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Improvements in Quadriceps Force and Work Efficiency are Related to Improvements in Endurance Capacity Following Pulmonary Rehabilitation in COPD Patients

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Abstract

Background and Objective The endurance time has been reported to be the most sensitive measure of improved exercise capacity in response to a variety of interventions for COPD. The aim of the present study was to determine whether the improvements in quadriceps force and measures obtained from a symptom-limited maximal test contributed to the improvements in endurance time following pulmonary rehabilitation.

Methods Fifty-seven consecutive COPD subjects completed a 10-week pulmonary rehabilitation program. The subjects completed a symptom-limited incremental cycle ergometry test and a constant work rate test before and after pulmonary rehabilitation. Peripheral and respiratory muscle strength was also measured. The relationships between the change in endurance time and the changes obtained from the incremental test and muscle strength test were investigated.

Results The endurance time showed the greatest improvement among the exercise capacity indices. The changes in endurance time were significantly correlated to changes in quadriceps force, peak work rate, anaerobic threshold and work efficiency on the incremental load test. In the multiple stepwise regression analysis, changes in quadriceps force and work efficiency measured on the maximal exercise test were selected.

Conclusion These findings suggest that the improvements in endurance time after pulmonary rehabilitation may be explained by increased quadriceps force and improvements in peak work rate and work efficiency.

Key words: endurance time, quadriceps force, work efficiency, chronic obstructive pulmonary disease, pulmonary rehabilitation

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Introduction

Pulmonary rehabilitation has a well-established role in the management of subjects with chronic obstructive pulmonary disease (COPD) (1). Evidence-based guidelines for pulmonary rehabilitation recommend that the exercise component of the program includes endurance and strength training, in particular of the lower limbs. Weakness of the peripheral muscles, particularly the quadriceps, often occurs in COPD, and this quadriceps muscle weakness has been shown to contribute to exercise limitations (2). Exercise training improves both exercise capacity and quadriceps muscle function; however, it is unclear whether the improvements in quadriceps function are related to improvements in exercise capacity.

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Exercise capacity has been evaluated in several ways, including field-walking tests such as the 6-minute walk test and shuttle walking test, and laboratory-based cardiopulmonary exercise tests. Examinations can be either incremental in nature to evoke a symptom-limited maximal performance, or at a constant work rate. In subjects with COPD, the endurance time using cycle ergometry has been shown to be more sensitive to interventions than measures obtained from a symptom-limited maximal test (3). Moreover, it has been reported that exercise training improved the quadriceps force, work efficiency and ventilation efficiency during exercise in COPD subjects, and these variables may be related to changes in endurance time obtained from a constant work rate test following pulmonary rehabilitation (4-6). The aim of the present study was to determine whether the improvements in quadriceps force and measures obtained from a symptom-limited maximal test contributed to the improvements in endurance time following pulmonary rehabilitation.

Materials and Methods

Subjects

Fifty-seven consecutive subjects with COPD who completed the pulmonary rehabilitation program at Tosei General Hospital between June 2003 and May 2007 were included in this study. All subjects reported exertional dyspnea, and were receiving a constant medication regimen without any history of an acute exacerbation for at least 3 months prior to recruitment. The diagnosis of COPD was based on the following criteria: 1) a history of smoking of more than 20 pack years, 2) a forced expiratory volume in 1 second (FEV_1) of less than 80% of the predicted value, 3) a FEV_1 / forced vital capacity (FVC) of less than 70%, 4) no obvious abnormal shadows on chest X-ray, and 5) no clinical diagnosis of asthma. The exclusion criteria included a history of lung surgery, the use of long-term oxygen therapy, or any comorbid conditions likely to reduce exercise capacity (e.g., musculoskeletal conditions, unstable heart disease, and neurologic impairment). At the beginning of the study, none of the subjects were current smokers. Informed consent was obtained from all who participated. This study was approved by the ethics committee of Tosei General Hospital (approval number 213).

Study design

Measures of body anthropometry, resting lung function, arterial blood gas tensions and quadriceps and respiratory muscle force were obtained. All subjects performed a symptom-limited incremental cycle ergometry test and a constant work rate cycle ergometry test. The subjects were evaluated one week before and immediately following the 10-week pulmonary rehabilitation program. The incremental cycle ergometry test was performed at the initial visit during the study. Two days following the initial visit, the subjects performed the constant work rate test.

Pulmonary function tests

Spirometry was performed (CHESTAC-55V; Chest, Tokyo, Japan), according to published recommendations (7). The total lung capacity was obtained using helium dilation, and the single-breath diffusion capacity for carbon monoxide was also measured. All values were expressed as a percentage of the predicted values reported by the Japan Society of Respiratory Diseases (8).

Muscle function tests

Quadriceps force was measured using a dynamometer (Cybex II; Lumex, NY, USA). The peak torque (Newton-meters, Nm) was measured in both legs during a maximal isokinetic knee extension maneuver with the hip in 90° flexion. The highest value from at least four maneuvers for each leg was recorded. The quadriceps force was then compared to the calculated predicted value based on age and gender (2).

All subjects underwent respiratory muscle testing to determine the maximal inspiratory pressure and maximal expiratory pressure. The former was measured at the residual volume, and the latter was measured at near total lung capacity, according to the method proposed by Black and Hyatt (9) (Vitalopower KH101; Chest, Tokyo, Japan). The highest value from at least three maneuvers was recorded and compared to the calculated predicted values (9).

Exercise tests

A symptom-limited incremental cycle ergometry test (Ergometer 232CXL; Combi, Tokyo, Japan) was performed on an electronically braked cycle ergometer in accordance with published guidelines (10) to evaluate the maximal exercise capacity and to determine the nature of the exercise limitation. Gas exchange and ventilatory variables were collected on a breath by breath basis using eight breath averaging (Centaura-1; Chest, Tokyo, Japan). The protocol required a 3-min unloaded phase followed by a 10-watt/min-stage at a pedaling rate of 60 rpm, until the subject could no longer continue because of severe dyspnea or leg fatigue. The maximum heart rate (HR_{peak}) was determined using the R-R interval from a 12-lead electrocardiogram (CardioStar; Fukuda Denshi, Tokyo, Japan). Peak values were defined as the values averaged during the last 30 seconds of the highest work load achieved. The peak values for oxygen uptake ($\dot{V}O_{2peak}$), work rate (WR_{peak}), tidal volume (VT_{peak}), and minute ventilation ($\dot{V}E_{peak}$) during the exercise were recorded. The anaerobic threshold was determined by the V-slope technique. The ventilatory efficiency and work efficiency were also calculated. The ventilatory efficiency was determined as the ventilatory equivalent for CO_2 ($\dot{V}E/\dot{V}CO_2$ slope). Work efficiency (oxygen consumption during exercise) was determined as the oxygen uptake to work rate ($\Delta\dot{V}O_2/\Delta WR$), which is the increase in oxygen uptake divided by the sum of the workloads (in watts) during exercise. The transcutaneous oxygen saturation (SpO_2) was monitored

Table 1. Anthropometric and Lung Function Data of the 57 Subjects at Baseline and Immediately Following Pulmonary Rehabilitation

	Baseline	Post-PR
Parameter		
Male / Female (n)	55 / 2	
Age (yrs)	68.3 ± 7.8	
BMI (kg/m^2)	20.1 ± 3.3	
MRC grade 2	21	
MRC grade 3	33	
MRC grade 4	3	
PaO_2 (torr)	75.2 ± 9.7	74.7 ± 9.5
$PaCO_2$ (torr)	39.8 ± 5.5	40.6 ± 7.1
Resting Lung function		
FEV_1 (L)	0.99 ± 0.36	1.01 ± 0.68
FEV_1 (% predicted)	43.5 ± 16.1	44.9 ± 15.5
FVC (L)	2.59 ± 0.72	2.72 ± 0.75†
FVC (% predicted)	80.6 ± 19.6	84.3 ± 20.1†
FEV_1/FVC (%)	38.1 ± 9.9	37.3 ± 9.6
IC (L)	1.77 ± 0.48	1.79 ± 0.51
FRC (L)	3.93 ± 0.77	3.90 ± 0.82
FRC (% predicted)	106.3 ± 16.4	106.0 ± 18.2
TLC (L)	5.75 ± 1.00	5.80 ± 1.00
TLC (% predicted)	112.4 ± 17.6	111.9 ± 14.9
RV (L)	2.85 ± 0.71	2.80 ± 0.67
RV (% predicted)	176.2 ± 45.4	173.4 ± 44.7
DLco (mL/min/mmHg)	10.50 ± 4.20	10.87± 4.27
DLco (% predicted)	72.7 ± 25.6	73.3 ± 27.2

All data are presented as means ± SD. †$p < 0.05$ compared with Baseline. PR, Pulmonary rehabilitation; BMI, body mass index; MRC, Medical Research Council; PaO_2, partial arterial oxygen concentration; $PaCO_2$, partial arterial carbon dioxide concentration; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; IC, inspiratory capacity; FRC, functional residual capacity; TLC, total lung capacity; RV, residual volume; DLco, diffusing capacity for carbon monoxide.

throughout the test using pulse oximetry (Pulsox-3Li; Minolta Inc., Tokyo, Japan). At the end of the test, the subjects were asked to estimate the severity of their breathlessness and leg fatigue using the modified Borg scale, and the symptom (dyspnea or leg fatigue) that caused them to terminate the test was recorded (11).

