

[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 infor-

mation; 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

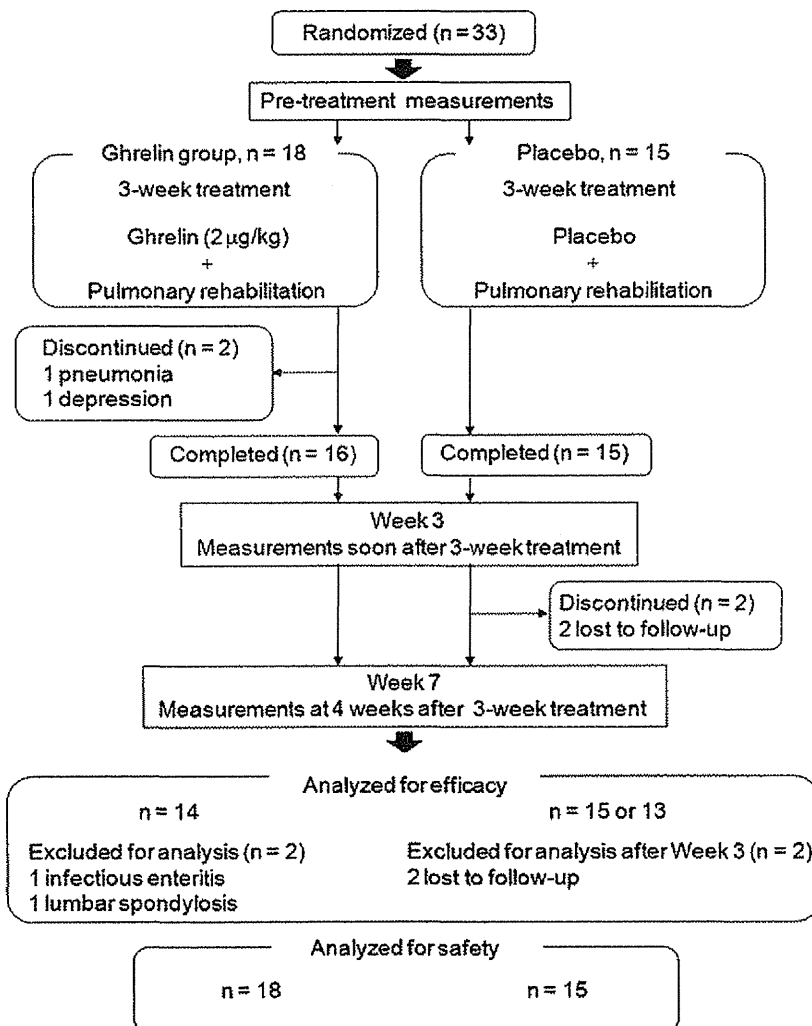


Figure 1. Trial profile.

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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/m²); 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 \geq 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 μ g/kg) or placebo twice a day for 3 weeks in hospital. The administration of ghrelin (2 μ g/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 μ g 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 [†]			
FEV ₁ , L	0.78 (0.20), 0.54–1.21	0.77 (0.21), 0.47–1.21	0.90
%FEV ₁ , % predicted	31.6 (8.1), 21.2–49.5	34.5 (9.1), 17.7–45.9	0.32
FEV ₁ /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 ICPEP [†]			
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; FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity; ICPEP = 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].

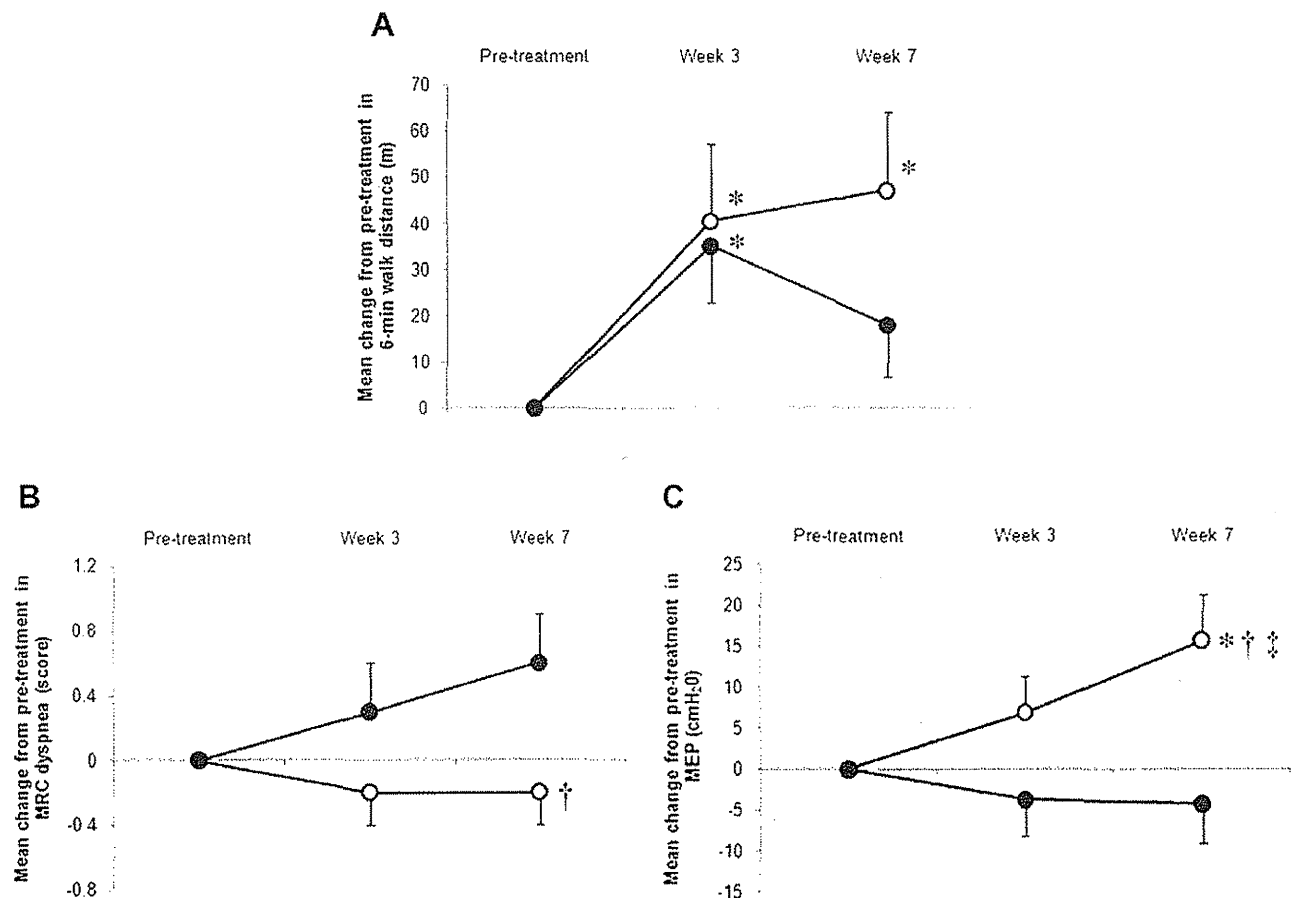


Figure 2. Change from pre-treatment in 6-min walk distance (6-MWD), Medical Research Council (MRC) score, and maximal expiratory pressure (MEP) over time. Open circles, ghrelin; closed circles, placebo. Data are presented as mean differences \pm SE. * $p < 0.05$: change between pre- and post-treatment (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 ($F(2, 51) = 4.17$, $p = 0.021$). doi:10.1371/journal.pone.0035708.g002

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 ₂ O	-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 ₂ O	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; FEV₁ = 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.

