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^{TM} 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].

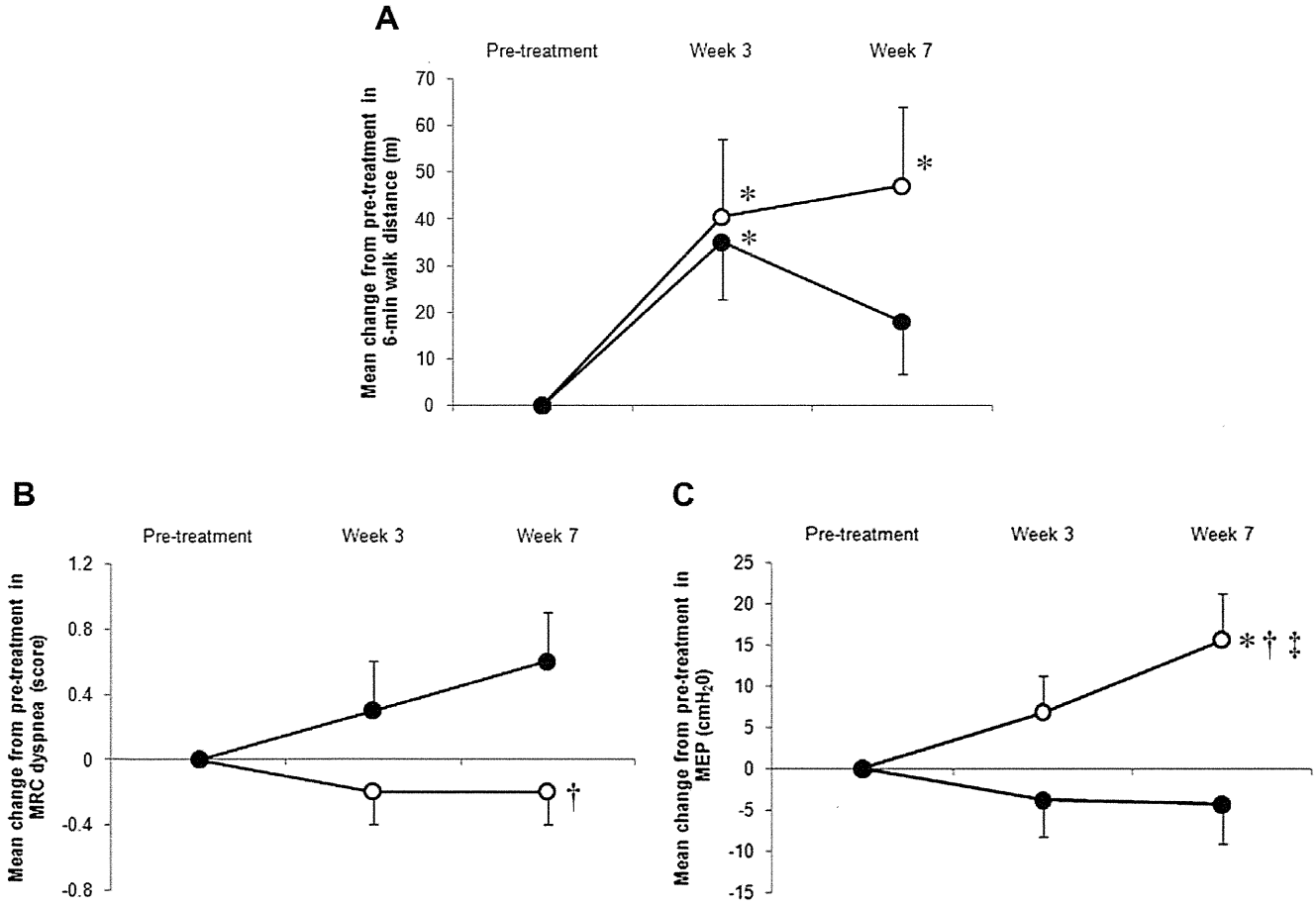

Figure 2. Change from pre-treatment in 6-min walk distance (6-MWD), Medical Research Council (MRC) score, and maximal expiratory pressure (MEP) over time. Open circles, ghrelin; closed circles, placebo. Data are presented as mean differences±SE. * $p<0.05$: change between pre- and post-treatment (within-group 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