A symptom-limited constant work rate test was performed to determine the endurance time using the same cycle ergometer used for the incremental test. The protocol required 2-min of unloaded cycling followed by cycling at 60 rpm with a work rate equivalent to 80% of the peak WR obtained in the incremental test (3). The identical work rate was used to measure the endurance time at the end of the pulmonary rehabilitation program. At the end of the test, the subjects were asked to estimate the severity of their breathlessness and leg fatigue using the modified Borg scale and the symptom (dyspnea or leg fatigue) that caused them to terminate the test was recorded (11).

Pulmonary rehabilitation program

The program consisted of twice-weekly supervised exercise training visits and three unsupervised sessions at home for a period of 10 weeks (12, 13). The supervised sessions lasted 60 minutes, and consisted of respiratory care, subject education, endurance and strength training. The subjects performed supervised training on a braked cycle ergometer, with a target of 20 minutes of continuous cycling. The target intensity was 80% of the WR_{peak} obtained from the incremental exercise test. Peripheral muscle strength training included upper and lower limb resistance training with weight machines, hand weights, or elastic bands, four lower limb exercises (step-ups, sit-to-stand, leg press, and knee extension), four upper limb exercises (lying triceps extension, dumbbell bench press, dumbbell fly, and shoulder shrugs) and three trunk exercises (trunk flexion, trunk rotation, and pelvic tilt). The respiratory muscle training was performed using an inspiratory threshold device (Threshold IMT; Respironics, Cedar Grove, NJ, USA). The subjects trained their breathing at a resistance that required 30% of the maximal inspiratory pressure for 15 minutes (14). The home exercise program consisted of walking and respiratory muscle training. The subjects were instructed to walk continuously for 20 minutes at a target dyspnea intensity of between 4 and 5 (11). The respiratory muscle training was performed twice daily for sessions of 15 minutes each. Patients recorded the time of walking and respiratory muscle training in a diary and the diary was checked at each supervised session.

Statistical analysis

The data for all subjects were analyzed followed by subgroup analysis with subjects stratified according to the symptom (i.e. dyspnea or leg fatigue) that limited their performance on the baseline incremental cycle ergometry test. Student's paired *t*-test and Wilcoxon signed rank tests were used (on parametric and nonparametric data, respectively) to compare the measures before and after the pulmonary rehabilitation program. The Δvalue in the outcome measures was expressed as the percent change before versus after the program. The significance of the differences in the Δvalues observed for two exercise tests was determined with a repeated measures analysis of variance. When a significant difference was found, post hoc analysis was performed with the Bonferroni adjustment method to identify which differences were significant. Pearson correlation coefficients (r) were used to examine the relationships between measured variables. For the variables significantly ($p<0.05$) related to the Δendurance time, a forward stepwise multiple regression analysis was performed using Δendurance time as the dependent variable. The variables used in the regression model were those that significantly ($p<0.05$) improved following pulmonary rehabilitation. A *p* value of less than 0.05 was considered statistically significant. All data were expressed as means ± SD. All analyses were performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, USA).

Results

The baseline characteristics of the 57 subjects together with their lung function data measured at baseline and immediately following pulmonary rehabilitation are summarized in Table 1. All subjects achieved high-intensity training. No major complications occurred during the training. The practice rate of home rehabilitation program was 73.6±

Table 2. Muscle Strength and Exercise Test Data at Baseline and Immediately Following Pulmonary Rehabilitation

	Baseline	Post-PR
Muscle strength test		
MIP (cmH_2O)	85.4 ± 34.0	101.5 ± 33.6 ‡
MIP(% predicted)	81.1 ± 32.3	96.4 ± 31.9 ‡
MEP (cmH_2O)	156.1 ± 55.5	161.6 ± 59.8
MEP (% predicted)	79.0 ± 27.8	81.9 ± 30.5
Quadriceps force (Nm)	84.7 ± 25.9	91.7 ± 25.1 ‡
Quadriceps force (% predicted)	70.8 ± 18.0	77.4 ± 18.0 ‡
Incremental cycle ergometry test		
$\dot{V}O_{2peak}$ (mL/min)	715.3 ± 254.9	728.3 ± 242.4
$\dot{V}O_{2peak}$ (% predicted)	48.1 ± 15.4	49.7 ± 16.8
WR_{peak} (w)	63.1 ± 20.7	68.8 ± 21.4 ‡
AT (mL/min)	537.7 ± 175.7	633.7 ± 210.1 ‡
AT (% predicted)	42.5 ± 12.0	50.3 ± 15.2‡
Work efficiency (mL/min/w)	5.6 ± 2.4	6.6 ± 2.4‡
Ventilatory efficiency	40.0 ± 8.5	39.8 ± 10.7
VT_{peak} (L/min)	1.25 ± 0.40	1.30 ± 0.40 †
$\dot{V}E_{peak}$ (L/min)	40.9 ± 14.7	42.3 ± 14.9
$\dot{V}E_{peak}$ / MVV (%)	105.8 ± 23.6	107.1 ± 22.1
HR_{peak} (bpm)	124.8 ± 19.5	125.4 ± 21.0
Lowest SpO_2 (%)	88.4 ± 5.2	88.4 ± 5.2
Dyspnea score	7.4 ± 1.9	7.4 ± 1.8
Leg fatigue score	6.3 ± 2.7	6.1 ± 2.6
Dyspnea / leg fatigue *	32 / 25	34 / 23
Constant work rate test		
Endurance time (seconds)	520.9 ± 396.9	1004.8 ± 516.5 ‡
Lowest SpO_2 (%)	89.7 ± 4.5	87.4 ± 6.0
Dyspnea score	7.9 ± 2.0	7.8 ± 2.0
Leg fatigue score	7.7 ± 2.5	6.9 ± 2.5
Dyspnea / leg fatigue **	12 / 45	24 / 33

All data are presented as means±SD. MIP, maximal inspiratory pressure; MEP, Maximal expiratory pressure; $\dot{V}O_{2\,peak}$, peak oxygen uptake; WR_{peak}, peak work rate; AT, anaerobic threshold; Work efficiency, oxygen uptake to work rate; Ventilatory efficiency, ventilatory equivalent for CO_2; VT_{peak}, peak tidal volume; $\dot{V}E_{peak}$, peak minute ventilation; HR_{peak}, peak heart rate; SpO_2, arterial oxygen saturation; *The source of the exercise limitation at the end of the incremental load test. **The source of exercise limitation at the end of the constant work rate test. ‡$p < 0.01$, †$p < 0.05$

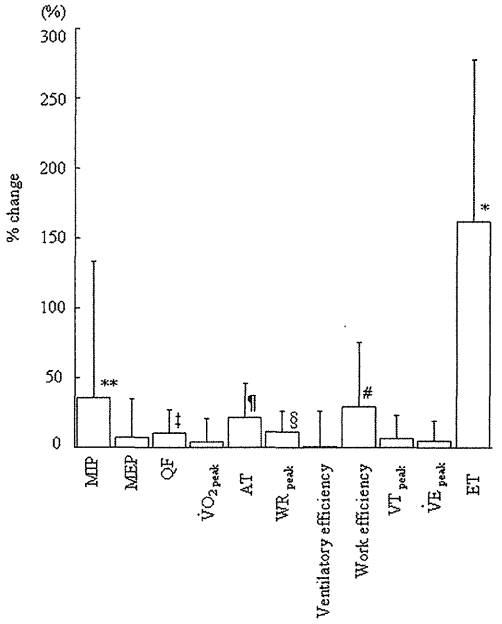

Figure 1. Changes in various measures of muscle function and exercise capacity on two exercise tests after pulmonary rehabilitation.

All values are expressed as means±SD. The changes are expressed as the percent change from baseline. Anaerobic threshold (n=51), MIP: maximal inspiratory pressure, MEP: Maximal expiratory pressure, QF: Quadriceps force, $\dot{V}O_{2\,peak}$: peak oxygen uptake, AT: Anaerobic threshold, WR_{peak}: peak work rate, VT_{peak}: peak tidal volume, $\dot{V}E_{peak}$: peak minute ventilation, ET: Endurance time. *: $p<0.05$ compared with all measures; #: $p<0.05$ compared with MEP, QF, $\dot{V}O_{2\,peak}$, WR_{peak}, Ventilatory efficiency, VT_{peak}, $\dot{V}E_{peak}$, ET; ¶: $p<0.05$ compared with MEP, QF, $\dot{V}O_{2\,peak}$, WR_{peak}, Ventilatory efficiency, VT_{peak}, $\dot{V}E_{peak}$, ET; §: $p<0.05$ compared with $\dot{V}O_{2\,peak}$, AT, Ventilatory efficiency, $\dot{V}E_{peak}$, ET; **: $p<0.05$ compared with $\dot{V}O_{2\,peak}$, Ventilatory efficiency, VT_{peak}, $\dot{V}E_{peak}$, ET; ‡: $p<0.05$ compared with $\dot{V}E_{peak}$.

16.6%. The subjects had moderate to very severe airflow limitation (FEV_1 43.5±16.1 % of %predicted). The FVC increased significantly following pulmonary rehabilitation in the lung function parameters. Other parameters, including the FEV_1 and inspiratory capacity, did not change significantly. Table 2 shows the muscle strength and exercise test data at baseline and immediately following pulmonary rehabilitation. No subjects stopped any tests due to severe desaturation. In the muscle strength tests, the maximal inspiratory pressure and quadriceps force were significantly improved. Following pulmonary rehabilitation, significant changes occurred in the WR_{peak}, anaerobic threshold, work efficiency, VT_{peak} and endurance time. However, the $\dot{V}O_{2\,peak}$ remained unchanged (Table 2). The changes in the measures of exercise capacity after the pulmonary rehabilitation program are shown in Fig. 1. The endurance time showed the most striking improvement, increasing by 162%, ($p<0.01$).

