conducted to determine the effectiveness of ghrelin in cachectic COPD patients, considering a synergistic interaction between ghrelin and PR, the data of this study need to be interpreted with caution, because, especially in advanced stage patients, excessive exercise training may partially worsen the anabolic and catabolic balance [1,20]. In the present study, which included patients with a lower exercise capacity and pulmonary function than those in the pilot study [13] and more cachectic patients than those in other studies on PR [21], the 6-MWD after 3-week PR in the placebo group was decreased in 3 (20%) of the 15 patients. Since 5 patients (33%) in the placebo group found the initial training work rate intolerable, the initial training work rate remained at its initial setting. In addition, at Week 3, outcome measurements showed no improvements with ghrelin compared with placebo. These findings may represent patients' variable responses to PR, which might have an influence on the effects of ghrelin. Of note, however, there were significant treatment effects of ghrelin in both SGRQ symptoms and MRC score. In addition, the treatment tended to improve the total SGRQ score by more than 4 points; a clinically meaningful improvement. These effects were not observed soon after the 3 week-treatment, but were seen 4 weeks after treatment, maintaining the improvement obtained in 6-MWD at Week 3. Similarly, 4 weeks after treatment, the effect of ghrelin on respiratory muscle strength was confirmed, though it has been reported that GH alone does not increase strength in healthy elderly [22,23,24]. Furthermore, repeated-measures ANOVA indicated significant time course effects of ghrelin versus placebo in SGRQ symptoms and MEP. Our data suggest that improving of the respiratory muscle strength, the O_2 pulse, and the ventilatory equivalents for oxygen may serve as a mechanism by which ghrelin-PR combination treatment improved symptoms, though further examination is needed to understand the precise mechanism. These findings suggest that repeated ghrelin administration may have beneficial, sustained effects after administration on symptoms through GH-dependent and/or -independent mechanisms.

Cachectic elderly patients with COPD who were given intravenous ghrelin showed a continuous increase of pulsatile GH secretion in the present study. There is evidence that insufficiency of sarcopenia-related hormones, such as GH and IGF-1, may contribute to cachexia [25,26]. Observational studies in cachectic COPD patients have found decreased levels of these hormones [27,28]. In the present study, despite significant increases in GH secretion levels throughout the 3-week treatment and respiratory muscle strength, ghrelin provided only a significant within-group increase in exercise performance, and a relative within-group increase in IGF-1 levels and body weight. Furthermore, ghrelin did not affect food intake, grip strength or plasma norepinephrine levels at rest in the present study. Although DEXA should be performed a greater number of times during the trial, at Week 3 ghrelin did not show any effects on whole lean body mass. Meanwhile, previous studies showed that ghrelin administration induced a positive energy balance and weight gain [8], increased food intake [9,13], and decreased sympathetic nervous activity [10,11,13]. The discrepancy may be explained by the fact that the intensity of exercise training for some cachectic participants counteracted the effects of ghrelin, though lower extremity exercise training at higher intensity produces greater benefits than lower intensity training [4]. As one of the reasons, the patients treated with both ghrelin and exercise training gained at Week 1, which was not seen in the placebo group. However, this weight gain reduced by Week 3. At Week 7, the weight was regained (Table 2). The days of attending PR in the ghrelin group was negatively correlated with the increase in body weight from Week 3 to Week 7 ($r = -0.710$, $p = 0.003$). We speculate that the unintended excessive exercise permitted by ghrelin administration with antidepressant-like effects [29] might prevent the obtained results. Nevertheless, these findings suggest that clinical interventions with ghrelin may help cachectic COPD patients via inhibiting somatopause and regulating metabolic balance.

The participants in the present study tolerated daily administration of ghrelin for 3 weeks (Table 4); the most frequent ghrelin-related side effects were mild and similar to those of previous reports [13,30,31], as well as with those of GH administration by injection [22]. However, given that the previous studies of the responses of ghrelin in proliferation, including tumor development, have demonstrated conflicting findings [32,33,34,35], more studies of the safety of ghrelin treatment are necessary before clinical application.

This study had some limitations. First, the number of participants was small, and few females were included in this trial. Second, the duration of the study was short. A more effective exercise training program, considering its intensity and frequencies, should have been conducted. Additional studies are needed to evaluate a more suitable regimen of ghrelin-PR.

In conclusion, ghrelin administration provided sustained improvements in symptoms and respiratory strength in cachectic COPD patients. Development of ghrelin administration methods may offer potential advantages over the currently approved treatment options for COPD. The lack of a significant between-group difference in exercise tolerance may result from the exercise training program conducted as the combination therapy. Careful examination is needed to develop more effective administration methods of ghrelin and combination therapy with ghrelin.

Supporting Information

Methods S1
(DOC)

Protocol S1
(DOC)

Checklist S1
(DOC)

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Conceived and designed the experiments: NN KK MN HK RM MY KM SM SO NM K. Yamahara. Performed the experiments: RM KM HK MN NN TH MM SK K. Yoshimura YT MY SM YA NM SO. Analyzed the data: KM RM HK MN NN TH MM SK K. Yoshimura YT MY SM YA NM SO K. Yamahara KK. Wrote the paper: KM.