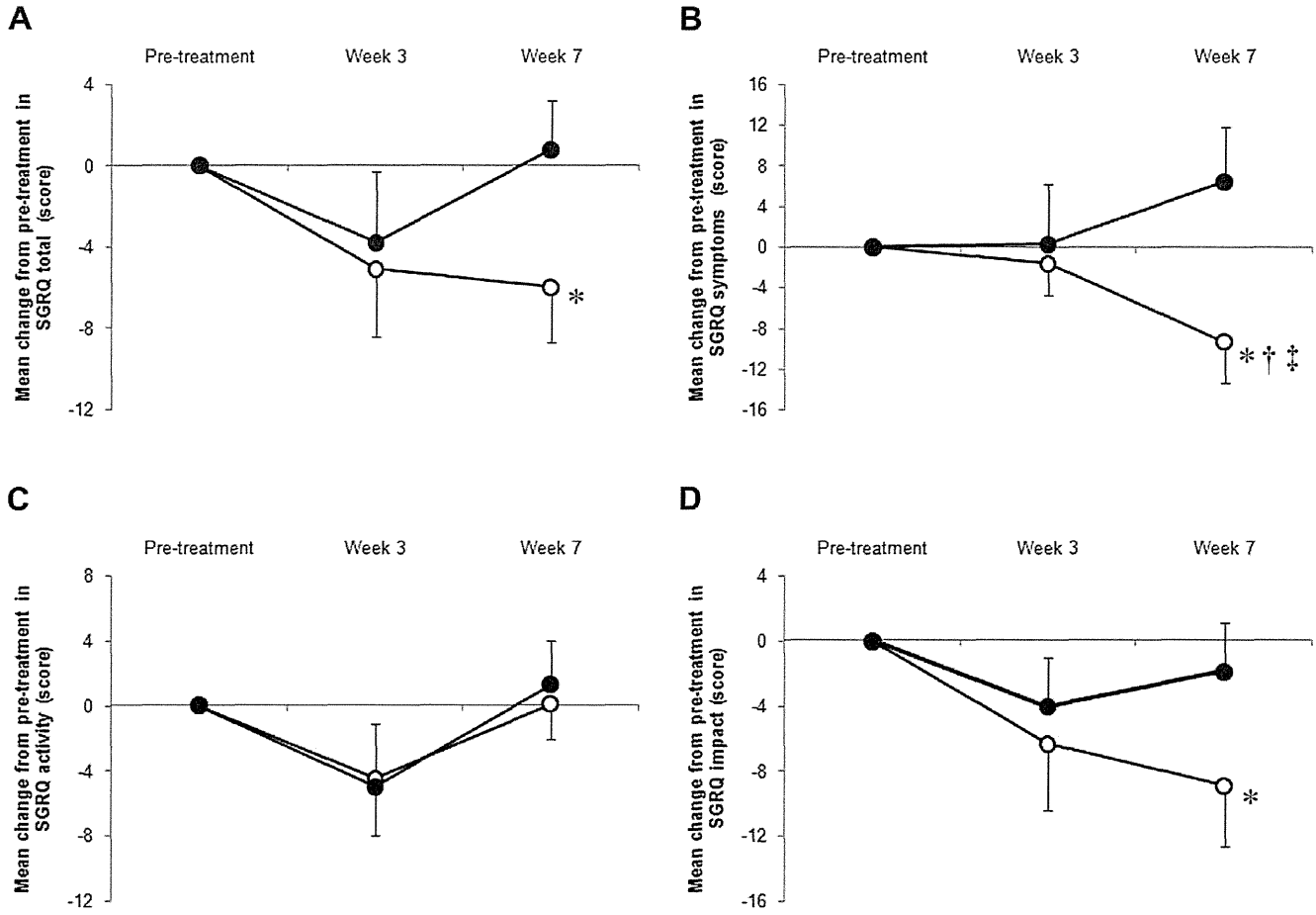

Figure 3. Change from pre-treatment in St. George Respiratory Questionnaire (SGRQ) scores over time. Open circles, ghrelin; closed circles, placebo. Data are presented as mean differences±SE. * $p<0.05$: change between pre- and post-treatment (within-group difference). † $p<0.05$: change between pre-treatment 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.
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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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SHORT COMMUNICATION

Differential effects of diazepam, tandospirone, and paroxetine on plasma brain-derived neurotrophic factor level under mental stress

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Objectives Serum brain-derived neurotrophic factor (BDNF) levels are reduced in depressed patients, and successful antidepressant treatment leads to increases in BDNF levels. However, little is known about how psychotropic drugs affect the mechanism of the human response to mental stress. We investigated the influence of psychotropic drugs on plasma BDNF levels under mental stress using a driving simulator (DS) task.
Methods Fourteen healthy male volunteers received one of four drugs, diazepam (5 mg), tandospirone (20 mg), paroxetine (10 mg), and matched placebo, in a double-blind, crossover manner. Subjects were asked to perform the DS task 4 h post-dosing. Plasma BDNF levels were measured before and after the DS task.
Results Plasma BDNF levels under the placebo, diazepam, and tandospirone conditions significantly decreased after the DS task compared with before the task. Conversely, no significant differences in plasma BDNF levels were detected under the paroxetine condition.
Conclusion As these three psychotropic drugs have differential effects on plasma BDNF levels under mental stress after 4 h post-dosing, antidepressants, unlike anxiolytics, might have a prompt positive effect on the mental stress response.

KEY WORDS—antidepressant; anxiolytic drug; brain-derived neurotrophic factor; mental stress

INTRODUCTION

Stress is common in everyday life and is believed to affect happiness, health, and cognition (Caspi *et al.*, 2003). A role for brain-derived neurotrophic factor (BDNF) in the effects of stress and the response to antidepressant treatment is supported by studies demonstrating opposing regulation of this neurotrophic factor (Charmey, 2004). BDNF, the most abundant neurotrophin in the brain, enhances the growth and maintenance of several neuronal systems and serves as a neurotransmitter modulator (Shimizu *et al.*, 2003). BDNF is present in blood and can pass through the blood–brain barrier carried by a high-capacity, saturable transport system (Pan *et al.*, 1998). Although the source and function of blood BDNF remains unknown, recent reports have shown that more than 99% of blood BDNF proteins are stored in platelets and can be released in serum (Radka *et al.*, 1996) and that blood levels of BDNF might in part reflect BDNF levels in the brain (Karege *et al.*, 2002, Mitoma *et al.*, 2008).

The "neurotrophin hypothesis of depression" is based largely on two observations: a decrease in hippocampal BDNF levels is correlated with stress-induced depressive behavior, and antidepressant treatment enhances the expression of BDNF (Martinowich *et al.*, 2007). Recent studies suggested that serum BDNF levels are reduced in depression (Sen *et al.*, 2008, aan het Rot *et al.*, 2009). Antidepressants are thought to upregulate the expression of BDNF and its receptor and to promote adult neurogenesis, which might be the core pharmacological effect of antidepressants (Martinowich and Lu, 2008); successful antidepressant treatment leads to an increase in plasma BDNF levels (Lee and Kim, 2008).

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Stress can decrease the expression of BDNF in the hippocampus (Duman and Monteggia, 2006). However, little is known about how psychotropic drugs affect the human response to mental stress. In our previous study, we examined the effects of antidepressants and anxiolytic drugs using driving simulator (DS) tasks (Iwamoto *et al.*, 2008, Takahashi *et al.*, 2010). Here, we adapted the DS task as the psychological stressor in order to examine how mental stress influences plasma BDNF levels and to investigate the effect of psychotropic drugs on plasma BDNF levels under mental stress conditions.

MATERIAL AND METHODS

Fourteen healthy male volunteers (32–44 years old, mean ± SD, 37.2 ± 3.6 years) were included. All subjects had had a driving license for at least 10 years and regularly drove a car for a minimum of 5000 km per year. Health interviews and the Structured Clinical Interview for DSM-IV conducted at the time of the study indicated that none of the participants had any physical or psychiatric disorders. The study was approved by the Nagoya University Graduate School of Medicine and Nagoya University Hospital ethics review committee, and written informed consent was obtained from each subject prior to participation.

The schedule of this study is shown in Figure 1. The study was a double-blind, placebo-controlled, crossover study with four periods of treatment, each separated by a washout period of at least 7 days. Each subject was assigned to receive four treatments in a randomized, counterbalanced order set by laboratory personnel, who did not test subjects and analyze results. The random allocation sequence of each subject was concealed until the study termination. During each treatment period, the subjects received a single dose of each of the study drugs: diazepam (5 mg), tandospirone (20 mg), paroxetine (10 mg), and matched placebo. The doses selected were based on general clinical recommendation for starting dose. All treatments were supplied in identical capsules for the double-blind design.

Each subject took one of the four drugs at 11:00 AM. The DS task was conducted 4 h after drug administration when the plasma concentration of paroxetine reaches its maximum (Doyle *et al.*, 1989, Ghose, 1989). Blood samples (10 mL) were collected in anticoagulant tubes before and after the DS task. Blood sample was immediately centrifuged at 1700 g for 10 min, and plasma sample was stored at −30 °C until used. Plasma BDNF levels were determined by enzyme-linked immunosorbent assay (Promega Co., Madison, WI, USA).

The car-following task in the DS task was used as the mental stressor. The details about this simulator (Toyota Central R&D Labs, Inc., Japan) are available elsewhere (Uchiyama *et al.*, 2003, Iwamoto *et al.*, 2008). The weighted average scores [adaptive weighted workload (AWWL)] (Miyake and Kumashiro, 1993) in the abridged Japanese version of the National Aeronautics and Space Administration Task Load Index (NASA-TLX) (Haga and Mizukami, 1996) was used to evaluate the mental stress of the car-following task. Seventeen healthy male volunteers completed the following two mental stress tasks using the DS in random order. One was a standard driving task, which required the subjects to drive the car freely on the road, and another was the car-following task that required the subjects to maintain a constant distance between the cars without the discretion of subjects. The time needed for the completion of both tasks is 5 min. The subjects were asked to rate the NASA-TLX after finishing each task, and the AWWL scores for each condition were calculated for subsequent analysis.

Statistical differences were determined with the paired *t*-test. Significance levels were set to 5% for all tests.