Univariate correlation analysis showed significant correlations between the Δendurance time and the Δquadriceps force, ΔWR_{peak}, Δanaerobic threshold, and Δwork efficiency for all subjects (Table 3). Restricting the analysis to subjects who stopped exercising because of leg fatigue (n=25) produced similar correlations. In contrast, in the subgroup limited by dyspnea (n=32), only the Δquadriceps force and Δwork efficiency were significantly correlated with the Δendurance time.

Forward multiple stepwise regression analysis using data from all subjects showed that the Δquadriceps force and Δwork efficiency were independent predictors of the Δendurance time ($r^2=0.38$ $p<0.01$) (Table 4). In those subjects who terminated the exercise because of dyspnea, the Δwork efficiency was a significant independent contributor to the Δendurance time ($r^2=0.24$ $p<0.01$). In subjects who stopped because of leg fatigue, the ΔWR_{peak} was a significant contributor to the Δendurance time ($r^2=0.53$ $p<0.01$).

Discussion

In the present study, the endurance time showed the largest increase of all exercise measures following the pulmonary rehabilitation program. A constant work rate test measures the ability to sustain submaximal exercise, which can

Table 3. Univariate Correlation Coefficients for the Δendurance Time with Physiological Measures

Variable	Δendurance time		
	Total (n=57)	Dyspnea* (n=32)	Leg Fatigue* (n=25)
ΔFVC	0.05	0.02	0.25
ΔMIP	- 0.13	- 0.17	- 0.01
ΔQuadriceps force	0.50 ‡	0.44 †	0.51 ‡
ΔWR_{peak}	0.42 ‡	0.09	0.72 ‡
ΔAT	0.28 †	0.02	0.44 †
ΔWork efficiency	0.44 ‡	0.39 †	0.43 †
ΔVT_{peak}	0.24	0.13	0.31

All variables included in this table improved significantly following PR. ‡$p < 0.01$. †$p < 0.05$; Δ, Changes are expressed as the percent change from baseline value; *Dyspnea, leg fatigue, the source of exercise limitation at the end of the incremental load test of Baseline

Table 4. Results of the Stepwise Multiple Regression Analysis

Variable	Δendurance time		
	All subjects (n=57)	Dyspnea* (n=32)	Leg Fatigue* (n=25)
ΔQuadriceps force	0.41 ‡	0.37	0.34
ΔWR_{peak}	0.28	0.06	0.73 ‡
ΔAT	- 0.01	0.06	0.15
ΔWork efficiency	0.41 ‡	0.49 ‡	0.34
Cumulative r^2	0.38	0.24	0.53

‡ $p < 0.01$. *Dyspnea, leg fatigue, the source of the exercise limitation at the end of the incremental load test of the Pre-PR

improve when there is no significant increase in the maximal exercise capacity (4). O'Donnell et al reported that exercise endurance is both reproducible and responsive to change (15). In the present study, although the $\dot{V}O_{2peak}$ and $\dot{V}E_{peak}$ measured on the incremental test did not increase, the improvements in the anaerobic threshold and endurance time on the constant work rate test demonstrated the efficacy of the pulmonary rehabilitation program. Previous studies (15, 16) also failed to show a statistically significant improvement in the $\dot{V}O_{2peak}$ after exercise training in subjects with COPD. This is the first report that indicates that endurance time has the advantage of being a more responsive measure than maximal exercise capacity for evaluating the outcomes of a pulmonary rehabilitation program. ET may be a responsive measure following pulmonary rehabilitation than the six-minute walk test and shuttle walking test, because the change in ET was the most striking improvement, increasing by 162%.

To our knowledge, improvements in quadriceps force and work efficiency were independent predictors of improvements in the endurance time. Quadriceps force contributes to exercise limitations in COPD (2). The addition of a muscle strength training component to a program of pulmonary rehabilitation increases both muscle strength and muscle mass (17). High-intensity endurance exercise training has been shown to improve leg muscle function (6, 7). In the present study, exercise training improved the quadriceps force. The addition of strength training to aerobic training was accompanied by greater improvements in quadriceps muscle mass and strength than aerobic training alone (18). Maltais et al reported that the oxidative enzymes in the peripheral muscles increased after high-intensity training (19). Therefore, the improvement in quadriceps force might reflect the improved aerobic function of the peripheral muscles, as well as the reduced lactate production during exercise.

In the present study, the Δwork efficiency was a significant contributor to the Δendurance time, in particular, subjects stopped exercising because of dyspnea. High-intensity exercise training resulted in improvements in work efficiency. Work efficiency during exercise has been noted to decrease in subjects with COPD (20). Lower work efficiency often indicates slow $\dot{V}O_2$ kinetics. The $\dot{V}O_2$ kinetics were significantly improved after exercise training in subjects with COPD (21). It is possible that the improved work efficiency also improved the dyspnea and muscle fatigue during endurance exercise, which was followed by an improved endurance time. Improvements in exercise capacity have been found to be accompanied by physiological changes, such as improved quadriceps force and more rapid $\dot{V}O_2$ kinetics following exercise onset (7). In the forward multiple stepwise regression analysis, the Δwork efficiency correlated significantly with the Δendurance time in subjects who terminated their exercise due to dyspnea, but not in those who stopped due to leg fatigue. This is consistent with the concept that oxygen debt contributes to a limitation in exercise capacity in subjects who stopped the test because of dyspnea. In subjects with COPD, in particular, there is exercise limitation due to dyspnea and pulmonary rehabilitation is necessary, including high intensity endurance training.

In the present study, the ΔWR_{peak} was a significant contributor to the Δendurance time in subjects who stopped because of leg fatigue. High-intensity exercise training improved the WR_{peak} indicating maximal exercise capacity. WR_{peak} is related to the limitation of lower limb $\dot{V}O_2$ during cycling exercise in COPD (22) Improvement in WR_{peak} following exercise training may increase lower limb $\dot{V}O_2$ during cycling exercise. In COPD subjects who stopped because of leg fatigue, pulmonary rehabilitation is necessary, including lower limb endurance training.

There are several limitations to the present study. Ventilatory gas analysis such as oxygen consumption during the constant work rate test was not performed. The relationships between the parameters of the gas analysis during the constant work rate test and the effect of the pulmonary rehabilitation should have been studied in further detail. We did not investigate the oxidative capacity of the quadriceps, and we did not examine the compliance with the home exercise program. No assessment was made of local muscle endurance or muscle fatigability. Quadriceps force is related to quadriceps muscle endurance assessed using repetitive magnetic stimulation (23). In the future, it will be necessary to examine the relationship improvements in quadriceps muscle endurance and exercise capacity. In this study, COPD patients did not receive any dietary supplementation. Combined dietary supplementation and exercise training may improve exercise capacity compared to exercise training alone in COPD patients (24).

The validity of using the endurance time obtained from a constant work rate test to evaluate changes following exercise training in subjects with COPD was also confirmed by the present findings. This conclusion is based on the fact that the endurance time was a very responsive measure, and also that the improvements in the endurance time were significantly associated with other exercise measures obtained from an incremental test. Although the endurance time from a constant work rate test is a sensitive parameter in pulmonary rehabilitation and is easily adopted, it is important to understand the relationship with parameters from the incremental load test, which assesses real exercise capacity. The endurance time from the constant work rate test is the most sensitive test for detecting the short-term effects of exercise training; the change in endurance time after pulmonary rehabilitation was significantly correlated with changes in quadriceps force, WR_{peak} and work efficiency on the incremental load test.

The authors state that they have no Conflict of Interest (COI).

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ORIGINAL ARTICLE

Selection of optimal epoch duration in assessment of rodent sleep–wake profiles

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Abstract

One of the major inconveniences encountered in sleep studies is the time-consuming labor involved in electroencephalography (EEG) analysis. The choice of epoch duration is of prime importance and is highly related to the outcome of EEG analysis. This study was designed to find the optimal EEG epoch duration that accurately reveals the sleep–wake profiles of animals and relieve researchers of the laborious analysis of rodent sleep–wake parameters. We analyzed mouse and rat EEG signals with commonly used epoch durations (4, 8, 10, 20, and 30 s) and compared the resulting sleep–wake profiles in terms of amounts of sleep and wakefulness, number and duration of episodes, number of sleep–wake stage transitions, and EEG power spectra. There were no statistical differences in the amount and EEG power density of wakefulness, rapid eye movement (REM) and non-REM sleep among the 5 epoch durations we used. However, the shorter the epoch, the more numerous the stage transitions and the larger the episode number. The purpose of an experiment should be a main criterion for determining the optimal epoch length for the experiment. When the amount of each stage and power density of EEG signals are desired, a 30-s epoch duration is appropriate, to save time; and the results obtained are no different from those found by analysis of other epoch durations. If stage transition and duration and number of episodes are to be considered, a 4-s epoch duration is suggested as the best choice for analysis.

Key words: electroencephalography, epoch duration setting, rodents, sleep parameters.

