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REPEATABILITY AND RESPONSIVENESS OF EXERCISE TESTS IN PULMONARY ARTERIAL HYPERTENSION

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Running Head: Repeatability and Responsiveness in PAH

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ABSTRACT

Exercise tolerance in pulmonary arterial hypertension (PAH) is most commonly assessed by the 6-min walk test (6MWT). Whether endurance exercise tests are more responsive than the 6MWT remain unknown.

Twenty stable PAH patients (mean age: 53[15]; mean pulmonary arterial pressure: 44[16]) already on PAH monotherapy completed the 6MWT, the endurance shuttle walk test (ESWT) and the cycle endurance test (CET) before and after the addition of sildenafil citrate 20 mg tid or placebo for 28 days in a randomized double-blind crossover setting. Pre/post-placebo tests were used to assess repeatability of each exercise test, whereas pre/post-sildenafil citrate tests were used to assess their responsiveness.

Sildenafil citrate led to placebo-corrected changes in exercise capacity of +18(25)meters (p=0.02), +58(235)seconds (p=0.58), and +29(77)seconds (p=0.09), for the 6MWT, the ESWT and the CET, respectively. The 6MWT was associated a lower coefficient of variation between repeated measures (3 vs. 18 vs. 13%), resulting in a higher standardized response mean compared to endurance tests (0.72, 0.25 and 0.38 for the 6MWT, the ESWT and CET, respectively).

The 6MWT had the best ability to capture changes in exercise capacity when sildenafil citrate was combined to patients' baseline monotherapy, supporting its use as an outcome measure in PAH.

Key Words: pulmonary hypertension, reproducibility of results, six-minute walk test.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is characterized by the progressive increase in pulmonary vascular resistance leading to right heart failure, poor exercise tolerance and early death. Because of its relationship with functional class and survival, exercise capacity is generally considered as a surrogate marker of disease severity in PAH [1]. As a result, the 6-min walk test (6MWT) has been the primary endpoint in the majority of the clinical trials [2].

In PAH, the 6MWT has been assumed to be repeatable and more responsive to clinical changes than the incremental cardiopulmonary exercise test (CPET) on ergocycle [3]. Based on these results, the 6MWT has become, by far, the most popular exercise testing modality in PAH [2]. However, the repeatability of the 6MWT has never been formally assessed in PAH, and its responsiveness to therapeutic intervention is questionable. Indeed, recent meta-analyses documented that the increase in 6MWT following monotherapy and combination therapy averaged 35 meters [2] and 22 meters [4], respectively, corresponding to ≈10% and ≈7% of baseline values. Moreover, the 6MWT may suffer from a ceiling effect in patients with mild PAH [5]. These observations suggest that the responsiveness of the 6MWT to interventions may be insufficient in PAH, leading to underestimation of the real treatment benefit and potentially to impeding drug development in this disease. The validation of a reliable exercise testing modality that is repeatable and responsive to interventions is therefore clearly needed in PAH. The endurance shuttle walk test (ESWT) and the cycle endurance test (CET) have been used in other chronic diseases like chronic obstructive pulmonary disease (COPD) and were shown to be more sensitive to clinical changes than the 6MWT or the CPET [6, 7]. CET in now the primary endpoint in most controlled trials evaluating bronchodilators and exercise training in COPD.

Conversely, endurance exercise tests have been rarely used in PAH and their responsiveness remains unknown.

The objective of this study was to compare the test-retest repeatability and responsiveness of the 6MWT, the ESWT and the CET in PAH. We hypothesized that the endurance tests would be as repeatable as the 6MWT, whereas their responsiveness following the addition of sildenafil citrate over initial monotherapy would be superior to that of the 6MWT.

METHODS

Ethics statement

The institutional ethics committee (Comité d'éthique de la recherche de l'Institut Universitaire de cardiologie et de pneumologie de Québec, protocol number: CÉR 20414) approved the research protocol and all patients gave written consent prior to study enrolment.

Subjects

Consecutive PAH patients on monotherapy but naive to phosphodiesterase type-5 inhibitors were recruited at the Institut Universitaire de cardiologie et de pneumologie de Québec. PAH diagnosis was made according to recent guidelines [8]. Recent right heart catheterization (<6 months) was used to describe hemodynamic severity. Only patients with stable clinical condition and medication over the last 4 months were eligible. Exclusion criteria were as follow: (1) unstable PAH defined as recent syncope or World Health Organization (WHO) functional class IV; (2) left ventricular ejection fraction < 40%; (3) significant restrictive (more than minimal lung

fibrosis on CT scan or total lung capacity < 70% of predicted) or obstructive ($FEV_1/FVC < 70\%$) lung disease; (4) systemic blood pressure <100/60 mmHg.

Study design

The design of this randomized, double blind, crossover study is shown in **Figure 1**.

Familiarization phase (Visits 1 and 2)

On day 1, a CPET was performed on an electrically braked ergocycle (Corival, Lode B.V., Groningen, The Netherlands) [9]. After 3 minutes of rest and 1 minute of unloaded pedalling, patients exercised using an individualized progressive RAMP protocol until exhaustion. Dyspnoea and leg fatigue were assessed at the end of the test using a 10-point modified Borg scale [10]. Patients then performed a practice CET. After 1 min of unloaded pedalling, the workload was set at 80% of peak workload achieved during the CPET. Patients were told to pedal as long as possible at a minimum rate of 60 rpm until exhaustion. The endurance time was defined as the total exercise duration, excluding unloaded pedalling.