RESULTS

The AWWL scores for the car-following task condition were significantly higher than the normal driving task condition (mean ± SD: 55.2 ± 16.9 vs. 38.2 ± 20.8; $p < 0.01$).

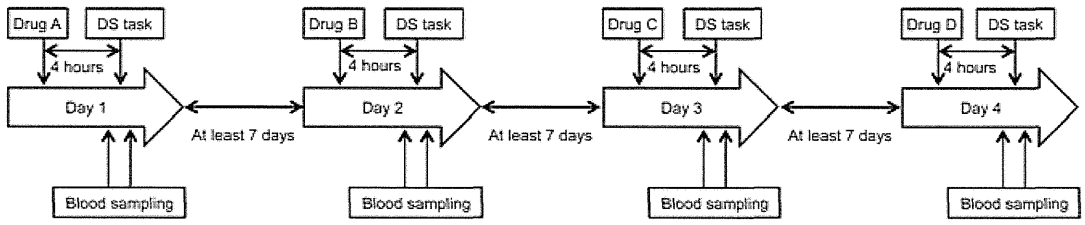

Figure 1. The figure shows the schedule of the study. Days 1, 2, 3, and 4 are treatment periods; each is separated by a washout period of at least 7 days. During each treatment period, the subjects received a single dose of one of the study drugs (drugs A, B, C, and D): diazepam (5 mg), tandospirone (20 mg), paroxetine (10 mg), and matched placebo. Each subject took one of the four drugs at 11:00. The DS task was conducted 4 h after drug administration. Blood samples were collected before and after the DS task

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The effects of psychotropic drugs on plasma BDNF levels before and after the car-following task are shown in Figure 2. Under the placebo condition, plasma BDNF levels after the car-following task were significantly decreased compared with the plasma BDNF levels before the task (mean ± SD: 0.64 ± 0.31 vs. 0.34 ± 0.21, $p < 0.01$). We also found that under the diazepam and tandospirone conditions, plasma BDNF levels after the car-following task were significantly decreased compared with plasma BDNF levels observed before the task (mean ± SD: 0.49 ± 0.23 vs. 0.34 ± 0.21, $p < 0.05$ and mean ± SD: 0.59 ± 0.36 vs. 0.31 ± 0.14, $p < 0.01$, respectively). Conversely, these changes were not observed under the paroxetine condition (mean ± SD: 0.57 ± 0.27 vs. 0.79 ± 0.63, $p = 0.19$).

DISCUSSION

From the AWWL scores, we considered the car-following task as a mental stress condition. In the present study, we investigated the effect of psychotropic drugs on plasma BDNF levels under mental stress using a DS task as the stressor. Although the task associated with increased mental stress significantly decreased plasma BDNF levels under the diazepam, tandospirone, and placebo condition, the same effect was not observed under the paroxetine condition.

Regarding psychological stress, a previous study of healthy subjects demonstrated that levels of perceived mental stress in the workplace were inversely correlated with serum BDNF levels (Mitoma *et al.*, 2008). Both acute and chronic mental stress may reduce serum BDNF levels. According to these findings, mental stress might negatively affect stress-vulnerable depressed patients in whom serum BDNF levels are already decreased.

A previous report indicated that antidepressants could enhance BDNF gene expression by activating cyclic adenosine monophosphate response element binding protein (Martinowich and Lu, 2008). Furthermore, a recent study showed that antidepressants directly promote BDNF release from platelets in rats (Watanabe *et al.*, 2010). Considering that plasma BDNF levels did not significantly decrease under mental stress following acute administration of paroxetine in our result, paroxetine might promote short-term (several minutes) BDNF release from platelets in human models, although further examination would be needed.

Although anxiolytic drugs such as diazepam and tandospirone can relieve stress-related symptoms, there are no reports indicating that anxiolytic drugs influence plasma BDNF levels. One study showed that stimulation of the gamma-aminobutyric acid system (i.e., diazepam) in adult Wistar rats results in an immediate decrease in hippocampal BDNF mRNA levels (Zafra *et al.*, 1991). To our knowledge, the effects of diazepam on plasma BDNF levels have not been examined in humans. The present results suggest that benzodiazepine had no influence on plasma BDNF

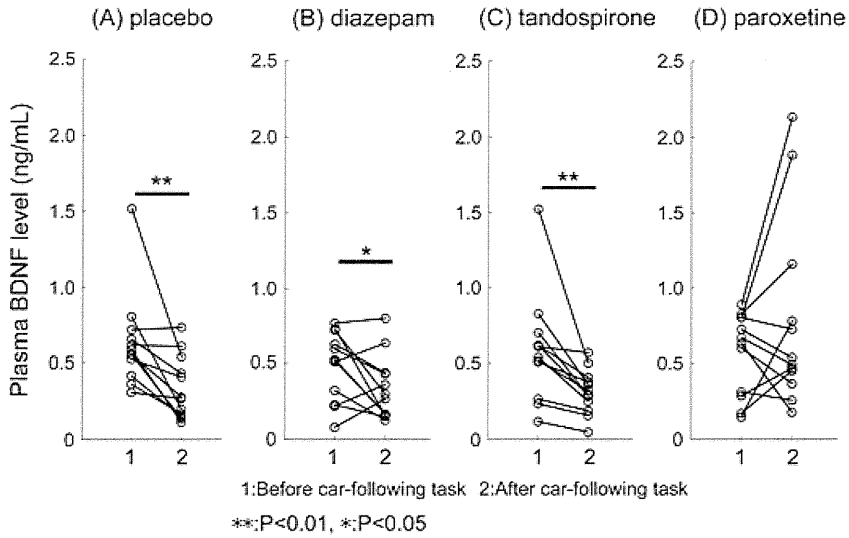

Figure 2. The effects of psychotropic drugs on plasma BDNF levels before and after the car-following task. Panel (A) shows the change in plasma BDNF levels during the placebo condition. Plasma BDNF levels after the car-following task are significantly decreased compared with levels before the task (p 0.01). Panels (B) and (C) show plasma BDNF levels following diazepam and tandospirone conditions. Plasma BDNF levels are significantly decreased after the car-following task compared with levels before ($p < 0.05$ and $p < 0.01$, respectively). Panel (D) shows plasma BDNF levels under the paroxetine condition. There is no significant difference in plasma BDNF levels before and after the car-following task ($p = 0.19$)

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levels immediately following stress. In terms of the "neurotrophin hypothesis of depression," antidepressants, but not anxiolytic drugs, can ameliorate the symptoms of depression and prevent stress-related recurrence of depression.