INTRODUCTION

The discovery of electroencephalography (EEG) in 1929 by the German psychiatrist Hans Berger was a historical breakthrough and a milestone to the sleep research community.[1] One of the major inconveniences encountered in sleep studies is the time-consuming labor involved in EEG analysis. Conventional analysis of EEG signals for sleep scoring is based on the time-domain assessment of wave patterns. The rules for visual sleep scoring are provided by the recommendations of Rechtschaffen and Kales, published in 1968.[2] According to this manual, it is possible to distinguish between wakefulness and sleep. Sleep states were sub-classified as either rapid eye movement (REM) sleep if there was rhythmic theta oscillation (6–8 Hz) or non-rapid eye movement (non-REM, NREM) if there was moderate delta (1–4 Hz) oscillation in the EEG. Although visual sleep-stage scoring according to these guidelines has been challenged in recent years,[3,4] it is the only criterion for

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sleep classification that has found worldwide acceptance. However, scoring procedures for the selection of epoch length are little standardized in rodents.

Sleep scoring is performed in time segments known as "epochs". The choice of epoch length can manipulate the duration of episode. Selection of epoch length is an important but often overlooked issue in EEG analysis. In the early studies, when the technology for EEG analysis was not widely deployed, polysomnographic variables were monitored, and sleep was scored in 30-s epochs by standard criteria regardless of the subject investigated;[5–8] some researchers even went to the length of utilizing 60-s epochs.[9] As a natural outgrowth of the technology, shorter epochs were introduced for analysis. These include 15-s,[10] 10-s, [11] 5-s,[12] 4-s,[13] and even 2-s[14] epochs according to the nature of the investigation. There seem to be many choices available, but there were two questions that remained: "Why is one epoch duration chosen while the others are abandoned?" and "Which epoch length can provide accurate results for sleep analysis and yet save time?"

The optimal epoch duration should not only accurately illustrate animals' sleep–wake profiles but also liberate researchers from tedious work. To find the optimal epoch duration for EEG analysis, we analyzed EEG signals of mice and rats by frequently used epoch durations (4, 8, 10, 20, and 30 s) and compared the resulting sleep–wake profiles in terms of sleep amounts, number and duration of episodes, number of sleep–wake stage transitions, and EEG power spectra. The results indicated that the purpose of an experiment should be a main criterion for deciding on the optimal epoch length. If the amounts of each stage and power density of EEG signals are desired, the 30-s epoch duration is the best selection to obtain accurate amounts and save analysis time, whereas when the number of stage transitions or the duration and number of episodes are needed, the 4-s epoch duration should be used for the analysis.

METHODS

Animals

Experiments were performed on 8 male Sprague-Dawley rats (weighing 280–300 g, 10–12 weeks old) and 8 male inbred C57BL/6 strain mice (weighing 22–26 g, 11–13 weeks old), which were provided by Shanghai SLAC Laboratory Animal Co., Ltd. The animals were housed in an insulated and sound-proof recording chamber that was maintained at an ambient temperature of 22 ± 0.5°C with a relative humidity of 60 ± 2% and that was on an automatically controlled 12 h light/12 h dark cycle (light on at 8:00 AM, illumination intensity, about 100 lux). The animals had free access to food and water. Experimental protocols were approved by the Medical Experimental Animal Administrative Committee of Shanghai. Every effort was made to minimize the number of animals used and any pain and discomfort experienced by them.

EEG and electromyogram recording

Under pentobarbital anesthesia (50 mg/kg, i.p.), the mice and rats were chronically implanted with EEG and electromyogram (EMG) electrodes for polysomnographic recordings. For rats, two stainless steel screw (1 mm in diameter) EEG electrodes (the first screw: anteroposterior (AP), +2 mm; left–right (LR), −2 mm; and the second: AP, −2 mm; LR, −2 mm, AP from bregma, LR from lambda, according to the stereotaxic coordinates of Paxinos and Watson[15]) and a reference electrode (opposite to EEG screw side, AP, +3 mm; LR, 3 mm) were surgically implanted as previously reported,[16] along with three stainless steel screws for anchorage to the skull. For mice, the implant consisted of two stainless steel screws (1 mm in diameter) that were inserted through the skull of the cortex (anteroposterior, +1.0 mm; left-right, −1.5 mm from bregma or lambda) according to the atlas of Franklin and Paxinos[17] and that served as EEG electrodes. Two insulated stainless steel, Teflon-coated wires bilaterally placed into both trapezius muscles served as EMG electrodes for rats or mice. All electrodes were attached to a micro connector and fixed to the skull with dental cement.

The EEG and EMG recordings were carried out by means of a slip ring designed so that behavioral movement of the animal would not be restricted. After a 10-day recovery period, the animals were housed individually in transparent barrels and habituated to the recording cable for 3–4 days before polygraphic recording. Each animal was recorded for 24 h beginning at 8:00 AM, the onset of the light period.

First, cortical EEG and EMG signals were amplified and filtered (EEG, 0.5–30 Hz) and then digitized at a sampling rate of 128 Hz. EEG and EMG were recorded using SleepSign, as previously described (Kissei Comtec, Nagano, Japan).[18,19]

Epoch selection, vigilance state analysis, and power spectral measurement

To investigate the effects of epoch length, we repeatedly analyzed polygraphic recordings by using epoch lengths of 4, 8, 10, 20, and 30 s. Computer methods for period amplitude analysis (PAA) were used for vigilance-state scoring. The vigilance states were divided into wakefulness, REM, and NREM sleep by SleepSign according to the standard criteria.[20,21] Waking is defined by a low-amplitude and high-frequency EEG with a high activity of EMG. NREM sleep is generally typified by a high-amplitude EEG associated with a low-voltage EMG. The presence of high EEG delta activity (0.65–4 Hz) is also employed to characterize this state. REM sleep is commonly defined by a low-amplitude, high-frequency EEG associated with the absence of EMG activity; the presence of EEG theta-activity (6–9 Hz) in the recording can be used to confirm this state. When one long epoch contained two different vigilance states if scored at a short epoch, the long one was judged according to the predominant state. As a final step, defined sleep–wake stages were examined visually and corrected if necessary.

Off-line power spectral analysis was performed with a Fast Fourier Transformation (FFT) analysis. The signals were subjected to simple analysis in the time domain. As for 10-s epochs, the EEG signal was separated into five 2-s regions per epoch (10 s). Each region was FFT calculated by using 256 data points (2 s) and the Hanning window, before the 5 spectra were averaged. The spectrum has a resolution of 0.5 Hz. As we have described previously,[18] on-line power spectral analysis was performed with a FFT using a 4- or 20-s epoch producing a power spectrum with a resolution of 0.25 Hz. The power spectrum resolution for 10-s and 30-s epochs is 0.5 Hz, whereas it is 0.125 Hz for the 8-s epoch. Because the sampling rate used in the study is 128 Hz, the FFT size should be $128 \times (2^N)$ ($N = 0,1,2,3,4. . . .$) data points for analyzing power spectral density. The data points for an epoch at 1, 2, 4 and 8 s are 128, 256, 512 and 1024, respectively. Accordingly, 10-s and 30-s are 1280 and 3840 points, but 1280 and 3840 are not $128 \times (2^N)$, so they could not be used for FFT analysis. Therefore, we select 5×2 s (256 points) for the 10-s epoch and 15×2 s (256 points) for the 30-s epoch. The data points for both 10 s and 30 s are selected as 256. Parameters applied for EEG signals automatically scored offline by SleepSign software are provided in Table 1.

Statistical analysis

All results were expressed as the means ± SEM (standard error of mean). Comparisons of sleep–wake profiles were made by one-way ANOVA per vigilance state with factor epoch duration followed by Tukey's test to determine whether or not the difference was significant in the total amount of time spent in each state, stage transition number, and episode number of each state. Power spectrum density and brief awakening were evaluated by using Student's *t*-test. The total amount of time spent in each state was expressed in minutes, and the stage transition number, episode number of each state, and power spectrum density were calculated for both the light period and the dark period. In all cases, $P < 0.05$ was taken as the level of significance.

RESULTS

Figure 1 shows the hypnogram of a rat for 2 min after the lights were turned on. The 4-s epoch analysis

Table 1 Parameters applied for EEG automatic scoring by SleepSign

Epoch duration (s)	FFT size	Average	Frequency resolution (Hz)
4	512	1	0.25
8	1024	1	0.125
10	256	5	0.5
20	512	5	0.25
30	256	15	0.5

FFT size, fast Fourier transformation size; Average, numbers of regions per epoch equal division from EEG signal.

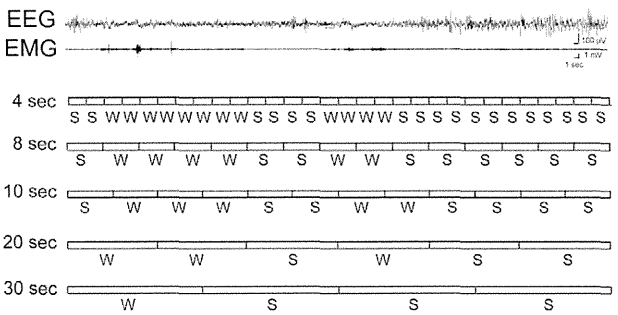

Figure 1 Typical example of vigilance-state scoring for 4-, 8-, 10-, 20-, and 30-s epochs of one raw EEG signal of a rat for 2 min in the morning after the light was turned on. The horizontal bar of time represents 1 s, and upper and lower vertical bars represent EEG 100 μV and EMG 1 mV, respectively.