On day 2, a 6MWT was performed on a flat 30-meter-long course according to recommendations [11]. An incremental shuttle walk test (ISWT) was also performed in an enclosed corridor on a flat 10-meter-long course delimited by two cones positioned 0.5 meters from either end [12]. Patients had to follow the rhythm dictated by an audio signal. The initial walking speed was set at 0.5 $m \bullet s^{-1}$ and subsequently increased every minute by 0.17 $m \bullet s^{-1}$ until exhaustion. Finally, a practice ESWT was performed on the same flat 10-meter-long course [13]. Following a 1.5 min

warm-up period, the walking speed was set at 85% of the peak walking speed achieved during the ISWT. The rhythm was dictated by an audio signal. The tests were performed 2 hours apart.

Treatment phase (Visits 3-4 and 5-6)

At Visit 3, the 6MWT, ESWT and CET were performed 2 hours apart [9]. To minimize any confounding effect based on test sequence, the order of the tests for each subject was randomly determined using the Latin Square design. Each patient then kept his individualized order for subsequent visits. Following this visit, patients were randomized to receive sildenafil citrate 20 mg tid or a matching placebo in a double-blind manner for 28 days. To ensure safety, patients were asked to measure their systemic blood pressure once a day during the first three days under both treatments. Blood pressure measurements were reported to a research nurse and were not reported to the study physician. Treatment was stopped in case of persistent headaches, systemic hypotension (<100/60mmHg) or any significant side effect. Patients were re-evaluated at Visit 4, after 28 ± 3 days of treatment with sildenafil citrate or placebo, in a similar manner than Visit 3. Then, after a 4-week washout period, the 6MWT, ESWT and CET were performed again before and after 28 ± 3 days of the alternate therapy (Visits 5-6). Pre/post-placebo tests were used to assess the repeatability of each exercise test, whereas pre/post-sildenafil citrate tests were used to assess the capacity of each test to detect changes in exercise capacity following PAH pharmacological treatment.

Physiological monitoring and measurements during exercise tests

During all exercise tests, cardiac parameters using a 12-lead ECG, breath-by-breath respiratory parameters and pulse oximetry were continuously monitored with a portable telemetric device

(Oxycon Mobile, Viasys Healthcare, Hoechberg, Germany). While encouragements were provided throughout incremental tests, no verbal encouragements were made during the 6MWT and endurance tests, as recommended [9, 11]. Patients finally rated their perception of exercise performance after each treatment phase using a 7-point Likert scale. This scale ranged from -3 to +3 and included the following ratings: -3 (large deterioration), -2 (moderate deterioration), -1 (slight deterioration), 0 (no change), 1 (slight improvement), 2 (moderate improvement) and 3 (large improvement).

Statistical analysis

The main outcome of interest was the capacity of each test to detect a significant improvement following the addition of sildenafil citrate to the current monotherapy. The primary outcome was thus the placebo-corrected change in exercise duration/length for each exercise test. Patients without post-baseline assessments were excluded from this analysis. A crossover statistical analysis, corrected for baseline values (covariable) was used. Repeatability of each exercise test was assessed using Pearson correlations and coefficients of variation between pre/post-placebo results. The coefficients of variation were calculated by dividing the SD of the change by the mean change of exercise duration/length between post-placebo versus pre-placebo and multiplied by 100 [14]. A modified Bland-Altman plot showing the relative change from mean according to the mean duration/length of the test was also used to appreciate the limits of agreement for each exercise test. The standardized response mean of each test was also computed as a marker of both the responsiveness and repeatability. This parameter was obtained by dividing the mean placebo-corrected difference in the exercise capacity between baseline and post-treatment by the SD of the change of the corresponding exercise modality (magnitude of change/SD of change).

In secondary analyses, the repeatability/responsiveness of the physiological response during the 6MWT, the ESWT and the CET at peak exercise were compared. Pearson correlations were performed to explore the relationship between the changes in exercise capacity captured by each exercise test and the Likert scores using post treatment and post placebo data (2 measures per patient). For each exercise test, we constructed a receiver operating characteristic (ROC) curve at progressive changes in exercise capacity to estimate the optimal threshold for both specificity and sensitivity to detect a ≥1 improvement on the Likert score. The multivariate normality assumption was verified with the Shapiro-Wilk test after Cholesky factorization. Univariate normality and variance assumptions were verified using the Shapiro-Wilk and Brown-Forsythe tests, respectively. The results are expressed as mean (standard deviation) unless specified otherwise. A statistical level of significance of 0.05 was used. All analyses were run blinded using SAS 9.2v (SAS institute, NC, USA). The sample size was estimated using the 6MWT. Assuming a mean placebo-corrected difference in 6MWT of 25 (45) meters between baseline and post-treatment and a drop-out rate of 20%, 25 subjects were necessary to detect a significant treatment effect with a type I error of 5 percent and a type II error of 20 percent.