The present study has several limitations. First, the sample was restricted to a small number of healthy adult male volunteers. It is possible that the responses to mental stress in female, depressed, or elderly patients could differ widely from those of healthy, younger men. Second, the present study evaluated only the immediate effects of low-dose administration of the drugs on plasma BDNF levels. Third, a 5-min simulator task may be inadequate for assessing mental stress. Although AWWL scores showed that this task induced mental stress, there is a possibility of a type 1 error because of the small sample size. Therefore, future studies using a larger number of subjects with repeated drug administration over a range of doses need to be conducted for conclusions to be drawn regarding the effects on plasma BDNF level. From the AWWL score, we regarded the car-following task as a mental stress condition, although there is no significant difference in plasma cortisol levels before and after the car-following task (data not shown). Then it is necessary to examine how the duration of the DS task influences plasma BDNF levels in more detail. Fourth, the degree of stress associated with the DS task needs to be examined by measuring changes in other stress-related variables (e.g., heartbeat and skin electrical resistance). Fifth, we did not examine plasma BDNF level change at 4 h post-dosing without DS task to elucidate whether drug treatments without DS task could affect plasma BDNF levels. Finally, we evaluated only 4-h time point for DS task when plasma concentration of paroxetine reaches its maximum. Because three drugs have different pharmacokinetic and pharmacodynamic profiles, we need to examine plasma BDNF level change at a time when plasma concentrations of diazepam and tandospirone reach their maximum in future study.

Our findings should be interpreted with following caveat. The treatments for depression, such as antidepressants (Shimizu *et al.*, 2003), electroconvulsive therapy (Okamoto *et al.*, 2008), and sleep deprivation (Gorgulu and Caliyurt, 2009) increase expression of BDNF. Although this is suggesting that there is an etiological link between the development of depression and BDNF, scientific studies have found that numerous brain areas show altered activity in depressed patients (Krishnan and Nestler, 2008), and it has not been possible to determine a single cause of depression.

In conclusion, diazepam, tandospirone, and paroxetine could have different effects on plasma BDNF levels under mental stress after 4 h post-dosing. Furthermore, antidepressants, unlike anxiolytics, might have immediate positive effects on the mental stress response.

CONFLICT OF INTEREST

None declared.

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Sexual dysfunction and hyperprolactinemia in Japanese schizophrenic patients taking antipsychotics

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ABSTRACT

This study aimed to estimate the prevalence of sexual dysfunction, evaluated by the Nagoya Sexual Function Questionnaire (NSFQ), and hyperprolactinemia in patients with schizophrenia and examine a relationship between sexual dysfunction and serum prolactin levels. This cross-sectional, comparative study was performed using a sample comprising 195 Japanese schizophrenic in- and outpatients treated with antipsychotics (117 males and 78 females). Data were collected from October 2009 to January 2010 using single, cross-sectional ratings of sexual function assessed by the NSFQ and concurrent measurement of serum prolactin levels. The prevalence of sexual dysfunction in patients with schizophrenia was high (males 66.7%; females 79.5%). Hyperprolactinemia (>25 ng/ml) was highly prevalent among schizophrenia patients, affecting 53.8% of females and 51.3% of males. Among female patients, 16.7% had prolactin levels>100 ng/ml. There was no relationship between sexual dysfunction and serum prolactin levels. The present study demonstrated a higher prevalence of sexual dysfunction and hyperprolactinemia in Japanese schizophrenia patients. Clinicians should keep these problems in mind and discuss potential solutions with patients to improve patients' quality of life and adherence to therapy.

1. Introduction

Despite evidence showing that sexual dysfunction is common in patients with schizophrenia (Cutler, 2003; Ghadirian et al., 1982; Kotin et al., 1976; Smith et al., 2002), physicians tend to overlook or disregard sexual dysfunction during the psychiatric evaluation of schizophrenic patients. For example, approximately 50–70% of male schizophrenic patients and 30–50% of female schizophrenic patients have sexual dysfunction (Fakhoury et al., 2001; Ghadirian et al., 1982). Sexual disturbances in such patients may be due to various factors, including the symptoms of schizophrenia (Aizenberg et al., 1995), secondary effects of living with a severe, chronic mental health condition, or adverse effects of antipsychotics or other medications (Smith et al., 2002). In particular, drugs that raise prolactin levels, such as risperidone, are associated with significantly higher rates of sexual problems (40–60%) compared with prolactin-sparing drugs (e.g., quetiapine, ziprasidone, and aripiprazol) (<30%) (Knegtering et al., 2004; Knegtering et al., 2006; Montejo Gonzalez et al., 2005; Montejo and Rico-Villademoros, 2008a; Montejo et al., 2010a, 2010b; Serretti and Chiesa, 2011).

The studies mentioned above present data about sexual dysfunction among patients with schizophrenia in Western countries. Conversely, there are few studies in Asian populations, including Japanese patients (Fujii et al., 2010). The prevalence of sexual concerns differs in healthy individuals according to their ethnicity. However, the accurate estimation of prevalence is complicated by the reluctance of psychiatric staff (i.e., psychiatrists and nurses) to discuss sexual concerns with patients (Withersty, 1976; Wolfe and Menninger, 1973). These problems are more pronounced in Far Eastern countries, perhaps due to socio-cultural reasons (Moreira et al., 2005, 2006). From a clinical point of view, it is important to be aware of sexual dysfunction in patients with schizophrenia and apply the knowledge of sexual dysfunction to the treatment of schizophrenia, because this symptomatology is relatively common among patients and may contribute to poor quality of life (QOL) and poor adherence with therapy (Gopalakrishnan et al., 2006; Olfson et al., 2005).

In Western countries, several instruments are used to assess sexual dysfunction, including the Arizona Sexual Experience Scale (ASEX)

Abbreviations: ASEX, Arizona Sexual Experience Scale; CLIA, chemiluminescence immunoassay; CP, chlorpromazine; CSFQ, Changes in Sexual Functioning Questionnaire; NSFQ, Nagoya Sexual Function Questionnaire; PRSexDQ-SALSEX, Psychometric Properties of the Psychotropic-Related Sexual Dysfunction Questionnaire; QOL, Quality Of Life; UKU, Udvalg for Kliniske Undersogekser Side Effect Rating Scale.

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(McGahuey et al., 2000), the sexual part of Udvalg for Kliniske Undersogekser Side Effect Rating Scale (UKU) (Lingjaerde et al., 1987), Psychometric Properties of the Psychotropic-Related Sexual Dysfunction Questionnaire (PRSexDQ-SALSEX) (Montejo and Rico-Villademoros, 2008b; Montejo et al., 2000), and the Changes in Sexual Functioning questionnaire (CSFQ) (Clayton et al., 1997). Although most instruments are useful (in particular, SALSEX is very brief, user-friendly, and reliable), some of them are rather long (CSFQ has 36 items for men and 35 items for women). In addition, the contents of ASEX, UKU, and PRSexDQ-SALSEX include vaginal dryness, vaginal lubrication, and orgasmic dysfunction, which may be intrusive for *patients and clinicians who feel embarrassed to talk about sexual concerns directly (Asian people in particular)*, and difficult to use (UKU involves a semi-structured interview). Therefore, it may not be easy to use these instruments among schizophrenic patients who are reluctant to discuss their sexual concerns, a topic that is sometimes considered taboo not only in Asian but also in Western countries. To address this problem, we recently developed and validated a short, minimally intrusive, and self-administered instrument called the Nagoya Sexual Function Questionnaire (NSFQ) (Kikuchi, et al., 2011).