Table 2 Total time spent in wakefulness, NREM and REM sleep for 12 h during light and dark periods in rats

Epoch duration (s)	Wakefulness (min)		REM sleep (min)		NREM sleep (min)	
	Light	Dark	Light	Dark	Light	Dark
4	199.04 ± 7.95	529.57 ± 10.19	77.94 ± 3.41	30.95 ± 2.37	443.01 ± 8.83	159.49 ± 9.61
8	208.75 ± 6.70	541.67 ± 9.72	74.93 ± 2.98	29.51 ± 2.46	436.32 ± 8.74	148.82 ± 8.49
10	210.78 ± 5.70	545.67 ± 10.81	76.31 ± 3.11	29.33 ± 2.38	432.93 ± 7.57	144.99 ± 9.33
20	200.87 ± 6.61	535.55 ± 10.33	73.37 ± 3.34	28.61 ± 2.35	445.74 ± 8.02	155.84 ± 8.84
30	193.37 ± 5.47	535.07 ± 11.28	71.49 ± 3.90	27.49 ± 2.35	455.13 ± 8.26	157.46 ± 9.70

Each value represents the mean ± SEM of 8 rats.

Table 3 Total time spent in wakefulness, NREM and REM sleep for 12 h during light and dark periods in mice

Epoch duration (s)	Wakefulness (min)		REM sleep (min)		NREM sleep (min)	
	Light	Dark	Light	Dark	Light	Dark
4	303.17 ± 5.11	495.67 ± 8.76	34.19 ± 2.97	13.93 ± 0.92	382.71 ± 3.42	210.39 ± 8.23
8	288.02 ± 4.44	495.61 ± 8.98	33.57 ± 2.68	14.76 ± 0.92	398.35 ± 3.11	209.57 ± 8.52
10	283.86 ± 4.87	490.99 ± 8.75	36.60 ± 2.87	13.40 ± 0.90	399.52 ± 2.82	215.61 ± 8.54
20	287.13 ± 4.59	486.18 ± 9.11	36.16 ± 2.34	14.38 ± 0.80	396.75 ± 3.60	219.43 ± 8.89
30	274.77 ± 3.87	487.10 ± 9.72	34.15 ± 2.13	12.85 ± 0.84	411.01 ± 3.05	220.07 ± 9.48

Each value represents the mean ± SEM of 8 mice.

provided the most accurate results for the sleep–wake profile. The first wakefulness (W) scored by 30-s epoch duration would be one NREM sleep state (S) and two wakefulness states if scored by 10-s epoch duration. The first wakefulness (W) scored by 20-s epoch duration would be two NREM sleep and three wakefulness states if scored by 4-s epoch duration. To study how epoch length affected the outcome of sleep–wake profiles, we used SleepSign software to analyze the rodent EEG signals at the commonly used epoch lengths of 4, 8, 10, 20, and 30 s and compared the results quantitatively and qualitatively.

Sleep amount and EEG power spectra

We compared the time spent in wakefulness, NREM, and REM sleep of rats and mice for 12-h light/dark and total 24-h periods when the different epoch durations were employed to analyze the EEG signals. As shown in Tables 2 and 3, and Figure 2, there were no significant statistical differences ($P > 0.05$) in the case of the 3 sleep stages in rats (Table 2, Fig. 2a) or mice (Table 3, Fig. 2b) during the 12-h light/dark periods or the total 24-h period, when the EEG was scored at a length of 4, 8, 10, 20 or 30 s.

Since the frequency resolution of the 4-s and 20-s epochs is 0.25 Hz, and that of the 10-s and 30-s ones is 0.5 Hz, we compared the power density of the 4 and and 20-s epochs, and of the 10 and 30-s ones. However, the frequency resolution of the 8-s epoch is 0.125 Hz, and so it could not be compared with the others. As shown in Figure 3, the power densities as analyzed by using the 4-s epoch and 10-s one was almost the same as those obtained with the 20-s epoch and 30-s epoch, respectively, in both rats and mice. The shape of the curve for rats showed the same peak at 3.5% and 2.9% of 0.65–4 Hz for NREM sleep between 4- and 20-s epochs and that at 5.8% and 7.2% of 6–9 Hz for REM sleep during light and dark periods, respectively; and the shape exhibited the same peak at 7.0% and 5.7% of 0.65–4 Hz for NREM sleep between the 10- and 30-s epochs and that at 11.2% and 13.6% of 6–9 Hz for REM sleep during light and dark periods, respectively. In mice, the shape of the curve peaked at 3.0% and 3.4% for NREM sleep and at 3.1% and 3.5% for REM sleep in the 4- and 20-s epochs during light and dark periods, respectively. It peaked at 5.8% and 6.0% for NREM sleep during light periods between the 10- and and 30-s epochs, respectively, and at 6.6% and 6.9% during dark periods ($P > 0.05$); for REM sleep, the curve peaked at 6.3 and 6.0 during light periods and at 7.0% and 6.0% during dark periods ($P > 0.05$) between the 10- and 30-s epochs, respectively. Therefore, analysis of the power density revealed that the choice of epoch

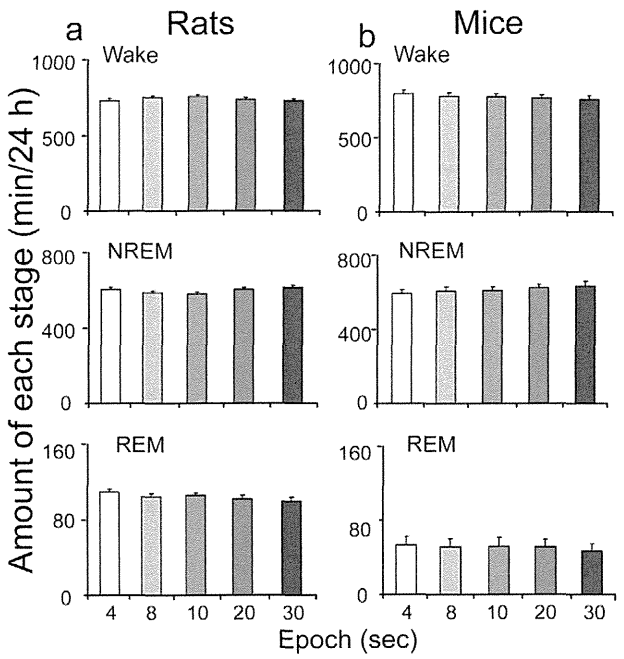

Figure 2 Effects of use of 4-, 8-, 10-, 20- and 30-s epoch scoring on total time spent in wakefulness, NREM and REM sleep for 24 h in rats (a) and mice (b). Each value represents the mean ± SEM of 8 animals. There was no significant difference between each group compared with the 4-s epoch duration group, as assessed by one-way ANOVA followed by Tukey's test.

duration between 4 and 20 s or 10 and 30 s did not affect the results of the EEG power density.

Numbers of state transition

To investigate how epoch durations affected the number of stage transitions, we analyzed the stage transition from wakefulness (W) to NREM sleep (N), from N to W, from N to REM sleep (R) and from R to W in both species (Fig. 4). In rats, in the light phase, data analyzed in terms of an epoch duration of 4 s displayed the highest number of the four stage transitions, as expected. With increasing epoch duration, the number of stage transitions became fewer and fewer; and among them the 30-s epoch analysis exhibited the lowest transition number. Tukey's test revealed significant epoch-dependent differences for all stage transition numbers except for N to W by 8-s analysis. During the dark period, compared with that for the 4-s group, the transition analyzed by using 8-, 10-, 20-, and 30-s epochs showed a statistical decrease in numbers from W to N and from N to W, but not from N to R; whereas the decrease from R to W was found only for the 30-s epoch (Fig. 4a). As shown in Figure 4b for mice, the data showed results similar to those obtained for the rats during either the dark or light phase. These findings indicate that the shorter the epoch duration, the greater the number of stage transitions.

Episode length of wakefulness and sleep

We further determined the effects of epoch duration on the occurrence of wakefulness and sleep bouts lasting 120, 240, 480, 960, 1920, and 3840 s. As shown in Figure 5a, during light or dark periods there was no statistical difference in the bouts of wakefulness episodes analyzed by using 4-, 10-, 20-, and 30-s epochs in rats. Since 8 s has no common multiple with the other epoch durations, it was unable to be included in the figure. Unlike its lack of effect on wakefulness, epoch duration significantly affected the measured bouts of NREM sleep episodes. During the light phase, the bouts of NREM sleep episodes over 120–240 and 240–480 s were dramatically decreased, compared with the bouts obtained with 4-s epochs, when epoch analysis was used at 20 and 30 s. Among them, when the 10-s epoch analysis was used, the bouts of NREM sleep episodes at 120–240 s were decreased in number, but those at 240–480 s were not statistically different from those obtained by the 4-s epoch analysis. On the contrary, the numbers of bouts of NREM sleep episodes at 480–960 s and at more than 960 s increased when the epoch analysis was set at 20- and 30-s epochs. Epoch duration at 10 s also resulted in an increase in the number of NREM sleep bouts of duration greater than 960 s. Similar results were found for the dark period. When epoch analysis at 20 and 30 s was used, the number of bouts of NREM sleep episodes at 120–240 s decreased, but those at 480–960 s increased. The increase in NREM sleep bout number for durations greater than 960 s was found only when the 30-s epoch was used for analysis. However, the bouts of NREM sleep episodes at 240–480 s showed no statistical difference across the four epoch analyses.