RESULTS

Patient's characteristics

A total of 22 patients were recruited from June 2009 to August 2011. We excluded one patient during the familiarization phase due to a significant ST segment depression during exercise and another patient withdrew consent during the second treatment phase (**Figure 2**). Baseline characteristics of the 20 PAH patients who completed the entire protocol are shown in **Table 1.**

Most patients had idiopathic PAH or PAH associated with connective tissue disease and were in functional class II (75%). They were treated with bosentan (n=15), ambrisentan (n=3) and, epoprostenol I.V. (n=2) for 22 (18) months.

Test-retest repeatability (**Figure 3**)

Following 4 weeks of placebo, the mean differences in the duration/length of the 6MWT, the ESWT and the CET were -5 (22) meters, +19 (144) seconds and -14 (35) seconds, respectively. The 6MWT showed less variation than the ESWT and the CET, and appeared to be the most repeatable test, as assessed by the coefficients of variation (**Table 2**), the Pearson correlations and the modified Bland-Altman analysis. The heart rate, the oxygen consumption ($\dot{V}O_2$) and the oxygen saturation by pulse oximetry were the most repeatable physiological parameters **(Table 2).**

Responsiveness of exercise tests (**Figure 4**)

The placebo-corrected changes in exercise capacity following the addition of sildenafil citrate to the baseline therapy were 18 (25) meters, p=0.02, 58 (235) seconds, p=0.58 and 29 (77) seconds, p=0.09 for the 6MWT, the ESWT and the CET, respectively. Despite relative increases in 6MWT were lower compared to endurance tests (+4%, +15% and +12% from baseline), the standardized response mean (95% CI) were 0.72 (0.23 to 1.21), 0.25 (-0.20 to 0.70) and 0.38 (0.07 to 0.83) for the 6MWT, ESWT and CET, respectively. Cardiac and ventilatory responses at peak exercise were unchanged following the addition of sildenafil citrate (data not shown). The mean Likert scores following sildenafil citrate and placebo were 0.5 (1.4) and 0.4 (1.1), p=0.77, respectively. The Likert scores correlated with the objective changes in duration/length for the 6MWT (r=0.32,

p=0.05), the ESWT (r=0.52, p=0.01) and the CET (r=0.35, p=0.04), respectively. The area under the ROC curve to detect a clinically perceptible improvement in exercise capacity (Likert score ≥1) was 0.63, 0.74 and 0.63 for the 6MWT, the ESWT and the CET, respectively. The increase in exercise capacity associated with a true positive rate (specificity) of 90% to be associated with a Likert score ≥1 were 32 meters, 66 seconds and 53 seconds for the 6MWT, the ESWT and the CET, respectively.

DISCUSSION

This study documented that amongst exercise tests currently used in chronic respiratory disorders, the 6MWT was the most likely to capture changes in exercise capacity induced by the addition of sildenafil citrate over baseline monotherapy in PAH. The enhanced responsiveness of the 6MWT was related to its better repeatability compared to the CET and the ESWT, supporting the use of this functional exercise test as an appropriate and clinically relevant endpoint in randomized controlled trials.

Exercise capacity is generally considered as a surrogate marker of disease severity in PAH [1]. Actually, the 6MWT and the CPET have excellent discriminative properties in this disease, and are closely correlated with functional class, disease severity and survival especially for idiopathic PAH patients [1, 15]. However, their responsiveness has been recently questioned. Indeed, previous randomized controlled trials failed to show improvement in exercise capacity when the $\dot{V}O_{2\,peak}$ assessed by CPET was the primary endpoint [3] or when the 6MWT was the outcome measure in less severely impaired patients [16].

Previous studies concluded that the ESWT [7] or the CET [6] were more responsive than the 6MWT to bronchodilators in COPD. In PAH, the relative increase in exercise tolerance was enhanced when assessed using the CET compared to the 6MWT following exercise training [17, 18]. In the present study, the relative increase in exercise capacity following the addition of sildenafil citrate was also higher for the ESWT and the CET compared to the 6MWT. However, only the increase in the 6MWT was statistically significant following the addition of sildenafil citrate to the baseline monotherapy. Using the standardized response mean, we also found that the ability to capture changes in exercise capacity was greater for the 6MWT than for the ESWT or the CET. Indeed, the standardized response mean for the 6MWT was near 0.8, a threshold considered "large" as compared to values between 0.2 and 0.5 considered as small to moderate for the ESWT and the CET, respectively [19]. This parameter was chosen rather than the t-statistic that provides no indication of random variation between tests or the Cohen's effect size that fails to recognize the relationship between placebo and treatment values. The standardized response mean is thus one of the most appropriate measures of the responsiveness of a measuring tool [20]. It has direct implication in the determination of sample size for clinical trials: the larger the standardized response mean, the smaller the sample size to demonstrate a treatment effect. The better repeatability of the 6MWT may account for its increased ability to capture changes in exercise capacity following the addition of sildenafil citrate. Indeed, the coefficient of variation of the 6MWT distance was well within 10%, a value arbitrary considered being optimal for this sort of assessment tool [14]. It is noteworthy that the enhanced repeatability of the 6MWT might have been influenced by the fact that this test is repeatedly performed by PAH patients as part of their routine follow-up. Indeed, exercise tests are associated with a learning effect [9]. However, special attention was made to ensure that any variability between the tests was related to random