From a pharmacological point of view, side effects of antipsychotics characterized by sexual dysfunction are related to hyperprolactinemia (Haddad and Wieck, 2004). More than 50% of schizophrenic patients treated with a prolactin-raising antipsychotic drugs experience hyperprolactinemia (Haddad and Wieck, 2004). Psychiatric staff tend to disregard symptoms of hyperprolactinemia that involve sexual dysfunction (loss of/decreased libido, erectile dysfunction [men], gynecomastia [men], amenorrhoea/oligomenorrhoea [women]), because clinical signs are subtle or even if the symptoms of hyperprolactinemia become obvious, patients and professionals are embarrassed to discuss them (Haddad and Wieck, 2004). Moreover, it is unclear to what extent sexual dysfunction is due to a direct effect of increased prolactin levels (Haddad and Wieck, 2004). Additionally, sexual dysfunction in Asian schizophrenic patients is a topic that has not been fully investigated due to the socio-cultural reasons stated above.

Considering the lack of the studies conducted in a Japanese population, and the implication for therapeutic intervention, our study aimed to estimate the prevalence of sexual dysfunction obtained from the measurement of NSFQ and hyperprolactinemia in Japanese schizophrenic patients and examine the relationship between sexual dysfunction and serum prolactin levels.

2. Methods

The present study was a cross-sectional, comparative trial. Data were collected from October 2009 to January 2010 using a single, cross-sectional rating of sexual function assessed by the NSFQ. Concurrently, measurement of serum prolactin levels was performed. Subjects were returning outpatients and inpatients with a diagnosis of schizophrenic disorder according to DSM-IV criteria. All patients had been stabilized on antipsychotic medication for more than 8 weeks. Patients were excluded if they had a general medical condition or a history of a surgical procedure known to cause sexual dysfunction. Psychotropic medications such as benzodiazepines, anticholinergics, antidepressants, and mood stabilizers were allowed if the patients were already receiving these medications prior to study enrollment. However, in this research, no patient was receiving antidepressants.

After obtaining demographic and medication/treatment information, an introductory presentation was made, during which the nature of the study was explained to the patients, and written informed consent was obtained from patients willing to participate. The study was approved by the Ethics Committee of the Nagoya University School of Medicine.

The NSFQ is a self-administered sexual function scale (Kikuchi, et al., 2011). The NSFQ was developed through the collaborative effort of specialists in psychiatry and urology. The NSFQ consists of seven items. Each item is evaluated on a six-point scale: (1) not at all; (2) almost never; (3) sometimes; (4) often; (5) always; and (6) unsure. The answers of (1) through (5) are assigned scores of 1 to 5 points, respectively, and (6) is assigned 1 point; total scores range from 5 to 35. The items for men are: 1) pulsating sensation in the breast/mammary area; 2) galactorrhea; 3) interest in women; 4) sexual interest; 5) sexual self-confidence; 6) erectile dysfunction; and 7) ejaculatory dysfunction. The items for women are: 1) menstrual irregularity; 2) pulsating sensation in the breast/mammary area; 3) galactorrhea; 4) interest in men; 5) sexual interest; 6) sexual self-confidence; and 7) sexual arousal. Subjects were asked to answer questions 6 and 7 if they gave scores of (2)–(5) for questions 1–5. To estimate the potential prevalence of sexual dysfunction, subjects with a score of 3 or higher on any relevant items in the NSFQ were considered to have sexual side effects. Subjects with a score of 3, 4, or 5 on each of the relevant items were considered to have mild, moderate, or severe sexual side effects, respectively. The prevalence of total sexual dysfunction was calculated by the following equation: the number of patients with a score of 3 or higher on any of the NSFQ items was divided by the total number of patients regardless of sexual dysfunction. Data for menstrual irregularity were recorded only for female subjects younger than 45 years. Prolactin levels were determined by chemiluminescence immunoassay (CLIA) (Siemens, Munich, Bayern, Germany). Blood was drawn for prolactin levels from 9:30 a.m. to 10:30 a.m. In this study, normal prolactin levels for female and male patients were $\leq$25 ng/ml and $\leq$20 ng/ml, respectively. All analyses were performed using JMP version 5.1.2 (SAS Institute, Inc., Cary, NC). The student *t*-test was used to compare: (1) mean prolactin levels between patients with and without sexual dysfunction, and (2) mean total score of the NSFQ and prolactin levels between men and women. We performed one-way factorial analysis of variance and multiple comparison tests (Tukey's Honestly Significant Difference test) to compare mean total NSFQ scores of among stratified prolactin levels (male: 0–20 ng/ml, 20–50 ng/ml, 50–100 ng/ml, female: 0–25 ng/ml, 25–50 ng/ml, 50–100 ng/ml, 100–150 ng/ml, 150–200 ng/ml), and mean total NSFQ scores and prolactin levels among groups receiving aripiprazole, olanzapine, risperidone, and polytherapy. The chi square test was used to test for differences between the frequency of total sexual dysfunction between men and women, and among groups receiving aripiprazole, olanzapine, risperidone, and polytherapy in men and women. We performed multiple regression analysis with the total NSFQ score as the dependent variable and prolactin level, age, sex, duration of illness, duration of treatment, number of antipsychotics, and dose of antipsychotics (chlorpromazine [CP] equivalent) as independent variables. A p value$<$0.05 was considered statistically significant.

3. Results

3.1. Characteristic of patients

Clinically relevant patient characteristics are presented in Table 1. Patients were divided into monotherapy groups receiving a single antipsychotic of risperidone, olanzapine, aripiprazole, or others, and the polytherapy group. The mean CP equivalent doses of antipsychotics and concomitant drugs (benzodiazepines and anticholinergics) in each group are shown in Table 1.

3.2. Prevalence of sexual dysfunction

Prevalence of sexual dysfunction is shown in Table 2. We observed gender-specific differences in the prevalence of sexual dysfunction. Specifically, the prevalence of moderate and severe sexual dysfunction in female patients was significantly higher than in male patients (chi square 6.633 p = 0.01).

Table 1
Characteristic of patients.

	Males	Females
N	117	78
Age (years)	43.9 ± 12.7	45.9 ± 12.1
Duration of illness (months)	247.2 ± 194.0	231.5 ± 162.9
Treatment of illness (months)	226.5 ± 187.2	199.9 ± 164.2
Dose of antipsychotics (mg/day)[a]	629.7 ± 406.7	595.4 ± 379.5
Dose of concomitant benzodiazepines (mg/day)[b]	7.0 ± 11.8	1.0 ± 1.9
Dose of concomitant anticholinergics (mg/day)[c]	1.2 ± 1.7	0.6 ± 1.8
Drug group		
Risperidone only		
N	42	27
Dose of risperidone (mg/day)[a]	539.5 ± 288.4	570 ± 349.5
Dose of concomitant benzodiazepines (mg/day)[b]	1.6 ± 3.1	1.6 ± 2.2
Dose of concomitant anticholinergics (mg/day)[c]	1.6 ± 1.8	0.7 ± 2.1
Olanzapine only		
N	15	9
Dose of olanzapine (mg/day)[a]	507.1 ± 233.5	722.2 ± 120.1
Dose of concomitant benzodiazepines (mg/day)[b]	3.3 ± 4.7	0.8 ± 2.1
Dose of concomitant anticholinergics (mg/day)[c]	0.2 ± 0.8	0
Aripiprazole only		
N	14	17
Dose of aripiprazole (mg/day)[a]	344.6 ± 297.8	411.8 ± 228.1
Dose of concomitant benzodiazepines (mg/day)[b]	8.6 ± 17.8	0.5 ± 1.0
Dose of concomitant anticholinergics (mg/day)[c]	0	0.4 ± 1.2
Other drugs (as monotherapy)		
N	3	7
Dose of antipsychotics (mg/day)[a]	165.5 ± 92.6	218.5 ± 107.6
Dose of concomitant benzodiazepines (mg/day)[b]	3.4 ± 4.7	0
Dose of concomitant anticholinergics (mg/day)[c]	0	3.1 ± 4.4
Polytherapy		
N	45	18
Numbers of antipsychotics	2.5 ± 0.7	2.44 ± 0.8
Dose of antipsychotics (mg/day)[a]	878.1 ± 464.9	889.4 ± 461.0
Dose of concomitant benzodiazepines (mg/day)[b]	9.1 ± 11.1	1.0 ± 1.0
Dose of concomitant anticholinergics (mg/day)[c]	0.21 ± 0.8	0