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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 \pm 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

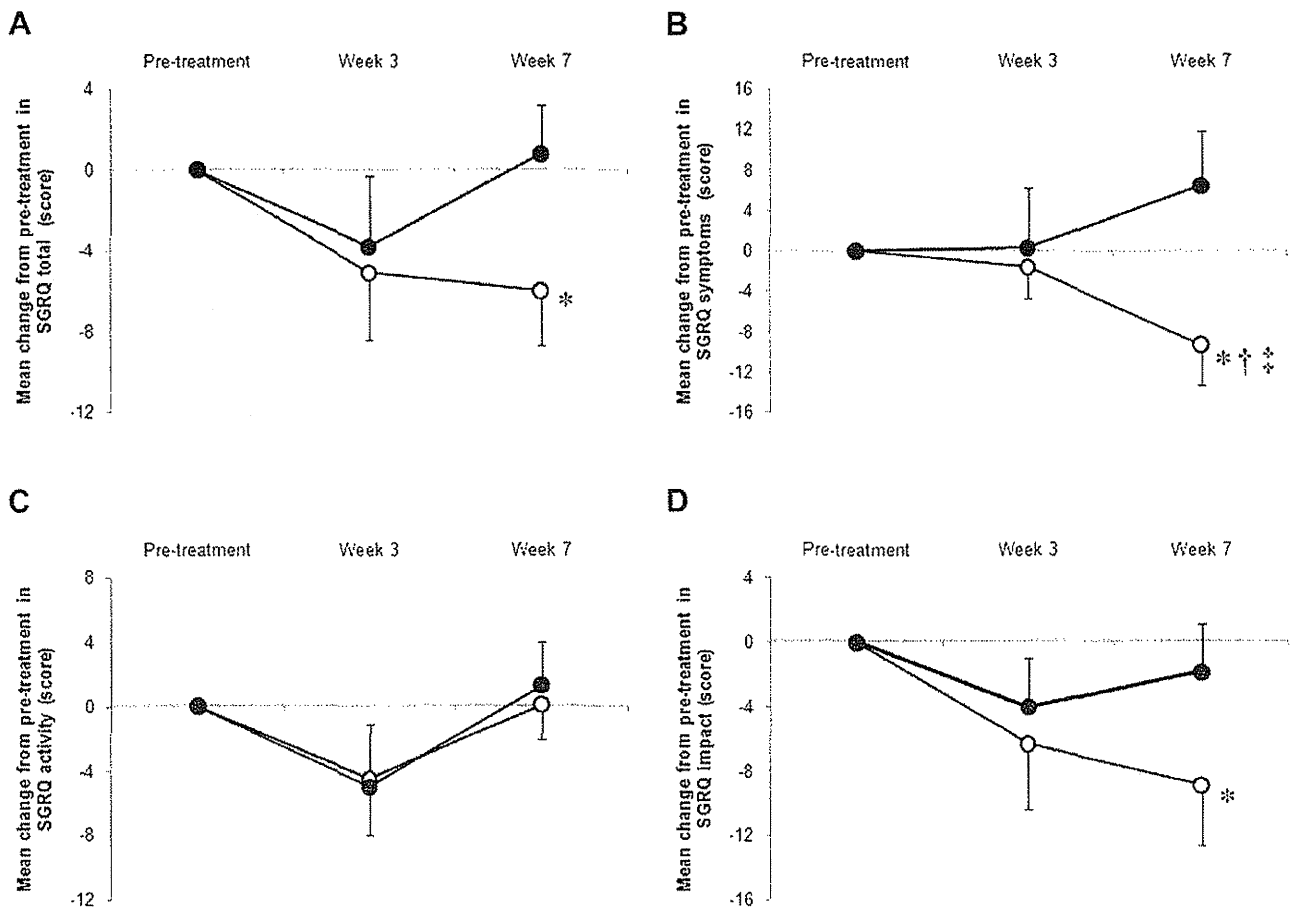


Figure 3. Change from pre-treatment in St. George Respiratory Questionnaire (SGRQ) scores over time. Open circles, ghrelin; closed circles, placebo. Data are presented as mean differences \pm SE. * $p < 0.05$: change between pre- and post-treatment (within-group difference). † $p < 0.05$: change between 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, 31 completed 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 \pm SD, $18.3 \pm 2.1 \text{ kg/m}^2$). 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 \pm SE: ghrelin group $46.4 \pm 6.2 \text{ ng/ml}$ at the mean peak time (35 min) versus the placebo group $1.1 \pm 0.5 \text{ ng/ml}$ at the mean peak time (55 min); between group $p < 0.0001$), the effect of which was maintained at Week 3 (mean change \pm SE: ghrelin group $15.8 \pm 2.1 \text{ ng/ml}$ at the mean peak time (30 min) versus the placebo group $0.4 \pm 0.2 \text{ ng/ml}$ 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 \pm SE: $12 \pm 6 \text{ ng/ml}$, 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 $+40 \text{ m}$, within group $p = 0.033$ versus placebo group $+35 \text{ m}$, 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.

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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 ($F(2, 51) = 1.10$, $p = 0.34$).

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 $p = 0.060$).

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.

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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 cmH₂O) 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 O₂ 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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Breathing irregularity during wakefulness associates with CPAP acceptance in sleep apnea

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Abstract

Purpose Individuals have different breathing patterns at rest, during wakefulness, and during sleep, and patients with sleep apnea are no different. The hypothesis for this study was that breathing irregularity during wakefulness associates with CPAP acceptance in obstructive sleep apnea (OSA).