errors instead of systematic bias as patients underwent practice tests to minimize any learning effect. Also, the order of the tests for each subject was randomly determined. Moreover, systematic bias was excluded using Bland-Altman analysis and crossover statistical analyses did not reveal any sequence, visit or time effect. Since a majority of patients were in WHO functional class II, it is also possible that most patients exercised near their maximal walking speed, which may have increased the 6MWT repeatability. Therefore, whether such repeatability is representative of its reliability in the setting of a multicentre study, with more severe patients and with a larger cohort of patients remains unknown. However, the capacity of the 6MWT to document clinical improvement in previous randomized controlled trials supports this concept [2].

Very few studies assessed the effect of a combination therapy on exercise capacity in PAH. When the previous randomized placebo-controlled trials were pooled in a recent meta-analyse, the mean increase in 6MWT was 22 meters when a second PAH therapy was added-on baseline therapy compared to placebo [4]. Only one study assessed the effect of sildenafil citrate 80mg tid in addition to baseline epoprostenol I.V. and found a 28.8-meter (95% CI, 13.9 to 43.8 meters) improvement in the 6MWT distance with the combination therapy compared to epoprostenol I.V. alone [21]. In the present study, a relatively low dose of sildenafil citrate was used for a shorter period of time (4 vs. 16 weeks) to assess responsiveness of exercise tests in PAH for the following reasons: 1) only sildenafil citrate 20mg tid is commercialized for the treatment of PAH in Canada; 2) in the pivotal randomized controlled trial evaluating the effect of sildenafil citrate monotherapy [22], most of the improvement in exercise capacity occurred during the first 4 weeks of treatment with no dose-effect relationship; and 3) four weeks of placebo was deemed to

represent an appropriate period to assess mid-term repeatability of exercise testing while minimizing the risk of clinical deterioration on placebo. Finally, a majority of our patients were in WHO functional class II. Therefore, the study design, potential drug-drug interaction between bosentan and sildenafil citrate [23] and patients' characteristics may have negatively affected the increase in exercise capacity observed in our study.

Previous studies suggested that the minimal detectable difference (assessed using distribution-based method) [24] and the minimal important difference (MID, assessed using anchor-based method) [25], of the 6MWT distance following the initiation of PAH therapy were 30 to 40 meters. Although significant, the improvement in walking distance observed following the combination of sildenafil citrate to baseline monotherapy was thus modest and below the suggested MID for this variable and for the perception threshold. However, changes in exercise capacity significantly correlated with the subjective perception of change in clinical status as assessed by a Likert scale. In an attempt to assess the minimal increase in exercise capacity that was perceptible by patients in our cohort, a ROC curve was constructed. Changes in 6MWT distance and in ESWT that had >90% chance (specificity) of being perceived by the patient were close to the MID of the 6MWT previously suggested in PAH [25], as well as previously reported in COPD for the ESWT [26]. Conversely, the perception threshold for the CET was far from the proposed MID of 105 seconds reported for CET in COPD [27]. One potential explanation is that our CET was too short in duration. We arbitrary chose to set the intensity at 80% of the peak workload to try to minimize the high variability that has been seen in COPD at lower relative workloads [28]. However, according to the American Thoracic Society, a constant workload test should last a minimum of 360 seconds [9], which was not the case in our study. The relatively

high intensity might have induced early peripheral muscle acidosis and fatigue. Therefore, the CET might have been less susceptible to track a beneficial effect on pulmonary hemodynamics following sildenafil citrate administration. This also reinforce that MID estimates obtained in one disease cannot be necessarily extrapolated to other conditions and that the MID might be disease-, severity- and intervention-specific [29].

CONCLUSION

Amongst the exercise tests commonly used to evaluate patients with chronic respiratory diseases, the 6MWT had the best ability to capture changes in exercise capacity when sildenafil citrate was combined to patients' baseline monotherapy in PAH. This enhanced responsiveness was related to a better repeatability of the 6MWT compared to the ESWT and the CET. Changes in exercise capacity also correlated with changes in patients' perception of exercise tolerance. These results support the use of the 6MWT as an outcome measure, especially in the setting of controlled trials in PAH.

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FIGURE LEGEND

Figure 1 **Study design** During the first two visits, patients performed an incremental cardiopulmonary exercise test (CPET) and an incremental shuttle walk test (ISWT). A practice 6-min walk test (6MWT), endurance shuttle walk test (ESWT) and cycle endurance test (CET) were also performed as part of a familiarization phase. On visit 3, patients performed baseline 6MWT, ESWT and CET before they received sildenafil citrate or a matching placebo of identical appearance in a double-blind manner for 28 days. Patients were reevaluated at Visit 4, keeping the same test order throughout the study. After a 4-week washout period, the 6MWT, ESWT and CET were performed again before and after 28 ± 3 days of the alternate therapy (Visits 5-6). *Figure legend: CPET, Cardiopulmonary exercise test; CET, Cycle endurance test; 6MWT, 6-min walk test; ISWT, Incremental shuttle walk test; ESWT, Endurance shuttle walk test*