In mice, as shown in Figure 5b, comparison with the 4-s epoch analysis showed that 10-, 20-, and 30-s epoch analyses revealed significant decreases in the number of bouts of wakefulness for the ranges of 120–240, 240–480, and 480–960 s in the light, and 120–240, 240–480, 960–1920, and 1920–3840 s in the dark, as indicated in the figure. However, there were no statistical differences among the 4 epoch analyses for the bouts

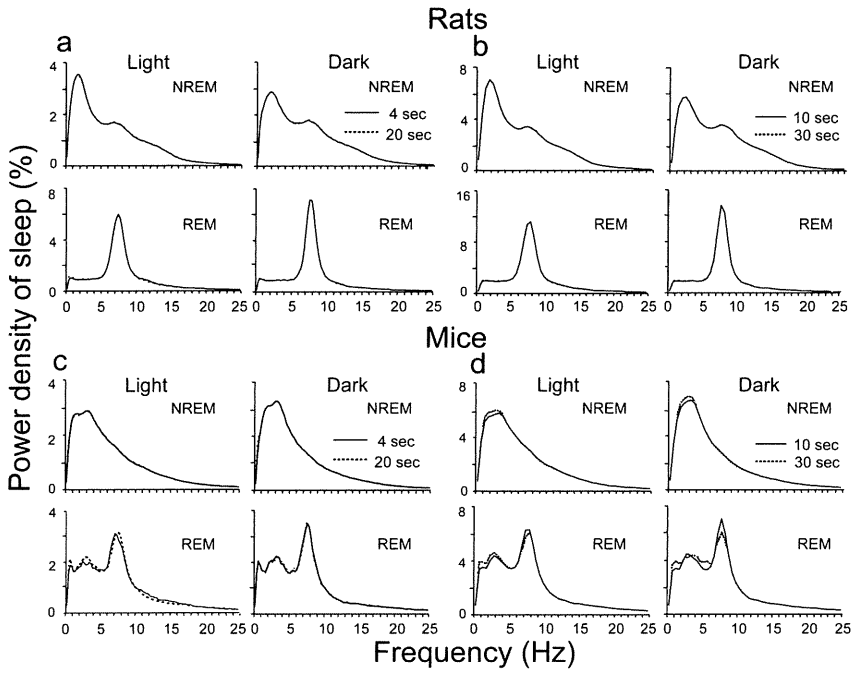

Figure 3 Effects of 4-, 10-, 20-, and 30-s epoch scoring on power density analysis of NREM and REM sleep of rats and mice during a 12-h period of light or darkness. Comparison of sleep power density produced by analysis of 4- and 20-s epoch (a) and of 10- and 30-s epoch duration (b) in rats. (c) 4-s epoch-duration analysis compared with 20-s epoch-duration analysis in mice. (d) 10-s epoch-duration analysis compared with 30-s epoch-duration analysis in mice. The power of each 0.25- or 0.5-Hz bin was first averaged across the sleep stages individually and then normalized as a group by calculating the percentage of the total power (0.25–25 Hz or 0.5–25 Hz) represented by each bin for the individual animal. There was no significant difference between each 2 groups as assessed by Student's *t*-test.

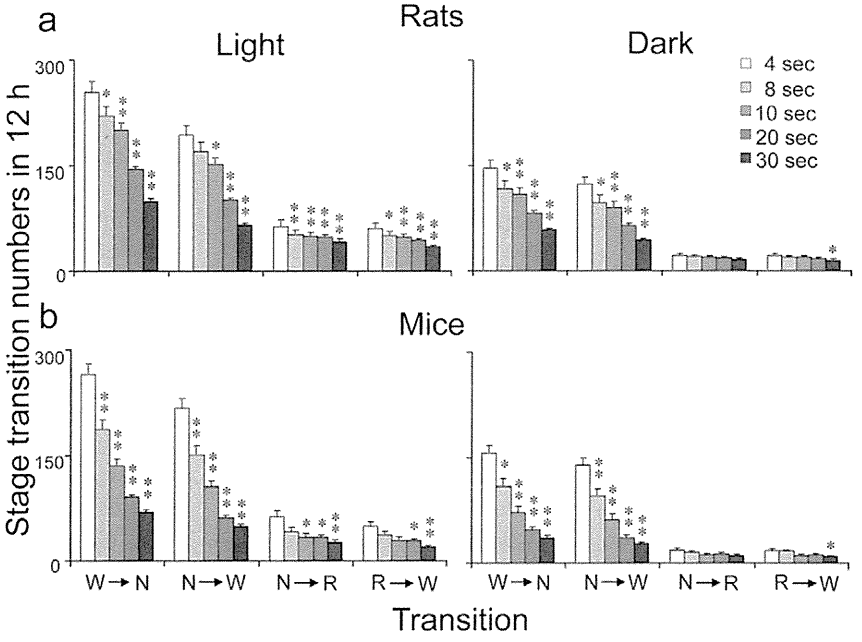

Figure 4 Effects of using 4-, 8-, 10-, 20-, and 30-s epochs on analysis of the numbers of state transitions from wakefulness (W) to NREM (N) sleep, from N to W, from N to REM sleep (R), and from R to W in rats (a) and mice (b). Each value represents the mean ± SEM of 8 animals. $*P < 0.05$; $**P < 0.01$, compared with 4-s epoch-duration group, as assessed by one-way ANOVA followed by Tukey's test.

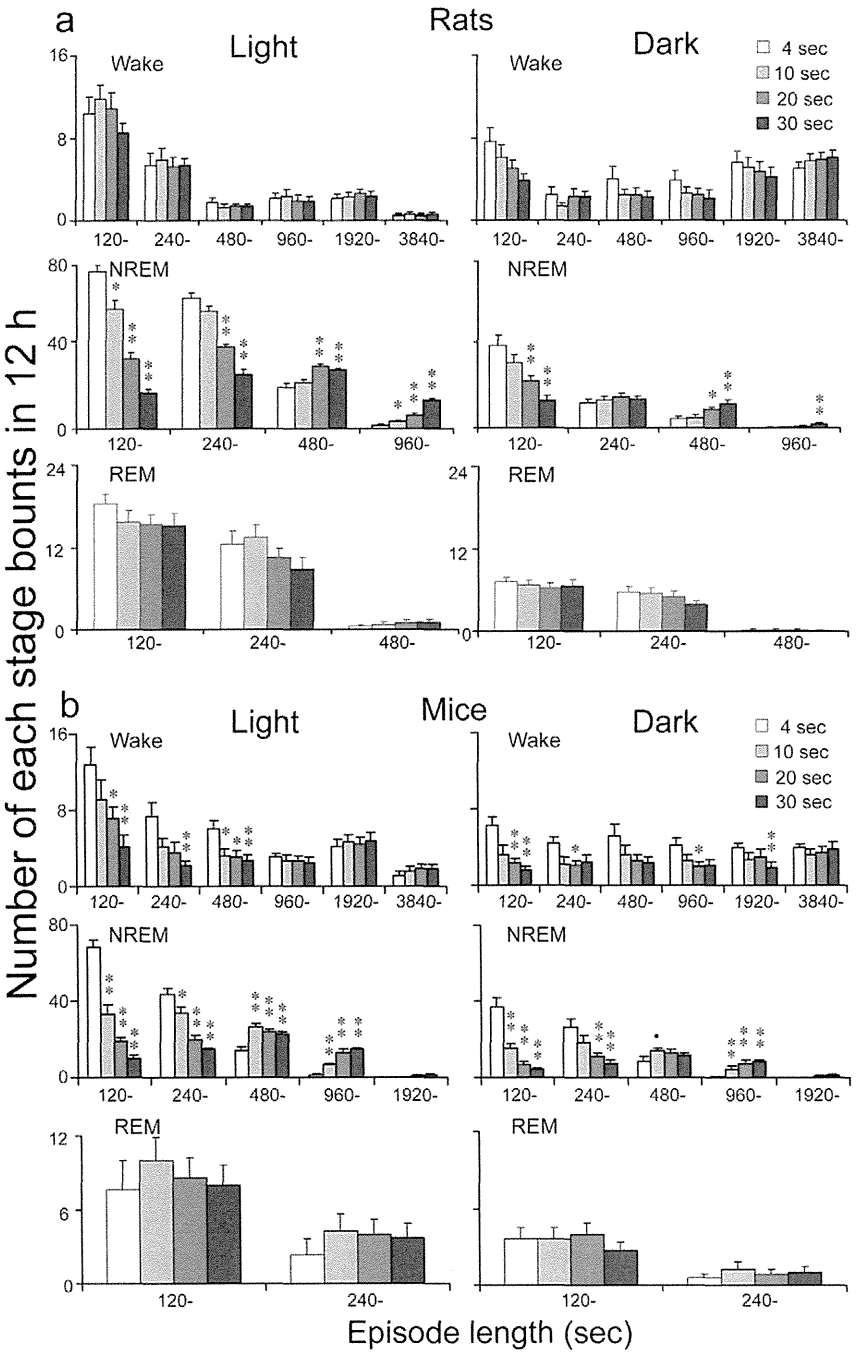

Figure 5 Effects of using 4-, 10-, 20-, and 30-s epochs on analysis of the numbers of bouts of wakefulness, NREM and REM sleep episodes in 12 h in rats (a) and mice (b). Each value represents the mean ± SEM of 8 mice and 8 rats. $^{*}P < 0.05$; $^{**}P < 0.01$, compared with 4-s epoch-duration group, as assessed by one-way ANOVA followed by Tukey's test.

of wakefulness over the ranges of 960–1920, 1920–3840, and more than 3840 s during the light and the dark phase. On the other hand, compared with the 4-s epoch analysis, the longer epochs of 10, 20, and 30 s resulted in a decreased number of the bouts of NREM sleep episodes at 120–240 s and 240–480 s, but in an increased number at 480–960 and 960–1920 s during both light and dark periods. These four epoch analyses did not alter the occurrence of NREM sleep episodes of duration greater than 1920 s. There was no statistical difference among the bouts of REM sleep episodes, as shown in Figure 5.