Methods From a 2007–2010-database of patients with a diagnostic polysomnography (PSG) and prescribed CPAP ($n=380$), retrospectively, 66 patients who quit CPAP treatment at 6 months were identified. Among them, 27 OSA patients quit despite having no side effects for discontinuing

CPAP (Group A) and were compared to a matched group (age, body mass index, and apnea–hypopnea index) with good 6-month CPAP adherence (Group B; $n=21$). Five minutes of respiratory signal during wakefulness at the initial PSG were extracted from respiratory inductance plethysmography recordings, and measured in a blinded fashion. The coefficients of variation (CV) for the breath-to-breath inspiration time (T_i), expiration time (T_e), T_i+T_e (T_{tot}), and relative tidal volume, as well as an independent information theory-based metric of signal pattern variability (mutual information) were compared between groups.

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Results The CV for tidal volume was significantly greater ($p=0.001$), and mutual information was significantly lower ($p=0.041$) in Group A as compared to Group B.

Conclusions Differences in two independent measures of breathing irregularity correlated with CPAP rejection in OSA patients without nasal symptoms or comorbidity. Prospective studies of adherence should examine traits of breathing stability.

Keywords Obstructive sleep apnea · CPAP adherence · Respiratory control · Nonlinear analysis

Introduction

Individuals breathe in different ways under strictly defined conditions during quiet wakefulness. There are also significant effects of different cognitive events such as thought, attention, and emotion on the basic breathing pattern, presumably through forebrain influences. Although forebrain activity is depressed during slow-wave sleep (non-REM), breathing individuality persists [1]. Moreover, identical twins breathe with a similar pattern [2, 3]. Thus, genetic background affects breathing pattern, and accounts for variation across individuals.

Breathing variability is measured not only through physiologic extractions of respiratory frequency and tidal volume but also using analyses of the breathing signal unrelated to the physiologic extractions. The latter includes methods such as sample entropy and mutual information that deconstruct the signal, and disclose features and patterns that reveal structural underpinnings and complexity. Such complexity occurs in expression of and differs among apnea types [4]. Previously, we reported a difference in an information theory-based metric of signal pattern variability (sample entropy) among patients with obstructive sleep apnea syndrome (OSAS) comparing those with many mixed apneas (>30 % of events being mixed apneas) to those with predominant obstructive apnea. Secondary observations in this data set suggested that breathing variability during wakefulness could be a predictor of acceptance of CPAP as a therapy [5]. While studies indicate that age, sex, severity of disease, symptoms of sleepiness, socioeconomic status, nasal symptoms, and psychological factors relate to CPAP adherence in OSAS [6–15], none have considered an individual's inherent features of respiratory control as quantified in terms of measures of variability.

Using a case-control design, we tested the hypothesis that the regularity of resting breathing during wakefulness might be a predictive feature of subsequent CPAP acceptance. Patients with nasal symptoms and comorbidity known to affect adherence were excluded, and our analysis focused on the breathing pattern during wakefulness prior to the diagnostic polysomnography (PSG). Ventilatory pattern variability was quantified using conventional (linear) statistical analysis (coefficient of variation (CV)) of breath-to-

breath tidal volume and frequency from noninvasive measures, as well as an information-based analysis of the respiratory signal using mutual information, an approach that uses the raw respiratory waveform data that does not depend on either breath depth or frequency identification.

Methods

Subjects

There was an initial exclusion of patients with an apnea-hypopnea index (AHI) <20 who had medical history of arrhythmia, cerebral infarction, and psychosomatic/psychogenic diseases or who used opioid, hypnotic medications, or antidepressants; all of which might have an influence on breathing irregularity. Figure 1 shows the ascertainment profile of the study that resulted in the final comparison

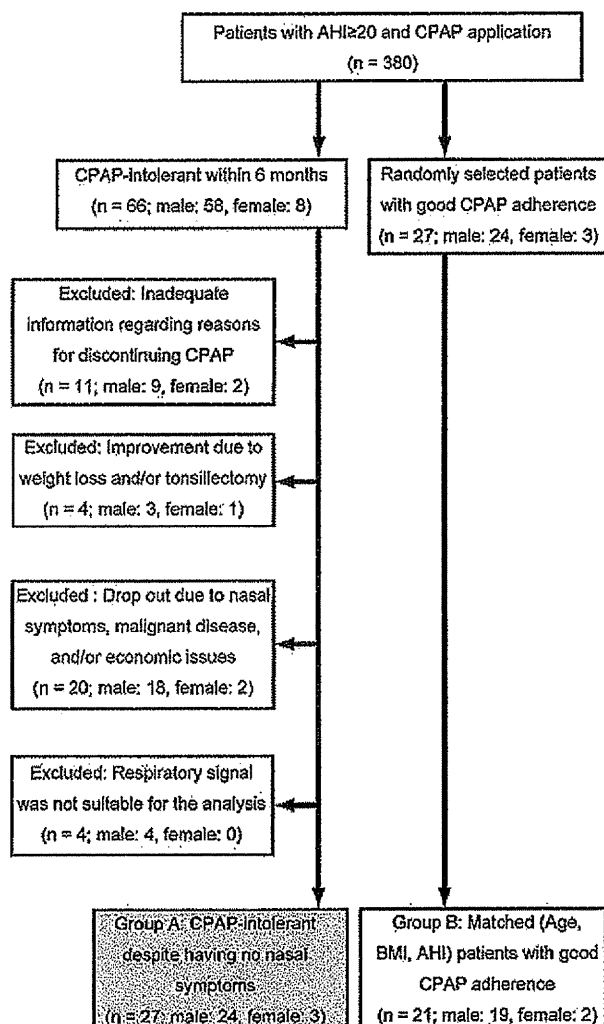


Fig. 1 Diagram for the comparison groups enrollment

groups. Among 380 patients with obstructive sleep apnea (OSA) who had a diagnostic PSG and then who were prescribed CPAP from 2007 to 2010, 66 patients quit CPAP treatment at 6 months. Among these 66 patients, 11 patients were excluded from further analysis due to loss of the information regarding reasons for CPAP dropout, 4 patients quit CPAP because sleep disordered breathing was improved by weight loss and/or tonsillectomy, 20 patients were CPAP-intolerant because of nasal symptoms, malignant diseases, and/or economic issues, and 4 patients were excluded as an adequate respiratory signal could not be obtained during wakefulness. Eventually, 27 patients were enrolled to this study analysis (Group A). We also randomly extracted 27 patients with good CPAP adherence from the same database. Fairly good adherence to CPAP was defined at a high threshold, i.e., more than 90 % of days with more than 5 h usage each night. A final group of 21 patients that were matched for age, body mass index (BMI), and AHI within the mean and standard deviation values for Group A were designated as Group B. Data were collected on the Epworth sleepiness scale (ESS) and current medications. Written informed consent was obtained from all patients, and the Human Subjects Ethics Committee of Nara Medical University approved the study.