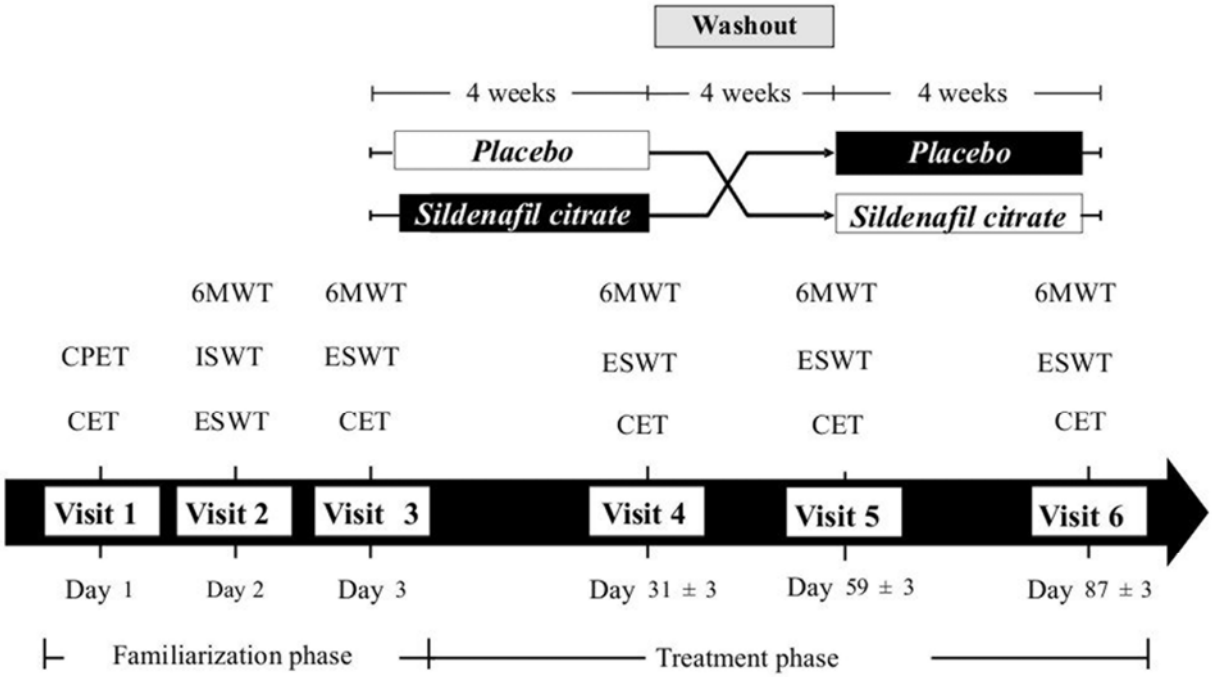

Figure 2 Study flow chart

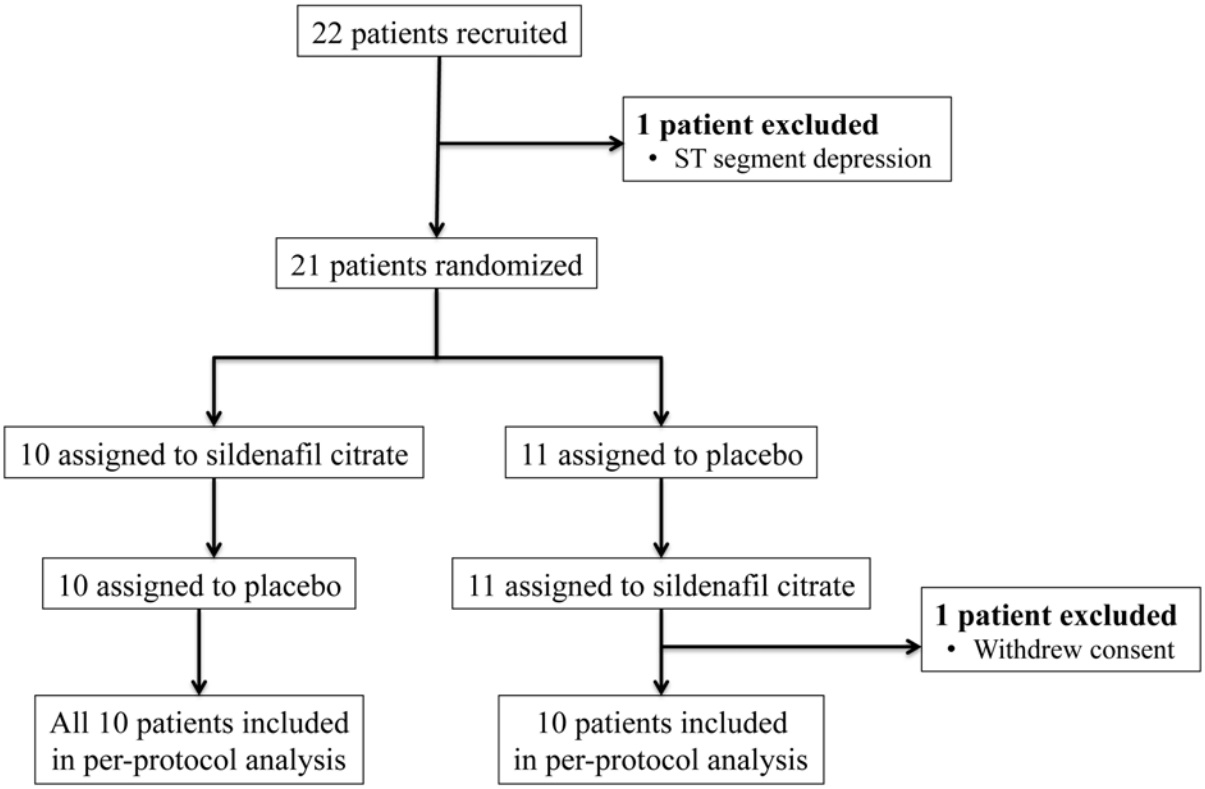
22 patients recruited
1 patient excluded
• ST segment depression
21 patients randomized
10 assigned to sildenafil citrate
11 assigned to placebo
10 assigned to placebo
11 assigned to sildenafil citrate
1 patient excluded
• Withdrew consent
All 10 patients included in per-protocol analysis
10 patients included in per-protocol analysis