Values are mean ± SD unless otherwise noted.
[a] Chlorpromazine-equivalent dose.
[b] Diazepam-equivalent dose.
[c] Biperiden-equivalent dose.

The most prevalent symptom of male sexual dysfunction was lack of sexual self-confidence in both the moderate and severe groups, whereas the most prevalent symptom of female sexual dysfunction was menstrual irregularity in the moderate group and lack of sexual interest in the severe group.

3.3. Total NSFQ score and serum concentrations of prolactin

The total NSFQ score and serum concentrations of prolactin are shown in Table 3. Results showed that hyperprolactinemia was highly prevalent among schizophrenia patients (males, 51.3%; females, 53.8%). Moreover, 16.7% of female patients showed extremely high concentrations of prolactin (>100 ng/ml). The mean prolactin level in female patients (45.3 ± 46.7 ng/ml) was significantly higher than in male patients (21.5 ± 16.7 ng/ml) ($t=5.0357$, $p<0.001$), but there was no significant difference between male patients (12.7 ± 5.5) and female patients (14.0 ± 5.1) in mean total NSFQ score ($t=1.6743$, $p=0.0957$). There were no significant differences in total NSFQ score among stratified prolactin levels in male patients ($F=1.219$ $df=116$, $p=0.299$) and female patients ($F=0.486$, $df=77$, $p=0.746$). Similar values of total NSFQ scores were observed in all stratified levels of prolactin.

3.4. Antipsychotic effect

Total NSFQ score, the frequency of total sexual dysfunction, and prolactin level of each antipsychotic treatment group are summarized by gender in Table 4. There were significant differences in the prolactin levels among the antipsychotic groups receiving aripiprazole, olanzapine, polytherapy, and risperidone in males ($F=13.251$, $df=114$, $p<0.001$; aripiprazole<polytherapy, $p<0.001$; aripiprazole<risperidone, $p<0.001$; olanzapine<polytherapy, $p=0.026$; olanzapine<risperidone, $p<0.001$) and females ($F=14.107$, $df=70$, $p<0.001$; aripiprazole<polytherapy, $p<0.01$; aripiprazole<risperidone, $p<0.001$; olanzapine<risperidone, $p<0.01$). As a whole, prolactin levels became higher in the following order: aripiprazole, olanzapine, polytherapy, and risperidone. However, the total NSFQ scores were not significantly different among the groups receiving aripiprazole, olanzapine polytherapy, and risperidone in males ($F=0.075$, $df=114$, $p=0.973$) and females ($F=1.537$, $df=70$, $p=0.213$). There was no significant difference in the frequency of total sexual dysfunction among groups receiving aripiprazole, olanzapine polytherapy, and risperidone (males, from mild to severe, chi square 1.5508 $p=0.82$; moderate and severe, chi square 4.3366 $p=0.36$; females, from mild to severe, chi square 9.0318 $p=0.06$; moderate and severe, chi square 7.6454 $p=0.11$). The frequency of total sexual dysfunction (from mild to severe) becomes higher in the following order: risperidone (64.3%), polytherapy (64.4%), olanzapine (66.7%), and aripiprazole (78.6%) in males, and olanzapine (55.6%), aripiprazole (82.4%), polytherapy (83.3%), and risperidone (92.6%) in females. Moderate and severe total sexual dysfunction becomes higher in the following order: polytherapy (33.3%), aripiprazole (35.7%), risperidone (45.2%), and olanzapine (53.3%) in males, and olanzapine (33.3%), aripiprazole (58.8%), risperidone (66.7%), and polytherapy (72.2%) in females.

3.5. Multiple regression analysis

We performed multiple regression analysis with total NSFQ score as the dependent variable and prolactin level, age, sex, duration of illness, duration of treatment, number of antipsychotics, and dose of antipsychotics (CP equivalent) as independent variables; however, no statistically significant correlations were found (R 0.2126, $p=0.2698$). No significant differences in mean prolactin levels were observed between the group with and without sexual dysfunction in males (prolactin level: 22.0 ± 17.0 in the group with sexual dysfunction, 20.6 ± 16.1 in the group without sexual dysfunction, $t=0.4245$, $p=0.6720$) and females (prolactin level: 50.0 ± 48.0 in the group with sexual dysfunction, 27.1 ± 37.1 in the group without sexual dysfunction, $t=1.7694$, $p=0.0808$).

4. Discussion

This study surveyed sexual dysfunction using a self-administered sexual functional scale, the NSFQ, and showed that sexual dysfunction is highly prevalent among Japanese patients suffering from schizophrenia. Our study revealed that patients' sexual dysfunction extended over multiple domains that were evaluated by different items of the NSFQ.

According to previous studies (Cutler, 2003; Ghadirian et al., 1982), the rate of sexual dysfunction was reported to be lower in female patients with schizophrenia than males with schizophrenia. However, Fujii et al. (2010) reported that sexual dysfunction in males is similar to that in females, and their results were consistent with our findings. As Fujii et al. noted, this tendency could be influenced by menstrual irregularities that are classified as sexual dysfunction. However, if menstrual irregularities (48.6%) were excluded from our dataset, the prevalence of sexual dysfunction in females did not change significantly (from 79.5% to 70.5%). The reason for the difference between the current study and prior research is that NSFQ reveals not only symptoms of sexual dysfunction that are obvious, such as menstrual irregularities, but also symptoms of sexual dysfunction that women are reluctant to discuss and therefore can be easily overlooked by medical professionals.

Table 2
Prevalence of sexual dysfunction in males and females.