Diagnostic PSG

Data acquisition started from 9 p.m. and continued until 6 a.m. on the following morning. Subjects were not informed that the respiratory signal before sleep onset was extracted and used for analysis. When sensors were applied and data acquisition could be initiated, subjects were instructed to close their eyes and the recording started.

The PSG was performed using a polygraph system (EEG7414; Nihon Kohden, Tokyo, Japan). electroencephalogram (EEG; C3-A2, C4-A1), bilateral EOG, submental electromyogram (EMG), ECG, and bilateral anterior tibial EMG were recorded. Airflow was monitored using an oronasal thermal sensor and/or nasal air pressure transducer. Thoracic and abdominal respiratory movements were monitored using respiratory inductance plethysmography (RIP) (Respirace; Ambulatory Monitoring Inc., Ardsley, NY, USA). Oxyhemoglobin saturation and pulse rate were monitored using pulse oximetry with a finger probe (OLV-3100; Nihon Kohden, Tokyo, Japan). All the signals were digitized and stored on a personal computer. Apneas were defined as an episode of complete airflow cessation measured from the thermal sensor lasting more than 10 s. Hypopnea was defined by ≥ 30 % reduction in amplitude of the RIP-sum signal lasting more than 10 s with ≥ 3 % oxygen desaturation. AHI was calculated as the average number of apnea-hypopnea events per hour over the total sleep period.

CPAP

All patients were initiated on nasal CPAP (REMstar Auto; Respicronics; Pittsburgh, PA, USA or GoodKnight 420E; Tyco Mallinckrodt Plaisir, France) with auto titrating mode. All patients under CPAP treatment visited our sleep laboratory every month, which is mandatory in the Japanese healthcare insurance system, and CPAP adherence was monitored every month using data extracted from the memory of the CPAP equipment. If necessary, CPAP settings including pressure range or CPAP mode (auto or fixed mode) were modified by an expert physician at the monthly visit to our laboratory. Eventually, most of the patients used CPAP with auto titrating mode during the follow up period.

Analysis of the respiratory signal

An investigator blinded to the groups chose approximately 5 min of artifact-free respiratory signal data before sleep onset, without a change in body position and scored as wakefulness, from the diagnostic polysomnography. Respiratory signals were generated by the sum of chest and abdominal signals using RIP. The sum was not calibrated to volume but adjusted to have a similar tidal displacement among subjects. The respiratory signal was identified, and the EMG (chin and limb) signal was used to detect body movements. When the amplitude of the EMG signal was high, that part of the respiratory signal was considered to be during movement and inappropriate for analysis. The part of respiratory signal in which eye movements without alpha rhythm in epochs not scored as sleep was also excluded from analysis. In the analytic phase of the study, investigators were also blinded to the group assignment, and each 5-min record of the respiratory signal during EEG-staged periods of wakefulness was analyzed for breath-to-breath inspiration time (T_i), expiration time (T_e), T_i+T_e (T_{io}), and relative tidal volume. To avoid the fluctuation of breathing due to drowsiness, we extracted 5 min of respiratory signal if EEG were considered to be fully awake for each epoch (30 s) over a 5-min period. To assess breathing irregularity, the CV ($[\text{standard deviation} / \text{mean}] \times 100$) for each parameter was calculated.

To further quantify breathing pattern variability, the mutual information of the raw respiratory signal (RIP-sum signals) sampled at 10 Hz was quantified. Mutual information is a measure of statistical dependence in a data set [16, 17]. This information theory-based metric reflects the decrease in uncertainty associated with a time-shifted data point $x(t+\tau)$ that results from knowledge of the coordinate $x(t)$. Mutual information (measured in bits) was computed as described previously [18, 19], and additional details are provided in an appendix. Due to the periodic nature of the respiratory pattern, mutual information was calculated over

multiple time delays (τ 's) from unity to one cycle length. Values were averaged across time lags excluding those with high linear correlations as defined by the first minimum of the mutual information function. Average mutual information (excluding small lags) was reported for each group. In practice, higher values of mutual information suggest increased statistical dependence (decreased variability and greater predictability) in the signal, while lower mutual information is associated with more variable (less predictable) patterns [20].

Statistical analysis

Comparison of continuous variables between the groups was done by the unpaired *t* test, and categorical variables were compared by the chi-squared test. Differences with *p* < 0.05 were considered significant. All results were expressed as means±standard deviation (SD). Statistical analysis was done with IBM SPSS Statistics 19 for Windows software (SPSS Inc., Chicago, IL).

Results

Subject characteristics

Table 1 shows subject characteristics for each group. There were no significant differences in ESS and the use of medications for hypertension, hyperlipidemia, and diabetes mellitus between groups. In Group A, the main reasons for poor CPAP acceptance were an uncomfortable feeling with CPAP or a sensation of it being hard to breathe and fall asleep. Some reported removing CPAP without awareness during sleep. Also, those who refused or could not tolerate CPAP treatment generally felt no significant improvement in presenting symptoms such as excessive daytime sleepiness,

Table 1 Subject characteristics

	Group A (n=27)	Group B (n=21)	<i>p</i> value
Age, year	51.6±10.1	51.3±10.0	N.S.
Sex, (male/female)	24/3	19/2	N.S.
AHI, /h	46.4±18.3	53.6±23.7	N.S.
ESS	10.4±5.7	11.4±6.0	N.S.
BMI, kg/m ²	25.2±3.2	26.8±2.4	N.S.
Hypertension	6/27 (22.2 %)	8/21 (38.1 %)	N.S.
Dyslipidemia	4/27 (14.8 %)	4/21 (19.0 %)	N.S.
Diabetes mellitus	3/27 (11.1 %)	0/21 (0.0 %)	N.S.

Data are shown as mean±SD or no. (%). Group A are the patients who dropped out of CPAP therapy; Group B are the patients with good CPAP adherence

AHI apnea-hypopnea index, ESS Epworth sleepiness scale, BMI body mass index, N.S. not significant

morning headache, and sleep quality. Regarding the 39 patients with poor CPAP acceptance excluded from the analysis, the AHI, age, and ESS were similar to 27 patients that were analyzed in Group A (data not shown).