Figure 3 **Test-retest repeatability of duration/length of the 6-min walk test (6MWT) the endurance shuttle walk test (ESWT) and cycle endurance test (CET)** The duration/length of post placebo tests significantly correlated with pre placebo tests for the 6MWT, the ESWT and the CET. The Pearson correlations (solid lines) and the identity lines (doted lines) are shown. A modified Bland-Altman plot showing the relative change between baseline and post-placebo tests according to the mean duration/length of each test is also shown. The solid line represents the mean relative difference between the two tests, whereas the dotted line corresponds to the coefficients of variation (CV). The coefficients of variation were similar for patients below and above the median value at baseline for the 6MWT (4 (3) versus 2 (2) %), the ESWT (12 (13) versus 15 (10) %) and the CET (16 (16) versus 11 (8) %). The limits of agreement (1.96 SD) were -48 to +38 meters, -263 to +301 seconds and -83 to +56 seconds for the 6MWT, the ESWT and the CET, respectively. *Figure legend: 6MWT, 6-min walk test; ESWT, Endurance shuttle walk test; CET, Cycle endurance test.*

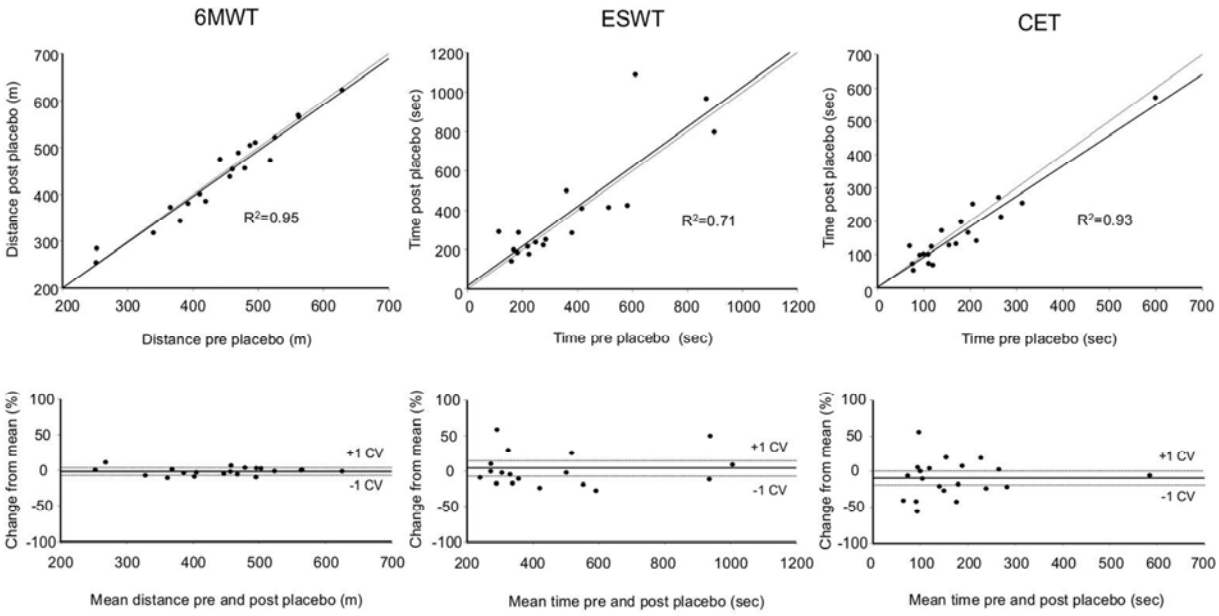
6MWT
ESWT
CET
Distance post placebo (m)
Time post placebo (sec)
Time post placebo (sec)
R²=0.95
R²=0.71
R²=0.93
Distance pre placebo (m)
Time pre placebo (sec)
Time pre placebo (sec)
Change from mean (%)
Change from mean (%)
Change from mean (%)
+1 CV
-1 CV
+1 CV
-1 CV
+1 CV
-1 CV
Mean distance pre and post placebo (m)
Mean time pre and post placebo (sec)
Mean time pre and post placebo (sec)