Gender	Category	≥3 (from mild to severe) n (%)	≤3 n (%)	≥4 (moderate and severe) n (%)	≥4 n (%)
Males	Total sexual dysfunction	78 (66.7)	39 (33.3)	47 (40.2)	70 (59.8)
	Pulsating sensation in the breast/mammary area	12 (10.3)	–	6 (5.1)	–
	Galactorrhea	2 (1.7)	–	0 (0)	–
	Interest in women	46 (39.3)	–	19 (16.2)	–
	Sexual interest	48 (41)	–	18 (15.4)	–
	Sexual self-confidence	53 (45.3)	–	25 (21.4)	–
	Erectile dysfunction	33 (28.2)	–	14 (12.0)	–
	Ejaculatory dysfunction	33 (28.2)	–	17 (14.5)	–
Females	Total sexual dysfunction	62 (79.5)	16 (20.5)	46 (59.0)	32 (41.0)
	Menstrual irregularity	17/35 (48.6)	–	7/35 (20)	–
	Pulsating sensation in the breast/mammary area	21 (26.9)	–	6 (7.7)	–
	Galactorrhea	6 (7.7)	–	3 (3.8)	–
	Interest in men	33 (42.3)	–	13 (16.7)	–
	Sexual interest	36 (46.2)	–	19 (24.4)	–
	Sexual self-confidence	26 (33.3)	–	13 (16.7)	–
	Sexual arousal	18 (23.1)	–	12 (15.4)	–

The prevalence of slight to severe and moderate to severe sexual dysfunction in females was significantly higher than that in males (mild and severe sexual dysfunction, chi square 3.900 $p = 0.0483$; moderate and severe sexual dysfunction, chi square 6.662 $p = 0.0098$).

Our results also showed that hyperprolactinemia was highly prevalent among schizophrenia patients, and mean prolactin levels were significantly higher in female patients than in male patients. In particular, 16.7% of female patients showed extremely high concentrations of prolactin (>100 ng/ml). Prior cross-sectional studies estimating the prevalence of hyperprolactinemia in schizophrenia patients treated with conventional antipsychotics or risperidone reported that approximately 60% of women and 40% of men had hyperprolactinemia (Haddad and Wieck, 2004; Halbreich, et al., 2003; Smith et al., 2002), with mean prolactin levels in women of 62.7 ng/ml and those in men of 32.4 ng/ml (Halbreich et al., 2003). Moreover, prior cross-sectional studies estimating the prevalence of hyperprolactinemia in schizophrenia patients treated with atypical antipsychotics (olanzapine, 29 patients; clozapine, 28 patients; risperidone, 19 patients) showed that 42% of women and 21% of men had hyperprolactinemia (Melkersson, 2005). In our study, many patients were treated with conventional antipsychotics or risperidone (men, 74.4%; women; 57.7%), so the results of this study were similar to previous studies in patients treated with conventional antipsychotics or risperidone.

Hyperprolactinemia can lead to various adverse hormonal effects, including sexual dysfunction, gynecomastia, amenorrhea, and galactorrhea (Cutler, 2003; Smith et al., 2002), and evidence from both medical and psychiatric populations supports an association between hyperprolactinemia and sexual dysfunction. However, it is still not clear to what extent sexual dysfunction is influenced directly by hyperprolactinemia (Haddad and Wieck, 2004). Several studies reported a relation between sexual dysfunction and prolactin levels (Arató et al., 1979; Bruke et al., 1994; Ghadirian et al., 1982; Smith et al., 2002). However, other studies, including the current findings, have failed to support an association between hyperprolactinemia and sexual dysfunction (Hamner, 2002; Kleinberg et al., 1999; Spollen et al., 2004). Several issues may explain these conflicting results. First, libido and orgasm are related to dopaminergic neuronal circuits, so dopamine blockade by antipsychotics may have an impact on libido and orgasm (Giuliano and Allard, 2001). Second, the secondary effects of prolactin elevation, that is, reduction of plasma levels of testosterone, estrogen, luteinizing hormone, or follicle-stimulating hormone, could lead to sexual side effects (Rinieris et al., 1989;

Table 3
Total NSFQ score and prolactin levels (ng/ml) in males and females.

Gender	Mean prolactin level (ng/ml)	Mean total NSFQ score	Stratified prolactin level (ng/ml)	n (%)	Mean total NSFQ score
					c
Males	21.5 ± 16.7 [a]	12.7 ± 5.5 [b]	0 - 20	57 (48.7)	11.9 ± 4.9
					13.4 ± 6.1
			20 - 50	55 (47.0)	
			50 - 100	5 (4.3)	14.4 ± 4.5
					d
Females	45.3 ± 46.7	14.0 ± 5.1	0 - 25	36 (46.2)	13.5 ± 6.2
			25 - 50	15 (19.2)	15.3 ± 3.1
			50 - 100	14 (17.9)	14.1 ± 3.5
			100 - 150	11 (14.1)	13.5 ± 5.3
			150 - 200	2 (2.6)	16.5 ± 0.7

In addition to the mean total score of NSFQ and prolactin levels (ng/ml) in males and females, the mean total NSFQ scores corresponding to stratified prolactin levels are presented. The mean prolactin level in females was significantly higher than in males ([a]$t = 5.0357$, $p < 0.001$), but there was no significant difference in mean total NSFQ score between males and females ([b]$t = 1.6743$, $p = 0.0957$). There were no significant differences in total NSFQ scores among stratified prolactin levels in males ([c]$F = 1.219$ $df = 116$, $p = 0.299$) and females ([d]$F = 0.486$, $df = 77$, $p = 0.746$).

Table 4
NSFQ total scores, total sexual dysfunction (%), and plasma prolactin levels (ng/ml) in each antipsychotic treatment group.

Males				
			Total sexual dysfunction	
Drug	Prolactin level a	NSFQ	≥3 (from mild to severe) n (%)	≥4 (moderate and severe) n (%)
Aripiprazole	5.2 ± 7.6	13.4 ± 5.0	11 (78.6)	5 (35.7)
Olanzapine	10.6 ± 7.4	13.1 ± 5.0	10 (66.7)	8 (53.3)
Polytherapy	23.2 ± 15.6	12.7 ± 6.1	29 (64.4)	15 (33.3)
Risperidone	29.8 ± 16.4	12.7 ± 5.4	27 (64.3)	19 (45.2)
Others	4.2 ± 0.4	8.0 ± 4.2	1 (33.3)	0 (0)

Females				
			Total sexual dysfunction	
Drug	Prolactin level f e	NSFQ	≥3 (from mild to severe) n (%)	≥4 (moderate and severe) n (%)
Aripiprazole	6.7 ± 7.6	13.5 ± 5.4	14 (82.4)	10 (58.8)
Olanzapine	23.4 ± 7.9	13.2 ± 3.2	5 (55.6)	3 (33.3)
Polytherapy	51.5 ± 45.2	12.7 ± 6.1	15 (83.3)	13 (72.2)
Risperidone	79.9 ± 48.9	13.9 ± 4.6	25 (92.6)	18 (66.7)
Others	17.6 ± 18.1	10.6 ± 6.1	3 (42.9)	2 (28.6)

There were significant differences in the prolactin levels among the antipsychotic groups of aripiprazole, olanzapine, polytherapy and risperidone in males ($F=13.251$, $df=114$, $p<0.001$, $^{a}p<0.001$, $^{b}p<0.001$, $^{c}p<0.026$, $^{d}p<0.001$) and females ($F=14.107$, $df=70$, $p<0.001$, $^{e}p<0.01$, $^{f}p<0.001$, $^{g}p<0.01$) subjects. The total NSFQ scores were not significantly different among the groups of aripiprazole, olanzapine polytherapy, and risperidone in males ($F=0.075$, $df=114$, $p=0.973$) and females ($F=1.537$, $df=70$, $p=0.213$). There was no significant difference in the frequency of total sexual dysfunction among the groups receiving aripiprazole, olanzapine polytherapy, and risperidone (males, from mild to severe, chi square 1.5508 $p=0.82$; moderate and severe, chi square 4.3366 $p=0.36$; females, from mild to severe, chi square 9.0318 $p=0.06$; moderate and severe, chi square 7.6454 $p=0.11$).