Breathing irregularity during rest before sleep onset

Figure 2 shows examples of RIP-sum signals during wakefulness for two subjects with poor CPAP acceptance (Group A) and two subjects with good CPAP adherence (Group B). These tracings highlight the more irregular breathing pattern, especially in amplitude rather than respiratory frequency, prior to sleep onset in Group A as compared to Group B. Although the CV values for T_i were significantly higher in Group A (22.6±10.2 vs. 15.9±7.8 %; *p*<0.05), the CV values for T_c and T_{tot} were similar between groups (T_c , 24.0±11.3 vs. 19.5±8.5 %; T_{tot} , 18.7±8.9 vs. 15.2±6.7 %, *p*>0.05). The CV values for tidal volume in Group A were significantly greater than in Group B (30.7±7.8 vs. 22.1±9.0 %; *p*<0.01) (Fig. 3). The independent analyses of the respiratory waveform also identified differences in breathing pattern. The mutual information was significantly lower in Group A as compared to Group B (0.94±0.16 vs. 1.16±0.52 bits, respectively, *p*<0.05) (Fig. 4). Mutual information is a measure of statistical dependence between points, so the lower value in Group A suggests a greater variability of the breathing pattern during wakefulness in subjects with poor CPAP acceptance. There were no correlations between severity of OSA (AHI) and parameters for breathing irregularity including CVs and the mutual information (data not shown).

Discussion

The present study supports the hypothesis of an association of breathing irregularity during wakefulness prior to the diagnostic sleep study, as quantified by two independent measures, to CPAP adherence. We observed that breathing irregularity is greater in the patients with OSA who could not tolerate CPAP therapy than in age-, AHI-, and BMI-matched patients with good CPAP adherence. While highly selected for the absence of nasal symptoms and confounding medical conditions, these findings suggest that during wakefulness, a pattern of individual resting breathing irregularity could be a predictive marker for CPAP acceptance.

Breathing irregularity during wakefulness is associated with genetic diseases such as RETT syndrome [21, 22], with certain environments such as high altitude [23, 24], with treatment with opioid medications [25, 26], and with medical conditions including heart failure [27–29] and cerebral infarction [30, 31]. These phenomena reflect particular features of the respiratory control system that involve respiratory rhythm generation and/or central and peripheral

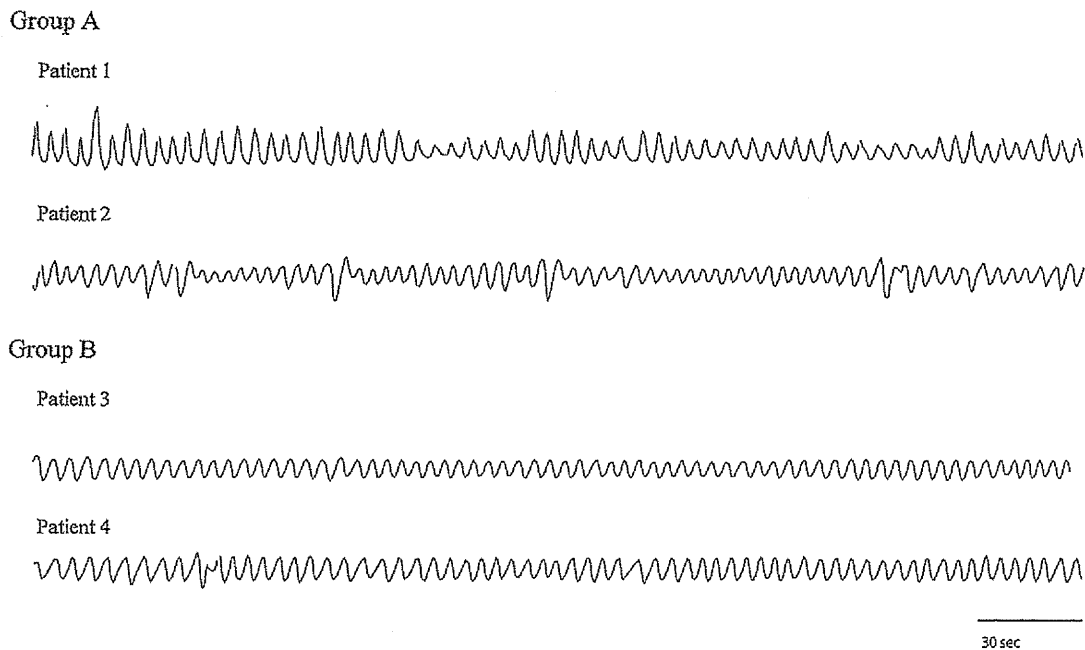


Fig. 2 Examples of RIP-sum tracings before sleep onset. Tracings of each two patients from Group A and B were presented, showing the breath-to-breath greater irregularity in tidal volume rather than

respiratory frequency in Group A. *Group A* is composed of the patients who dropped out of CPAP therapy. *Group B* is composed of the patients with good CPAP adherence

chemoreception. Breathing “stability” or “instability” is operationally defined from the output of the respiratory controller. We previously reported that breathing behavior during wakefulness even in room air and in the phase of post acute hypoxic exposure as well were different between mouse strains [32–35], thus we think that the patterning of breaths over time (periodic, chaotic, etc.) around eupnea is an important feature to begin to define not only operationally but mechanistically. In a previous study, findings suggested that the central respiratory control system in mixed apnea dominant OSAS is different from obstructive apnea dominant OSAS and closer to patterning in central apnea syndrome [5]. These results taken together with the present study indicate that patients with OSA who cannot tolerate CPAP and showed irregular breathing may have somewhat of a different respiratory control system from patients with good adherence to CPAP.

An interaction of respiratory output with the upper airway and diaphragm may determine the expression of apnea types, such as central and obstructive [4]. The relative proportion of these components would depend on individual factors, which may be genetic or secondary to a medical condition. We speculate that patients with poor CPAP acceptance may have a relatively high central gain rather than peripheral chemoreceptor component as compared to patients with good adherence to CPAP, and this difference may be reflected by the breath-to-breath variability in tidal volume. Previously, we had compared mixed apnea

predominant to obstructive apnea dominant, and in that comparison, the differences were in the variability in respiratory frequency and, to a lesser extent, tidal volume [5]. In the present study, we focused on obstructive apnea dominant patients; and, while variability was different in tidal volume, breath-by-breath variability in respiratory frequency was similar between groups. In both studies, however, analysis of the raw signal provided insight in the direction of difference, being lower in groups with less adherence.