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Impaired ghrelin signaling is associated with gastrointestinal dysmotility in rats with gastroesophageal reflux disease

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Nahata M, Muto S, Oridate N, Ohnishi S, Nakagawa K, Sadakane C, Saegusa Y, Hattori T, Asaka M, Takeda H. Impaired ghrelin signaling is associated with gastrointestinal dysmotility in rats with gastroesophageal reflux disease. *Am J Physiol Gastrointest Liver Physiol* 303: G42–G53, 2012. First published April 19, 2012; doi:10.1152/ajpgi.00462.2011.—Gastroesophageal reflux disease (GERD) is often associated with decreased upper gastrointestinal motility, and ghrelin is an appetite-stimulating hormone known to increase gastrointestinal motility. We investigated whether ghrelin signaling is impaired in rats with GERD and studied its involvement in upper gastrointestinal motility. GERD was induced surgically in Wistar rats. Rats were injected intravenously with ghrelin (3 nmol/rat), after which gastric emptying, food intake, gastroduodenal motility, and growth hormone (GH) release were investigated. Furthermore, plasma ghrelin levels and the expression of ghrelin-related genes in the stomach and hypothalamus were examined. In addition, we administered ghrelin to GERD rats treated with rikkunshito, a Kampo medicine, and examined its effects on gastroduodenal motility. GERD rats showed a considerable decrease in gastric emptying, food intake, and antral motility. Ghrelin administration significantly increased gastric emptying, food intake, and antral and duodenal motility in sham-operated rats, but not in GERD rats. The effect of ghrelin on GH release was also attenuated in GERD rats, which had significantly increased plasma ghrelin levels and expression of orexigenic neuropeptide Y/agouti-related peptide mRNA in the hypothalamus. The number of ghrelin-positive cells in the gastric body decreased in GERD rats, but the expression of gastric preproghrelin and GH secretagogue receptor mRNA was not affected. However, when ghrelin was exogenously administered to GERD rats treated with rikkunshito, a significant increase in antral motility was observed. These results suggest that gastrointestinal dysmotility is associated with impaired ghrelin signaling in GERD rats and that rikkunshito restores gastrointestinal motility by improving the ghrelin response.

gastric emptying; food intake; growth hormone; rikkunshito

GASTROESOPHAGEAL REFLUX DISEASE (GERD) is a condition in which regurgitation of gastric acid and other gastric contents into the esophagus causes discomfort and complications (49). Decreased the lower esophageal sphincter function and esophageal clearance are involved in impairment of the esophageal mucosa due to gastric acid (4), and proton pump inhibitors, which suppress gastric acid secretion, have been the drugs of choice for the treatment of GERD. Delayed gastric emptying occurs frequently among patients with acid reflux (3, 31), and treatment with prokinetic agents, which are agonists of the 5-hydroxytryptamine 4 (5-HT_4) receptor, partially improves symptoms in patients with GERD (16, 39). Thus it was concluded that gastric dysmotility is also a factor involved in acid reflux into the esophagus (3, 10, 29, 31). However, the reasons for gastric dysmotility remain unclear.

Ghrelin is a 28-amino-acid endogenous ligand of the growth hormone secretagogue receptor (GHS-R) and is primarily secreted from gastric endocrine cells (8, 26). In addition to its secretagogue action on growth hormone (GH), ghrelin is known to have a strong orexigenic effect (26, 34) and has been reported to enhance gastrointestinal motility (12, 20, 30). In vitro, ghrelin contracts muscle strips of rat forestomach and antrum (7, 15). Ghrelin administration induces dose-dependent phase III-like contractions in the antrum and increases the motility index (MI) in rodents (11, 45, 52) and humans (42). In addition, treatment with ghrelin and GH-releasing peptide-6, which is an agonist of ghrelin receptors, improves delayed gastric emptying in mice administered with cisplatin (28) and diabetic mice with gastroparesis (37). These effects of ghrelin are believed to act on the central nervous system via the afferent vagus nerve and promote gastrointestinal motility through an efferent pathway (1, 9, 30). However, whether impaired ghrelin signaling is involved in gastrointestinal dysmotility in GERD remains unknown.

Rikkunshito is a Kampo medicine (traditional Japanese medicine) that is widely prescribed to patients with various gastrointestinal symptoms. Clinically, it has been reported that rikkunshito improves gastrointestinal complaints related to functional dyspepsia (47, 51), and its efficacy has been proven in a multicenter double-blind study (17). Recently, it has also been reported that rikkunshito leads to a fundamental and clinical reduction in esophageal acid reflux and related symptoms in patients with GERD (21–23, 32). In addition, we and others have found that rikkunshito promotes ghrelin secretion in the stomach (13, 44) and increases ghrelin receptor sensitivity (14).

We hypothesized that impaired ghrelin signaling is involved in gastrointestinal dysmotility in GERD. To examine this hypothesis, we investigated endogenous ghrelin levels and the effects of exogenously administered ghrelin in an experimental model of GERD. Furthermore, we also examined whether rikkunshito improves ghrelin signaling in this GERD model.

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