Smith et al., 2002). Third, noradrenergic and histaminergic effects are also suggested to change aspects of sexual performance (Meston and Frohlich, 2000). Fourth, the influence of the primary disease, treatment duration, age, and gender make it even more difficult to interpret the data (Smith et al., 2002). Finally, the different methodologies used to reveal sexual dysfunction likely lead to a major underestimation of the frequency of sexual dysfunction (Peuskens et al., 1998) Thus, clinicians should take these facts into account and pay attention to symptoms of sexual dysfunction in the presence or absence of hyperprolactinemia.

Previous studies suggested that the prolactin-raising drugs (e.g., risperidone) provoke significantly higher rates of sexual problems (40–60%) compared with prolactin-sparing drugs (e.g., quetiapine, ziprasidone, and aripiprazol) (<30%) (Knegtering et al., 2004, 2006; Montejo Gonzalez et al., 2005; Montejo and Rico-Villademoros, 2008a; Montejo et al., 2010a, 2010b; Serretti and Chiesa, 2011; Baggaley, 2008), whereas both our findings and those of Fujii et al. (2010) did not reveal any difference in the prevalence of sexual dysfunction among different medications in Japanese schizophrenic patients. The inconsistency between previous studies and the present study may be related to: (1) the small number of subjects on monotherapy and different numbers of subjects in the drug groups in our study, (2) the use of different rating scales (NSFQ ascertains information about sexual concerns indirectly, whereas the similar items on other scales are ascertained in a more direct manner. As a consequence, NSFQ may not be a strong instrument to detect such differences.), (3) different demographic data (the duration and treatment of illness in this study were more than 200 months, so there were many chronic patients in this study.), and (4) ethnic or cultural differences, (5) no baseline data (e.g., previous sexual functioning, sexual life, partner) before treatment with antipsychotics. However, our study revealed that patients taking antipsychotics reported to induce less sexual dysfunction have just as much sexual dysfunction as patients taking antipsychotics reported to induce more sexual dysfunction, suggesting that clinicians should pay attention to the clinical signs of sexual dysfunction regardless of the drugs patients are taking.

Our study has several limitations. First, sexual dysfunction was evaluated at only single time point, and this study was a cross-sectional study using a small clinical sample which composed of many chronic patients. So other factors but drug could have impact on the result of

this study, and it was difficult to comprehend the factor of drug perceptively. Moreover the number of patients on monotherapy was small and differed among the antipsychotic groups. However, our sample had adequate statistical power to effectively accept or reject the null hypothesis (males, $F = 13.251$, $p < 0.01$, power of test $= 1.00$; females, $F = 14.107$, $p < 0.01$, power of test $= 1.00$). Therefore, the current study could detect differences of prolactin level among drug groups accurately. Although we collected data regarding patients' ages, duration of illness, and duration of medication, no baseline data (e.g., previous sexual functioning, sexual life, partner) before treatment with antipsychotics were available and no inclusion and exclusion criteria were used in this study. These limitations might have impact on the results in this study different from prior studies. Sexual life could be influenced by many different factors that methodology may need to be quite strict. In addition, NSFQ may not be sufficient for assessing the orgasm, satisfaction and arousal due to the specific cultural aspects related to the Asian population and the further studies using other instruments should be performed in order to address this issue.

To clarify the relationship between prolactin levels and sexual dysfunction, an interventional study that investigates the effect of lowering prolactin levels on sexual dysfunction by switching antipsychotics is needed. Second, we have no data on other characteristics that might influence sexual dysfunction, including smoking status and other endocrinologic factors (such as testosterone and estradiol levels) and metabolic parameters like obesity or diabetes (Bhasin et al., 2007). Considering the important role of endocrinologic and metabolic factors in sexual dysfunction, further studies are needed to evaluate the impact of these factors (Wu et al., 2010). Third, although the patients who participated in the study were in stable condition, the impact of the disease itself on sexual dysfunction was not assessed. This factor should be included in a future study. Fourth arousal disturbances and orgasmic dysfunction are very relevant in the investigation of sexual functioning and these results are needed to obtain and clarify accurate sexual information, but these factors were not asked directly at NSFQ (There is item of sexual arousal in female NSFQ). We think that this fourth limitation might be a strong limitation and have impact on the result in this study. In order to address these issues, we are planning to conduct study of sexual function using SALSEX or other scale together with NSFQ and in order to improve performance of NSFQ.

5. Conclusion

This study is the first survey using NSFQ to estimate sexual dysfunction. Results showed a high prevalence of sexual dysfunction and hyperprolactinemia in Japanese schizophrenic patients, as with previous studies. Clinicians should pay careful attention to patients' sexual dysfunction to improve their QOL and adherence to therapy.

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在宅中心静脈栄養を導入した６症例の神経性食欲不振症

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抄　録：６名の慢性遷延化した神経性食欲不振症患者に在宅中心静脈栄養法（Home Parenteral Nutrition; HPN）を導入した。全例女性で罹病期間は12.3±6.0年（平均±SD），制限型３例・むちゃ食い/排出型３例で，導入時のBody mass indexは11.3±0.8 kg/m^{2}であった。複数回の入院歴があり，導入理由は重症のやせ，外来治療で是正できない低カリウム血症や腎不全で，家族が再入院に消極的で，本人が在宅治療を希望した。投与エネルギーは860～1500 kcal/日で，12～81ヵ月の観察期間中，１名は体重増加を達成し，１名は点滴を途中で廃棄する行為があり，HPNを離脱した。他の４名はHPNを継続して，２名は体重を維持し，２名は合併症の悪化が阻止できている。HPNは，感染症などのリスクはあるが，再入院を阻止でき，患者や家族のQOLの改善に有益であると考えられた。

索引用語：神経性食欲不振症；在宅中心静脈栄養法（Home Parenteral Nutrition; HPN）；カテーテル関連血流感染；QOL

Experience of home parenteral nutrition in 6 patients with enduring anorexia nervosa

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Abstract: Some patients with anorexia nervosa (AN) develop intractable illness and are at high risk of death. They need multiple admissions to save their lives or treat serious complications including renal failure, hypokalemia or infectious diseases. In Western countries, home parenteral nutrition (HPN) has been employed for patients with anorexia nervosa since the 1980s. Recently, the Ministry of Health, Labour and Welfare in Japan has promoted home medical care to reduce medical expenses. We have used HPN to treat 6 patients with enduring AN and report herein the clinical profiles, reasons for application of HPN, treatment progress and outcomes. All patients were female, with restricting-type AN in 3 patients and binge-eating/purging-type AN in 3. Three patients had been diagnosed with depression or compulsive disorders in addition to AN. Mean age at start of HPN and duration of illness were 28.6 ± 5.4 years and 12.3 ± 6.0 years, respectively. The minimum body mass indices during illness and at start of HPN were 10.2 ± 2.7 kg/m^2 and 11.3 ± 0.8 kg/m^2, respectively. Patients had been hospitalized 3 – 9 times in our department, as well as 2 – 7 times in other medical institutions due to severe malnutrition, hypokalemia, dehydration or