Although the augmented breath, sigh, is considered an important component of normal breathing [36], Baldwin et al. concluded that sighs indicate maturity and functional integrity of the neurorespiratory feedback control and proposed sighs as being important for the regulation and resetting of the neurorespiratory controller [37]. Moreover, in general, sigh could occur both during stress and negative emotions, such as panic and pain, and during positive emotions, such as relaxation and relief [38–40]. In the current study, we did not exclude sighs from the 5-min segment of respiratory data. The number of sighs might affect the CV values for tidal volume. Thus, sigh was not discarded in the analysis, because it could contribute to greater tidal volume variability and poor CPAP acceptance. This is supported by previous reports that psychological factors may relate to adherence to CPAP [15]. Therefore, differences in the respiratory control system such as a high central component or an intrinsic psychological status can explain our results; however, exploring the exact mechanism for this variability

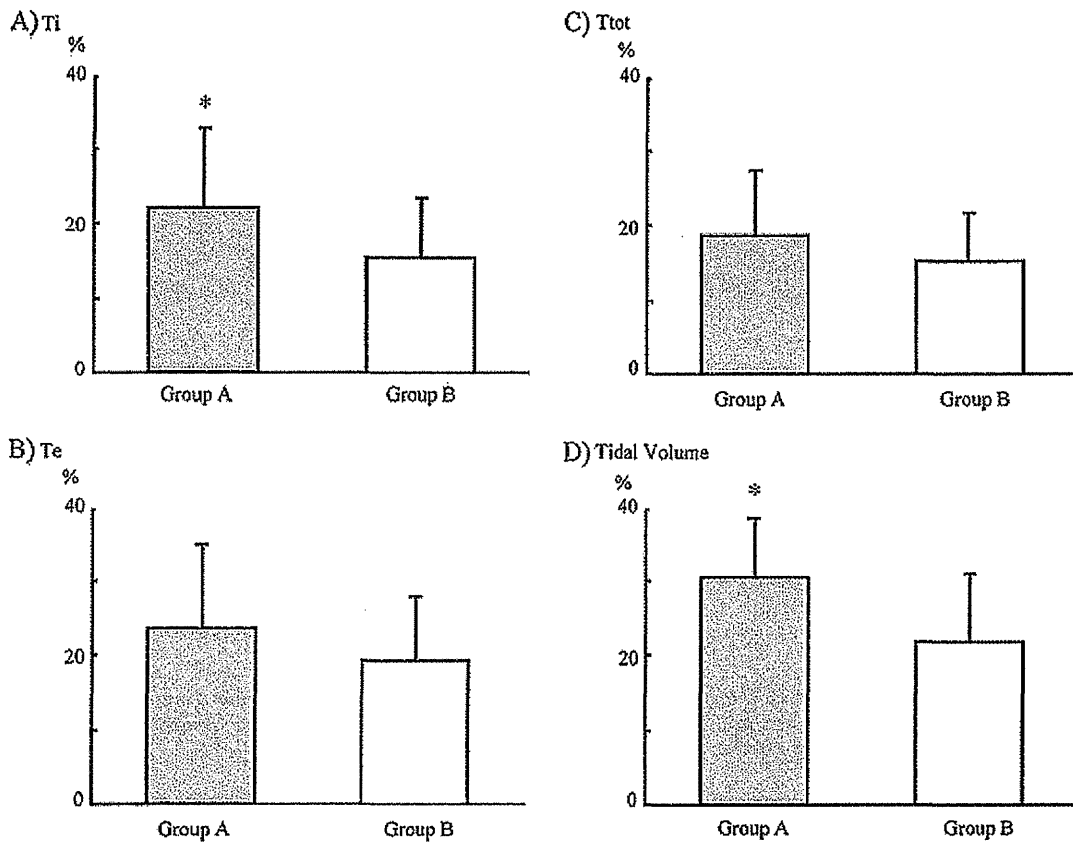


Fig. 3 Coefficients of variation for breath-to-breath respiratory variables during resting breathing before sleep onset. Values are mean±SD. a T_i , inspiration time; b T_e , expiration time; c T_{tot} , T_i+T_e , d Tidal

volume. Group A is composed of the patients who dropped out of CPAP therapy. Group B is composed of the patients with good CPAP adherence. The asterisk indicates significant difference between groups

is beyond the scope of this study. In addition, it has been recently demonstrated that arousability is one of the physiological traits that contribute to the pathogenesis of OSA [41]. Arousal due to positive airway pressure and/or CPAP discomfort may worsen CPAP adherence, thus the difference in individual arousal threshold may have contributed to our results, but further study would be needed to elucidate this issue.

A strength of the study was an ability to select a sufficient number of patients to match highly successful CPAP users to an extremely intolerant group and in both excluding known factors (stroke, opioid use, etc.) that might confound the comparisons. There are limitations in the present work. First, we cannot exclude a possible effect of hypocapnia in the poor CPAP acceptance group; however, if this were the case, then the differences between the groups would be based on a respiratory control factor such as hypercapnic responsiveness and/or apneic threshold of carbon dioxide. Second, although the statistics reveal that the CV values for tidal volume in the patients with OSAS who can not tolerate CPAP were significantly higher than in patients with good adherence, the average difference in CV values between the groups was less than 10; however, the fact that the independent measure of mutual

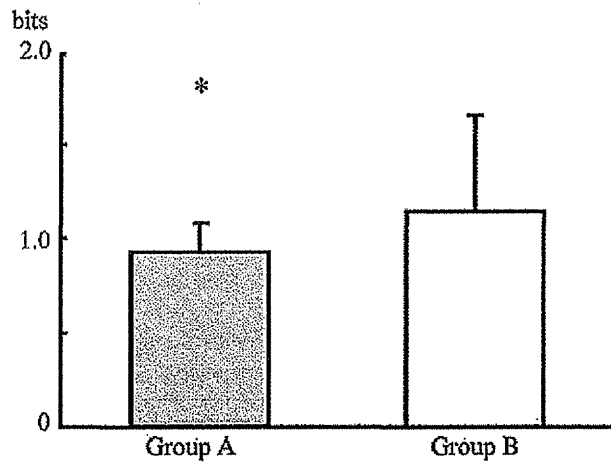


Fig. 4 The mutual information of the raw respiratory signal (RIP-sum signals). The mutual information was significantly lower in Group A as compared to Group B, suggesting a greater variability of the breathing pattern in Group A. Group A is composed of the patients who dropped out of CPAP therapy. Group B is composed of the patients with good CPAP adherence. The asterisk indicates significant difference between groups

information also showed such differences suggests that even small absolute differences could be important to consider. Third, although we successfully demonstrated a significant association between breathing irregularity during wakefulness and CPAP acceptance, this was a retrospective study. Thus, a prospective study will be needed to confirm that breathing irregularity predicts CPAP adherence. Lastly, the prescription of different commercial-based CPAP devices might be a confounding factor for CPAP adherence, but dropout rate was not different between these CPAP devices users.

In summary, we conclude that irregular breathing in terms of respiratory amplitude and temporal variability of the breathing signal during wakefulness may affect CPAP acceptance. This suggests that there are distinct features of respiratory control in patients who accept CPAP or cannot tolerate CPAP.

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Conflicts of interest None of the authors have financial conflicts of interest to declare as it relates to the contents of this manuscript.