Figure 4 **Responsiveness of the 6-min walk test (6MWT), the endurance shuttle walk test (ESWT) and the cycle endurance test (CET)** The mean change in duration/length following add-on sildenafil citrate (black bars) and placebo (white bars) are plotted for the 6MWT (A), the ESWT (B) and the CET (C). Panel D represents the standardized response mean of each test. *Figure legend: 6MWT, 6-min walk test; ESWT, Endurance shuttle walk test; CET, Cycle endurance test. *p=0.02*

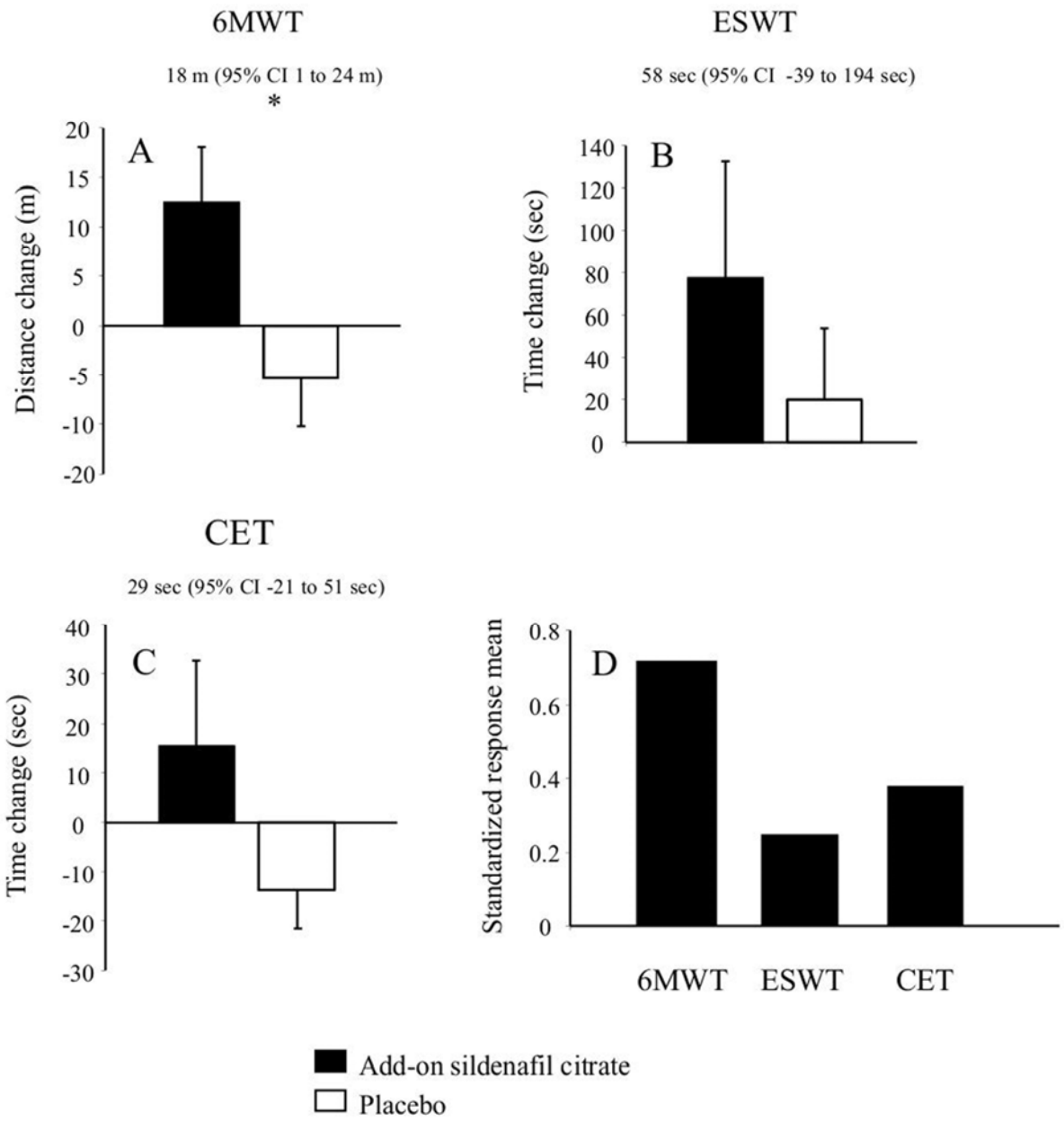
6MWT
18 m (95% CI 1 to 24 m)
*
A
Distance change (m)
20
15
10
5
0
-5
-10
-20
ESWT
58 sec (95% CI -39 to 194 sec)
B
Time change (sec)
140
120
100
80
60
40
20
0
CET
29 sec (95% CI -21 to 51 sec)
C
Time change (sec)
40
30
20
10
0
-10
-20
-30
D
Standardized response mean
0.8
0.6
0.4
0.2
0
6MWT
ESWT
CET
Add-on sildenafil citrate
Placebo

Table 1 Patients' characteristics (n=20)