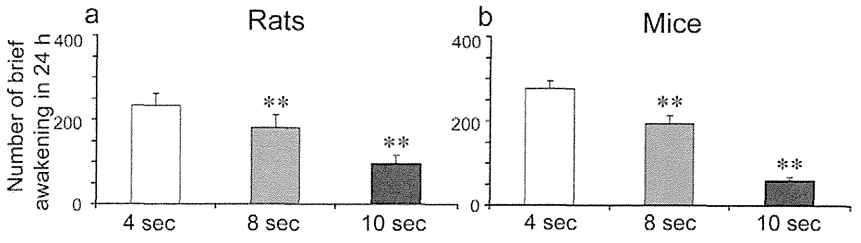

Figure 6 Effects of using 4-, 8-, and 10-s epochs on analysis of brief awakening in 24 h in rats (a) and mice (b). Each value represents the mean ± SEM of 8 mice or 8 rats. $^{**}P < 0.01$, compared with 4-s epoch-duration group, as assessed by Student's *t*-test.

These results indicate that the epoch-duration setting mainly affected the bouts of NREM sleep episodes, that is, the longer the epoch used for analysis, the fewer bouts of 120–240 s and 240–480 s, and the more bouts of 480–960 s and 960–1920 s. However, the epoch duration did not alter the number of bouts at more than 1920 s. Furthermore, the numbers of REM sleep bouts over 120 s was not affected by epoch duration.

Number of brief awakenings

We determined the effects of epoch duration on the occurrence of brief awakenings lasting less than 16 s. As shown in Figure 6, compared with the data analyzed by 4-s epoch, the numbers of brief awakenings decreased to 79% and 70% for the 8-s epoch, and to 41% and 21% for the 10-s epoch in rats and mice, respectively.

DISCUSSION

To find the optimal epoch duration in EEG analysis that satisfies the purpose of the experiment and relieves researchers from time-consuming labor, we compared the results obtained from five different commonly used epoch durations. Although analysis using epochs from 4 to 30 s did not affect the amount of each stage or power density, changes in epoch duration resulted in statistical differences in the five epoch analyses of the number of stage transitions and the duration and number of vigilance stages.

The epoch duration used for analysis affected the maximum value of the power spectrum density. As shown in Figure 3, as expected, the maximum value produced by 10- and 30-s epoch analysis was twice that produced with 4- and 20-s epochs, because the power density ratio of the fixed frequency (F_{fix}) can be computed from the following formula:

$$Ratio_{F_{fix}} = \frac{Power_{F_{fix}}}{\sum_{f=0}^{25} Power_f}\left(f{:}0{:}\frac{1}{N}{:}25\right)$$

$\frac{1}{N}$ is the frequency resolution. If the FFT size is doubled, $\frac{1}{N}$ is accordingly decreased to half its original value. Because of the continuity of signal frequency spectra, the power density value of consecutive frequencies can be viewed as nearly the same. Then, as the frequency resolution is decreased by half, the value of $\sum_{f=0}^{25} Power_f$ becomes twice as large. However, when the size of the FFT doubles, the value of the fixed frequency remains unchanged. That is why $Ratio_{F_{fix}}$ decreases to half its original value.

Robert *et al.*[22] reviewed automated sleep staging systems in rats. For each system, time is artificially segmented into elementary adjacent intervals referred to as epochs. According to Robert's opinion, the choice of the epoch length depends on various considerations, including the available memory of the computer data processing system and the resolution of the hypnogram required by the researchers, the time-scale of the phenomenon measured in the animal, and the destination of the investigation.[22]

Epoch length has to ensure both a frequency resolution high enough to provide a good accuracy for spectrum values and a total number of epochs high enough to provide statistically significant information. It has been reported that longer epochs provide higher frequency resolution and therefore higher accuracy in power computation than short epochs.[23] Our findings demonstrate that there was no essential statistical difference in the power density of EEG signals calculated by using short epochs of 4 and 20 s or 10 and 30 s.

The epoch durations in the literature vary from 1 to 30 s, with the majority having about a 10-s length.[24] As the major sleep parameters are the time spent in each stage and the power density for NREM and REM sleep, the stages transition and bouts of each stage episode are often not considered. If this is the case, an epoch duration of 20 s or even 30 s may well be used, thus saving a lot of time. Since spectral power density

analysis with a 20-s epoch can provide the same result as a 4-s one, a 4-s epoch for power density only is not necessary.[25]

An epoch duration as short as 4 s has been employed in EEG analyses, particularly when stage transitions and brief awakenings are studied. The brief awakening was first defined by Franken *et al.* as a period of wakefulness <16 s during an NREM sleep episode.[26] If a longer epoch duration was used for EEG analysis, the brief awakenings during an NREM sleep episode would not be detected or their number would be decreased. In the present study, we found that the number of brief awakenings significantly decreased in both rats and mice when epoch durations were set at 8 and 10 s, as compared with 4 s. Huang *et al.*[13] used 4-s epochs to analyze the EEG signals of histamine H_1 receptor knockout mice, and found that the knockout mice exhibit a significant decrease in the occurrence of brief awakening, suggesting that the H_1 receptor is involved in the regulation of behavioral state transitions from NREM sleep to wakefulness. If the sleep–wake profiles of H_1 receptor knockout mice were analyzed by using longer epoch durations, the phenotype of decreases in brief awakening would not be identified (data not shown). Also using a 4-s epoch, Tobler *et al.*[27] found that prion protein knockout mice exhibit an almost twofold increase in the number of brief awakening episodes, which was positively correlated with sleep fragmentation and light sleep and negatively correlated with slow-wave activity.[28]

In conclusion, there were no statistical differences in the amount and EEG power density of wakefulness, NREM sleep and REM sleep among the epoch durations of 4, 8, 10, 20, and 30 s. However, the shorter the epoch, the more numerous the stage transitions and the larger the episode number. For assessment of the amount and power density of sleep, a 30-s epoch duration might be adequate to save time and obtain the same results as obtained with shorter epochs. When data on the number of stage transitions, episode duration, and number of bouts, and numbers of brief awakenings occurrence are needed, the 4-s epoch duration should be chosen. The purpose of the experiment should be a main criterion used in choosing the optimal epoch length. When the amount of time spent in each stage and the power density of EEG signals are desired, a 30-s epoch duration is an appropriate option to save time, and the results obtained are not different from those found by analysis of other epoch durations. If stage transitions and durations, number of episodes, and numbers of brief awakenings are to be considered, a 4-s epoch duration is suggested to be the best choice for analysis.

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ORIGINAL ARTICLE
Pulmonary Circulation

A Phase III, Multicenter, Collaborative, Open-Label Clinical Trial of Sildenafil in Japanese Patients With Pulmonary Arterial Hypertension

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Background: There is evidence that phosphodiesterase type-5 is effective for the treatment of pulmonary arterial hypertension (PAH).

Methods and Results: A phase III, multicenter, open-label clinical trial of sildenafil 20 mg t.i.d. was conducted in 21 Japanese patients with PAH to examine its efficacy, safety, and pharmacokinetics. The present trial consisted of a screening period and 12-week treatment. Patients who were enrolled in the present trial increased their 6-min walking distance of administration increased at week 12 by 84.2 m from baseline. Hemodynamic parameters (eg, mean pulmonary artery pressure and pulmonary vascular resistance), Borg dyspnea scores, and plasma brain natriuretic peptide concentrations also improved compared to baseline. Most patients improved or sustained WHO functional class. Seven subjects, who were examined for the pharmacokinetics of sildefanil, showed relatively large interindividual variations in the C_{max}, AUC_{0-8}, $C_{ss,av}$, and C_{trough} of the drug. Any serious adverse events, severe adverse events, and deaths were not observed. Most of events of undeniable causality were mild or moderate in severity. Sildefanil was well tolerated by the subjects.

Conclusions: Sildenafil 20 mg t.i.d. was effective and safe for Japanese patients with PAH. (*Circ J* 2011; **75:** 677–682)

Key Words: Efficacy; Pharmacokinetics; Pulmonary arterial hypertension; Safety; Sildenafil

Pulmonary arterial hypertension (PAH) is a group of pathologies with a poor prognosis that are featured by progressive obliteration of the small pulmonary vascular bed and a progressive increase in pulmonary vascular resistance (PVR), eventually leading to refractory right heart failure and premature death.[1–3] A national prospective registry in the United States[4] has reported that the estimated median survival of patients with primary pulmonary hypertension (PPH) who were untreated following a definite diagnosis was 2.8 years and that the estimated 5-year survival rate was 34%. PAH is diagnosed when a mean pulmonary artery pressure (mPAP) is greater than 25 mmHg at rest.[5] The disease is classified the following into several categories: PAH of unknown etiology (idiopathic or familiar) and PAH associated with collagen vascular disease, congenital systemic-to-pulmonary shunts, portal hypertension, human immunodeficiency virus (HIV) infections, drugs/toxins, and others.[6] PAH provokes exertional dyspnea, easy fatigability, palpitation, chest pain, syncope, cough, and/or other symptoms and considerably deteriorates quality of life of the patients.