Appendix

Mutual Information: Mutual information (MI) is a measure of the statistical dependence between two time series, or two collections of points from a data set, that can arise from both linear and nonlinear sources [42]. The Mutual information between a given time series $x(t)$ and its time-shifted version $x(t+\tau)$ is computed from the joint probability distribution of $x(t)$ and $x(t+\tau)$, where τ represents a time lag. The joint probability distribution is defined as $P[x(t), x(t+\tau)]$, where $P[x(t)]$ and $P[x(t+\tau)]$ are the marginal distributions of the original and time-shifted time series, respectively. The MI can be computed as follows:

$$MI[x(t), x(t + \tau)] = \sum_i \sum_j P[x_i(t), x_j(t + \tau)] \log \left[\frac{P[x_i(t), x_j(t + \tau)]}{P[x_i(t)] \cdot P[x_j(t + \tau)]} \right]$$

Because the breathing pattern over long time periods is strongly periodic, we computed MI for τ values from one sample (adjacent points separated by 100 ms) to one cycle length. MI tends to decrease quickly as τ is increased from a lag of one and then becomes more uniform at higher time lags, and the average MI of a given epoch was quantified excluding small lags as defined by the first minimum of the MI function.

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呼吸器疾患に伴う肺高血圧症

Pulmonary hypertension due to lung diseases

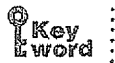


熊本牧子(写真) 木村 弘

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◎これまで呼吸器疾患に伴う肺高血圧症として、COPD を代表とする閉塞性肺疾患、肺線維症や結核後遺症などの拘束性肺疾患があげられていたが、2008年2月にアメリカ・ダナポイントで開催された第4回肺高血圧症ワールドシンポジウムでの討議を経て、あらたに拘束性および閉塞性の混合型パターンをとる呼吸器疾患が追加された。この新しいサブグループには気管支拡張症、嚢胞性肺線維症に加えて、上肺野優位の肺気腫に下肺野優位の肺線維症を合併した症例(CPFE)が含まれる。CPFEは予後が悪く、予後不良因子として約半数に肺高血圧症(PH)が関与していると報告されている。また、COPD患者において肺の機能的障害が軽度～中等度であるにもかかわらず顕著な呼吸困難と肺動脈圧の上昇を示す“out of proportion”な予後不良である一群の存在が認識されつつある。従来、呼吸器疾患に合併するPHは比較的軽度であることより、酸素療法以外の特異的治療法は行われてこなかった。しかし、肺動脈性肺高血圧症で用いられる肺血管拡張薬が適応となる可能性も考えられ、前向きに検討すべきである。シルデナフィルの有用性を示唆する報告もあり、さらなるデータの集積は今後の重要な課題である。



呼吸器疾患, 肺高血圧症(PH), 換気/血流バランス, out of proportion, 気腫合併肺線維症(CPFE)

慢性閉塞性肺疾患(chronic obstructive pulmonary disease: COPD)、間質性肺疾患などの呼吸器疾患において肺高血圧(pulmonary hypertension: PH)をきたすことは以前から知られていたが、呼吸器疾患に合併するPHはおおむね軽症～中等症であり、現時点では確立された治療は酸素療法や右心不全に対する薬剤療法が主体である。しかし近年、肺のフローボリューム曲線や画像所見に比べて高度のPHを有する症例が存在することも報告されており、このような病態に対するあらたな治療戦略の確立が必要となってきた。

COPDにおけるPHの有病率についてはいくつかの報告がある。一秒率の平均値が40%である175人のCOPD患者において、平均肺動脈圧(mean pulmonary arterial pressure: mPAP) >20 mmHgのPHの有病率は35%であった¹⁾。重症のCOPDにおいてはPH有病率が50%以上と高いもののおおむね軽症であった^{2,3)}。間質性肺疾患ではPHの有病率は32～39%と報告されており⁴⁾、肺線維症に肺気腫が合併した病態(combined pulmonary fibrosis and emphysema: CPFE)ではPH有病率は約50%程度とさらに高くなる⁵⁾。

COPDにおいては、PHの合併は生存期間の短縮³⁾(図1)および急性増悪の頻度の増加⁶⁾と関連する。肺線維症やCPFEにおいてもPHは予後不良因子である^{4,7)}。

呼吸器疾患の疫学

軽症のCOPDも含めた多数の症例に右心カテーテルを行うことは倫理上難しい。また、心エコーのみによる診断は正確さを欠くため、COPDにおけるPHの有病率については患者の全体像を表すデータはない。しかし、入院患者における

発症要因

呼吸器疾患に併発するPHの要因としてはさま

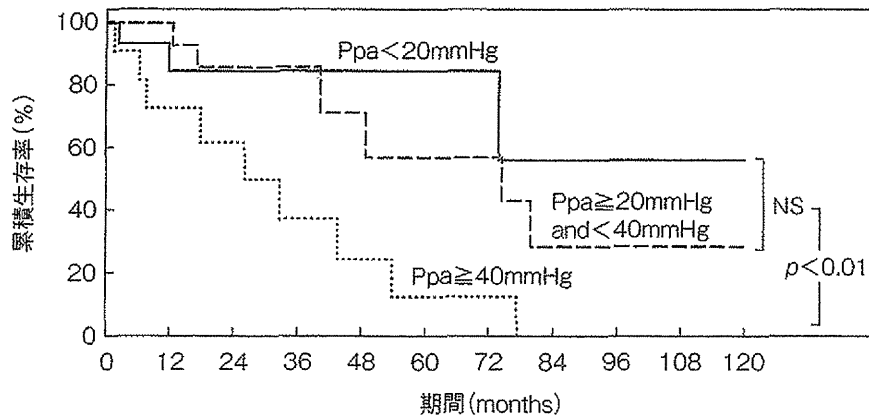


図 1 COPD患者におけるPHの重症度別の生存率³⁾
NS : not significant (有意差なし).

表 1 COPDにおいて肺血管抵抗を増強させる因子¹⁹⁾

COPDにおけるPHを起こす因子	肺血管への作用
気流の制限	血管への圧変動
気腫性病変	血管床の減少
肺泡低酸素	血管攣縮, リモデリング
高炭酸ガスによるアシドーシス	血管攣縮
赤血球増多	血液の粘性増加
肺および全身の炎症	リモデリング, 線維化

サイド
メモ

気腫合併肺線維症(CPFE)