PAH Type	
IPAH	n=9
PAH-Her	n=1
PAH-CTD	n=8
PAH-CHD**	n=2
Gender (F/M)	(16/4)
Age (years)	53 (15)
BMI ($kg \bullet m^{-2}$)	27 (5)
WHO Functional Class (II/III)	(15/5)
Pulmonary Hemodynamics	
RAP (mmHg)	6.4 (3.6)
mPAP (mmHg)	44 (16)
PCWP (mmHg)	10 (4)
CI ($l/min \bullet m^2$)	3.0 (0.6)
PVRi ($WU \bullet m^2$)	6.6 (3.1)
6MWD (m)	448 (98)
6MWD (% predicted)	78 (15)
CPET	
HR ($beat \bullet min^{-1}$)	138 (24)
$\dot{V}O_2$ ($ml \bullet min^{-1}$)	1008 (303)
$\dot{V}O_2$ ($ml \bullet kg^{-1} \bullet min^{-1}$)	14.65 (4.18)
RER	1.23 (0.12)
$\dot{V}_E$ ($L \bullet min^{-1}$)	59 (9)
SpO_2 (%)	89 (6)
$\dot{V}_E / \dot{V}CO_2$	51 (14)
$\dot{V}O_2$/HR ($ml \bullet beat^{-1}$)	7.4 (2.2)
Borg (Leg fatigue)	7 (2)
Borg (dyspnoea)	7 (2)

Values are n or mean (sd). ** Includes two patients with Eisenmenger physiology related to persistent arterial canal and ventricular septal defect. Predicted values are form Casanova et al. [30]

Table legend: IPAH, Idiopathic pulmonary arterial hypertension; PAH-Her, Heritable pulmonary arterial hypertension; PAH-CTD, Pulmonary arterial hypertension associated with connective tissue disease; PAH-CHD, Pulmonary arterial hypertension associated with congenital heat disease; BMI, Body mass index; WHO, World Health Organization; RAP, Right atrial pressure; mPAP, Mean pulmonary artery pressure; PCWP, Pre-capillary wedge pressure; CI, Cardiac index; PVRi, Pulmonary vascular resistance index; 6MWD, 6-min walk test distance; CPET, Cardiopulmonary exercise test; HR, Heart rate; $\dot{V}O_2$*, Oxygen consumption; RER, Respiratory exchange ratio;* $\dot{V}_E$*, Minute ventilation;* SpO_2*; Oxygen saturation by pulse oximetry;* $\dot{V}_E / \dot{V}CO_2$*, Ventilatory equivalent for carbon dioxide;* $\dot{V}O_2$*/HR, Oxygen pulse.*

Table 2 Test-retest repeatability of the physiological parameters

	6MWT				ESWT				CET			
	Pre placebo	*Post placebo*	*Pearson (R^2)*	*CV (%)*	*Pre placebo*	*Post placebo*	*Pearson (R^2)*	*CV* (%)	*Pre placebo*	*Post placebo*	*Pearson (R^2)*	*CV* (%)
Distance (m)	445 (97)	440 (98)	0.95	3	424 (312)	452 (342)	0.70	18	N/A	N/A	N/A	N/A
Time (sec)	N/A	N/A	N/A	N/A	373 (236)	392 (278)	0.71	14	179 (121)	165 (115)	0.93	13
HR (beats•min^{-1})	124 (22)	126 (22)	0.93	3	128 (19)	127 (20)	0.89	2	135 (22)	135 (23)	0.90	2
$\dot{V}O_2$ (ml•min^{-1})	924 (234)	932 (274)	0.94	4	965 (243)	977 (246)	0.77	4	943 (266)	938 (285)	0.80	4
$\dot{V}CO_2$ (ml•min^{-1})	1000 (303)	986 (328)	0.95	5	1061 (268)	1066 (287)	0.84	5	1183 (362)	1171 (391)	0.96	4
RER	1.08 (0.13)	1.05 (0.11)	0.69	4	1.11 (0.12)	1.10 (0.12)	0.57	4	1.26 (0.13)	1.25 (0.13)	0.74	4
$\dot{V}_E$ (L•min^{-1})	47 (10)	48 (10)	0.79	5	53 (8)	53 (8.11)	0.48	6	56 (10)	56 (12)	0.71	4
$\dot{V}_E/\dot{V}CO_2$	50 (17)	52 (20)	0.96	5	54 (18)	53 (18)	0.93	5	51 (14)	51 (15)	0.96	3
$\dot{V}O_2$ /HR (ml•beat^{-1})	7.5 (2.0)	7.6 (2.4)	0.89	5	7.8 (2.1)	7.8 (2.0)	0.92	4	7.1 (2.0)	7.0 (2.1)	0.92	4
S_PO_2 (%)	85 (8)	86 (8)	0.97	1	86 (7)	86 (8)	0.85	2	86 (9)	87 (8)	0.80	2

Table legend: HR, Heart rate; $\dot{V}O_2$, Oxygen consumption; $\dot{V}CO_2$, Carbon dioxide production; RER, Respiratory exchange ratio; $\dot{V}_E$, Minute ventilation; $\dot{V}_E/\dot{V}CO_2$, Ventilatory equivalent for carbon dioxide; S_PO_2; Oxygen saturation by pulse oximetry; $\dot{V}O_2$/HR, Oxygen pulse; CV, Coefficient of variation.