In Japan, PPH is designated for listing in the Specified Disease Treatment Research Program, and in 2004 there were 758 patients with identifiable PPH.[7] The number of patients with PAH, including patients with PAH associated with

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underlying disorders (eg, collagen vascular disease and congenital heart diseases), is estimated to be approximately 6,000 in Japan. Therapeutic drugs for PAH have been used in recent years in addition to treatment with conventional agents (anticoagulants, diuretics, inotropic agents, supplemental oxygen, calcium-channel blockers, vasodilators, antiproliferative agents, and endothelin-receptor antagonists).[8]

New insights have been obtained on the pathogenesis of PAH thorough the action of various enzymes. For instance, Rho-kinase activation was recently found to be involved in the pathogenesis of PAH. The inhibition of Rho-kinase reduces monocrotaline-induced PAH, and the phosphorylation of RhoA and prevention of its translocation to the plasma membrane mediate hypoxia-induced PH. Furthermore, there is direct evidence for Rho-kinase activation in PAH patients.[9–11]

Phosphodiesterase type-5 (PDE-5) is strongly expressed in the lung, and PDE-5 gene expression and activity are increased in chronic PH.[12–14] Based on these findings, the inhibition of PDE-5 has been researched as a mechanism of a new approach to the treatment of PAH. Sildenafil (Viagra®), a potent and highly selective inhibitor of PDE-5 that metabolizes cGMP,[15–18] was approved as a therapeutic drug for male erectile dysfunction. cGMP-specific PDE-5 is abundantly present in the pulmonary vasculature, and sildenafil leads to nitric oxide-mediated vasodilation and decreases PVR.[19,20]

The present study was designed as a small-scale clinical trial to clarify the features of sildenafil as a therapeutic drug for PAH in Japan, and its objective was to examine the efficacy, safety, and pharmacokinetics of sildenafil administered orally to Japanese patients with PAH at the regimen of 20 mg t.i.d. for 12 weeks.

Methods

Between April 2007 and February 2009, the phase III, multicenter, collaborative, open-label clinical trial of sildenafil 20 mg t.i.d. was conducted in 21 Japanese patients with PAH who met all inclusion criteria, who did not fall under any exclusion criterion, and who were enrolled at 8 medical institutions in Japan. The protocol of the present trial was approved by the institutional review board or ethical review board at each institution, and the trial was conducted in accordance with the protocol. All subjects provided written informed consent before their enrollment.

The present clinical trial consisted of the screening period and 12-week treatment period. Throughout the treatment period, one 20-mg tablet of sildenafil citrate was orally administered t.i.d. to each subject at intervals ≥6 h. Subjects visited the hospital 5 times (visit 1 at screening, visit 2 at the onset of administration, visit 3 at week 4 of administration, visit 4 at week 8 of administration, and visit 5 at week 12 of administration or at discontinuation), together with contact by phone at week 1 of administration.

The objectives of the present trial were as follows: 1) to verify the efficacy and safety of sildenafil 20 mg t.i.d. administered orally to Japanese patients with PAH for 12 weeks; and 2) to examine the steady-state pharmacokinetics of sildefanil and its metabolite under these conditions of administration.

The main inclusion criteria were as follows: the male or female patient should be aged ≥16 years, should be diagnosed with PAH, and should have a mean PAP >25 mmHg and a pulmonary capillary wedge pressure (PCWP) <15 mmHg in right heart catheterization at screening or baseline.

Assessment of Efficacy

The primary endpoints for efficacy were as follows: 1) change in 6-min walking distance at week 12 of administration from baseline; and 2) changes in baseline hemodynamic parameters [mean PAP, PVR, and cardiac output (CO)] at week 12 of administration from baseline.

The secondary endpoints for efficacy were as follows: 1) change in baseline 6-min walking distance at week 8 of administration from baseline; 2) changes in baseline WHO functional class at weeks 4, 8, and 12 of administration from baseline; 3) changes in baseline hemodynamic parameters [PAP (systolic and diastolic), systemic blood pressures (systolic, diastolic, and mean), PCWP, right atrial pressure, cardiac index, heart rate, PVR index, systemic vascular resistance, systemic vascular resistance index, arterial oxygen saturation, arterial oxygen tension, mixed venous oxygen saturation, and mixed venous oxygen tension)] at week 12 of administration from baseline; 4) changes in baseline Borg dyspnea scores at weeks 8 and 12 of administration from baseline; and 5) changes in baseline plasma brain natriuretic peptide (BNP) concentration at weeks 4, 8, and 12 of administration from baseline.

Assessment of Pharmacokinetics

Subjects in the present trial were assessed for the plasma concentrations and pharmacokinetic parameters [time to reach maximum concentration (T_{max}), maximum concentration (C_{max}), and area under the curve (AUC_{0-8})] of sildefanil and its metabolite at steady state after sildefanil administration, and for the average plasma concentration at steady state ($C_{ss,av}$) and plasma through concentration (C_{trough}) at the steady state of sildefanil.

Blood for the pharmacokinetic assessment was collected before administration and at 0.5, 1, 1.5, 2, 4, 6, and 8 h after administration on the specified visit days. The pretreated samples of the blood collected were used to measure the plasma concentrations of sildefanil and its metabolite at Covance (Indianapolis, IN, USA) according to the liquid chromatography–tandem mass spectrometry method. The lower limit of quantification was 1.00 ng/ml for both sildenafil and its metabolite.

Assessment of Safety

Subjects in the present trial were assessed for adverse events during history taking, physical examinations, laboratory tests [hematology: hemoglobin, hematocrit, red blood cell count, platelet count, white blood cell count, differential white blood count (neutrophils, eosinophils, basophils, lymphocytes, and monocytes), and prothrombin time; and blood chemistry (total bilirubin, direct bilirubin, AST, ALT, ALP, γ-GTP, albumin, total protein, BUN, creatinine, sodium, potassium, uric acid, and BNP)], vital signs (blood pressure, pulse rate, and body weight), 12-lead electrocardiography, and ophthalmology (examination, visual acuity, color sense, and funduscopy).

Statistical Analysis

SAS software version 8.2 (SAS Institute Inc; Cary, NC, USA) was used to perform all statistical analyses according to Student's t-test. Full analysis set (FAS) was analyzed for efficacy, and FAS and per protocol set (PPS) were analyzed for primary endpoints. FAS consisted of subjects who received at least 1 dose of sildenafil and who were assessed for efficacy at baseline and after sildefanil administration. A P value of <0.05 was considered statistically significant.

PPS consisted of subjects in FAS who met the following

Table 1. Subject Disposition and Analysis Sets

	No. of subjects
Enrolled	21
Medicated	21
Completed	19
Discontinued	2
Analyzed for efficacy	
Full analysis set	20
Per protocol set	16
Assessed for pharmacokinetics	7
Assessed for safety	21
Adverse events	21
Laboratory tests	20

Table 2. Demographic Characteristics of Subjects and Their Features at Baseline

Background factors	No. of subjects
Gender	
Male	4
Female	17
Age (years)	
<18	0
18–44	9
45–64	10
≥65	2
Mean±SD	47.1±14.7
Minimum to maximum	19–68
Body weight (kg)	
Mean±SD	58.5±10.6
Minimum to maximum	38.1–84.0
WHO functional class	
I	0
II	7
III	14
IV	0

Table 3. Types of PAH and Duration of Disease

	No. of subjects
Idiopathic PAH	
No. of subjects	6
Duration of disease (years)	
Mean	1.46
Minimum to maximum	0.1–4.0
Familiar PAH	
No. of subjects	5
Duration of disease (years)	
Mean	1.15
Minimum to maximum	0.3–4.0
PAH associated with other disorders (eg, collagen vascular disease, congenital systemic to pulmonary shunts, portal hypertension, HIV infection, and drugs/toxins)	
No. of subjects	10
Duration of disease (years)	
Mean	3.33
Minimum to maximum	0.1–15.0

PAH, pulmonary arterial hypertension; HIV, human immunodeficiency virus.

Table 4. Combination Therapies (Therapeutic Drugs for PAH and Basic Therapeutic Drugs for PAH)

	No. of subjects
No. of subjects	21
Therapeutic drugs for PAH	
Beraprost	9
Basic therapeutic drugs for PAH	
Warfarin	9
Cardiotonic drugs (eg, digoxin)	0
Calcium-channel antagonists	12
Diuretics	21
Oxygen therapy	14

PAH, pulmonary arterial hypertension.

Table 5. Six-minute Walking Distance at Baseline and at Weeks 8 and 12 of Administration, as Well as Its Changes From Baseline in Subjects

Endpoint	Six-minute walking distance (m)	
	Actual value	Change from baseline
Subjects		
Baseline		
No. of assessed subjects	20	–
Mean±SD (95%CI)	326.0±86.2 (285.7, 366.3)	–
Week 8 of administration		
No. of assessed subjects	19	19
Mean±SD (95%CI)	410.2±72.9 (375.0, 445.3)	87.5±75.3* (51.2, 123.8)
Week 12 of administration or discontinuation (LOCF)		
No. of assessed subjects	20	20
Mean±SD (95%CI)	410.2±66.6 (379.0, 441.3)	84.2±74.9* (49.1, 119.2)

CI, confidence interval; LOCF, last observation carried forward.
*P<0.0001.