2005年にCottinらが、気腫病変と線維化病変が合併している61例を気腫合併肺線維症(combined pulmonary fibrosis and emphysema: CPFE)という病名で報告したのを契機に、その存在が注目されるようになった⁵⁾。しかし、この病態はすでにわが国ではよく認知されており、1991年の特発性間質性肺炎の臨床的診断基準(第三次改訂案)において「特発性間質性肺炎本来の縮小性変化に気腫性変化が加わるもの」を「B群(非定型例)」として記載されている疾患群と同じであると考えられる²⁰⁾。CPFEの有病率は0.2~0.96%で、肺気腫症例の約6%と報告されている²¹⁾。①重喫煙者の男性、②肺活量や一秒率の低下は軽度であるにもかかわらず、高度の肺拡散能低下があり、労作時に著しい低酸素血症を生じる、③上肺野に小葉中心性肺気腫病変、下肺野に線維化病変の存在、④肺高血圧症の合併が約半数にあり、予後不良因子となる、⑤肺癌(とくに扁平上皮癌)の合併が多い、といった特徴がある。気腫病変と線維化病変という異なる病態が一個体に併存するのは、肺の炎症や損傷に対する修復過程においてアポトーシスの誘導、蛋白分解、線維化が生じるが、肺のそれぞれの部位で何らかの因子によって主体となる修復機転が異なることが原因ではないかと考えられている²²⁾。CPFEは肺気腫のみや肺線維症のみとは異なった臨床的特徴を有しており、予後不良因子となる肺高血圧や肺癌の合併にはとくに注意が必要である。

さまざまな見解があるが、もっとも重要な因子のひとつとして低酸素性肺血管攣縮(hypoxic pulmonary vasoconstriction: HPV)があげられる。HPVとは肺泡気の酸素分圧が低下するとその領域を走行する血管が限局的に収縮するという現象である。HPVは本来、低換気の肺泡領域の血流を下げ、換気血流比の不均衡分布による低酸素血症を予防するという生理的な反応であるが、重症のCOPDなどでは肺泡気酸素分圧が低い領域が著しく拡大し、広範な領域で肺血管収縮が起こり、肺動脈圧の上昇をきたす。

また、気腫性変化や線維性変化に伴う肺血管の破壊・閉塞・狭小化による肺血管床の減少も肺高血圧を助長する。さらに、肺泡低酸素や慢性炎症が継続すると、筋性肺動脈の内膜の増殖と、血管平滑筋の肥大といった不可逆的な形態学的変化、いわゆる肺血管のリモデリングが起こり、肺動脈血流抵抗の上昇をきたす。そのほか呼吸性アシドーシスや低酸素に起因する二次性の多血症による血液粘性の増加もPHを増強させる(表1)。

血管拡張作用のある一酸化窒素(nitric oxide: NO)や血管収縮作用のあるエンドセリン1もPH

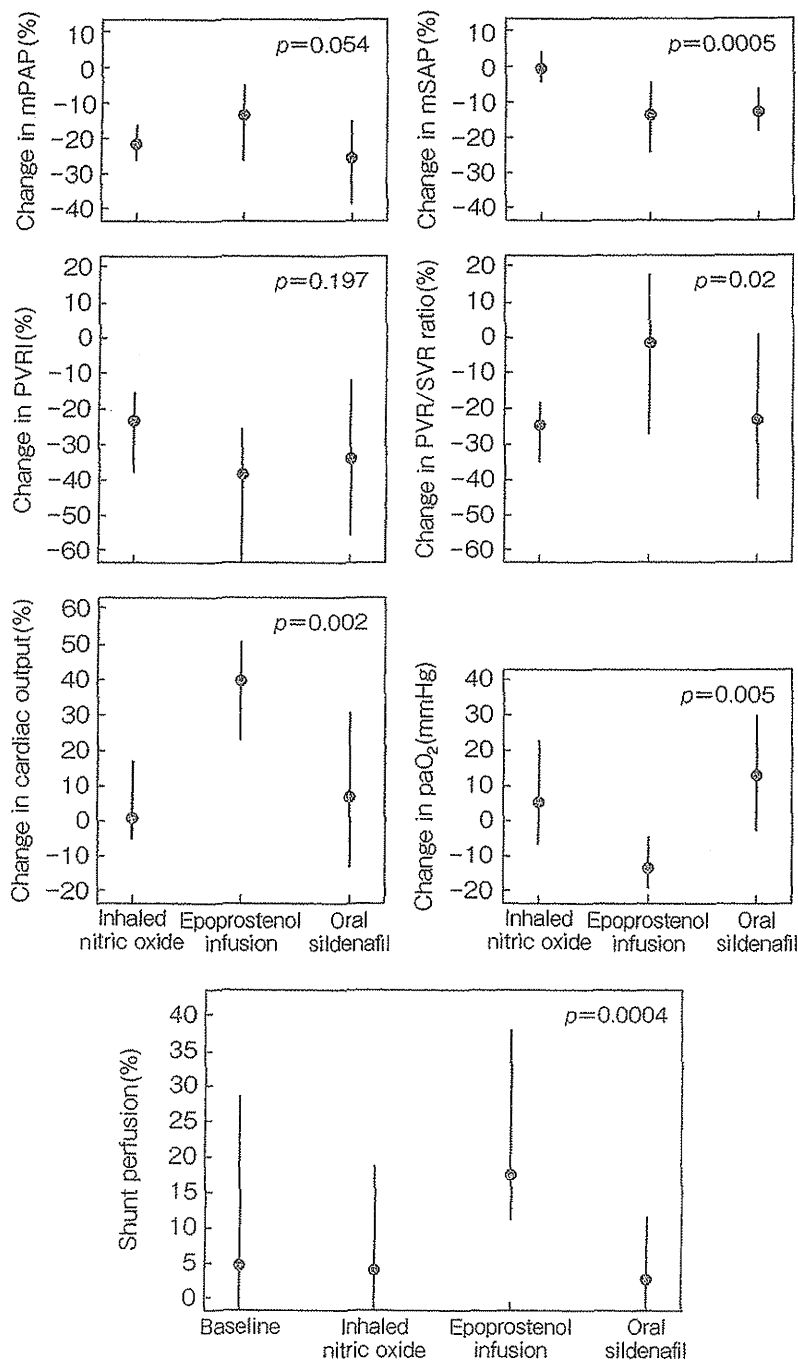


図 2 PH合併肺線維症に対するランダム化比較試験¹³⁾

一酸化窒素(NO)吸入 vs. エポプロステノール静注 vs. シルデナフィル経口。

mPAP: mean pulmonary arterial pressure(平均肺動脈圧),
 mSAP: mean systemic arterial pressure(平均全身血圧), PVRI:
 pulmonary vascular resistance index(肺血管抵抗係数), PVR/SVR
 ratio: ratio of pulmonary to systemic vascular resistance(肺血管抵抗/体血管抵抗比), paO₂: partial pressure of arterial oxygen(動脈血酸素分圧)。

の病態に関与していると考えられており、PH合併COPD患者における呼気中のNO低下や、呼気濃縮液や末梢血中のエンドセリン1の上昇が報告されている⁸⁾。

診断

PHと呼吸器疾患の臨床症状や身体的徴候は共通する部分が多く、呼吸器疾患がある患者ではPHの存在を予測することは困難なことがある。