ABSTRACT

Objective: To evaluate the construct validity and reproducibility of the six-minute step test (6MST) in individuals with obstructive sleep apnea (OSA) treated with continuous positive airway pressure (CPAP). Methods: We evaluated 48 volunteers diagnosed with OSA and treated with CPAP for at least two months. The volunteers underwent the six-minute walk test (6MWT) and the 6MST, in random order and on different days, with an interval of, at most, seven days between the two tests. Results: A moderate positive correlation was found between the distance walked on the 6MWT and the number of steps climbed on the 6MST (r = 0.520; p < 0.001). There was no significant difference between the two 6MSTs in terms of the number of steps climbed (121.7 ± 27.1 vs. 123.6 ± 26.7). Reproducibility for performance on the 6MST and for cardiovascular variables was considered excellent (intraclass correlation coefficient > 0.8). Regarding cardiovascular responses, the 6MST produced higher values than did the 6MWT for HR at six minutes, percent predicted maximum HR, and leg fatigue at six minutes, as well as for systolic blood pressure at six minutes and at one minute of recovery. Conclusions: The 6MST is valid and reproducible, producing greater cardiovascular stress than does the 6MWT. However, the 6MST is also characterized as a submaximal test for the assessment of exercise tolerance in individuals with OSA treated with CPAP.

Keywords: Sleep apnea, obstructive; Reproducibility of results; Exercise test; Exercise tolerance.

INTRODUCTION

Obstructive sleep apnea (OSA) is a disease characterized by airflow obstruction during sleep due to upper airway collapse. As a consequence, repeated episodes of hypoxia, hypercapnia, and acidosis, followed by reoxygenation, affect the cellular bioenergetic function in striated muscles. Therefore, there may be structural damage to muscle fibers, in association with comorbidities such as arterial hypertension, cardiac arrhythmias, and heart failure, all of which can affect functional exercise capacity (FEC) in the population with OSA.

Regarding the treatment of OSA, the adoption of a better lifestyle, weight reduction, physical exercise, lateral decubitus sleep posture, use of intraoral devices, and orthodontic or surgical correction can be used. However, the gold standard in the treatment of OSA is the use of continuous positive airway pressure (CPAP), which maintains upper airway patency during sleep, reducing the tendency toward upper airway collapse and toward successive periods of hypoxia/reoxygenation, thus helping to improve sleep quality. Therefore, the systemic effects of the disease are minimized and the chances of developing comorbidities are reduced, which result in the attenuation of potential changes in FEC.

The most reliable method for assessing FEC is cardiopulmonary exercise testing (CPET), which analyzes cardiovascular and respiratory behavior during maximal effort. However, in addition to not being widely tolerated by patients, CPET is costly and complex to perform, which makes its use difficult. Field tests, in contrast, are generally based on a submaximal assessment, which results in higher tolerability, are inexpensive, are easy to administer, and, in general, use a habitual form of effort as a form of assessment.

Regarding the use of field tests in OSA, including after CPAP treatment, several studies have used the six-minute walk test (6MWT). However, there have been no studies using the six-minute step test (6MST) to assess exercise tolerance in individuals with OSA. Therefore, the objective of the present study was to validate and evaluate the reproducibility of the 6MST in individuals with OSA treated with CPAP.
METHODS

This was a cross-sectional study approved by the Human Research Ethics Committee of the Federal University of Pernambuco, in the city of Recife, Brazil (Ruling no. 2,081,503) and registered with ClinicalTrials.gov (Identifier: NCT03334331).

The study included individuals aged 28 to 69 years who had moderate or severe OSA, had a body mass index greater than 18 kg/m² and less than 45 kg/m², and had been on CPAP for at least two consecutive months.

The study excluded pregnant women and individuals presenting with the following comorbidities: uncontrolled hypertension or diabetes; orthopedic or neurological disorders; respiratory disorders, such as COPD or asthma; or any cardiovascular or respiratory disease that prevented them from performing the tests.

Initially, all participants were informed about the study procedures and gave written informed consent. Subsequently, participants underwent history taking, in which they were interviewed regarding their personal and clinical data, and anthropometric assessment.

Some CPAP device usage data were obtained by accessing the device’s memory card.

The following data were collected: proportion of CPAP use > 4 h/night; mean hours of CPAP use; titrated pressure (cmH₂O); and mean apnea-hypopnea index (events/h). Questionnaires were then completed regarding excessive daytime sleepiness (Epworth Sleepiness Scale), sleep quality (Pittsburgh Sleep Quality Index), and level of physical activity (International Physical Activity Questionnaire Short-Form).

The 6MWT and the 6MST were performed on different days, with a minimum interval of two days and a maximum interval of seven days between the two tests, the order of which was determined by randomization (www.randomization.com). Therefore, on the first day of assessment, participants performed either the 6MWT or the 6MST, according to the randomization, twice at least 30 min apart, and, on the second day of assessment, they performed the other test under the same conditions. During the test-retest interval, participants were asked to rest in a sitting position.

The 6MWT was performed in accordance with the American Thoracic Society (ATS) guidelines, along a flat, 30-m corridor with turnaround points marked with a traffic cone. Participants were instructed to walk as far as possible (walking back and forth around the cones) without running for six minutes, at a pace they could maintain.

In order to standardize, for the purpose of reproducibility, the way the 6MST was to be performed, we followed the ATS guidelines for the 6MWT, including the use of standard phrases of encouragement every minute. For the 6MST, we used a wooden step (dimensions: 20 cm in height × 80 cm in length × 40 cm in width) with an anti-slip surface. Participants were instructed to step up and down the step, maintaining a pace that would allow them to climb the step as many times as possible during the six-minute test period. Participants could use alternate legs to step up with, without the support of their arms, which remained stationary at their sides.

Tests were conducted by two evaluators: one monitored the cardiopulmonary variables, and one kept track of the number of laps completed or steps climbed by the participant. The following physiological variables were measured at rest, immediately upon completion of the test, and at one minute of recovery: HR; systolic blood pressure (SBP); diastolic blood pressure (DBP); SpO₂; and leg fatigue. HR and SpO₂ were also monitored every minute during the tests. Tests were interrupted if the participant reached the predicted maximum HR, if SpO₂ dropped to less than 85%, or if the participant asked to stop the test. If a test was interrupted, the participant was instructed to stop and rest in a standing position; however, the timer was not stopped, because the participant could continue the test whenever able (i.e., when SpO₂ was ≥ 88% and HR was 10 beats below the maximum HR) until the end of the six-minute test period.

The sample size was calculated with G*Power statistical software, version 3.1.3 (G*Power Team; Heinrich-Heine-Universität Düsseldorf, Kiel, Germany) for moderate between-test correlations (r = 0.5) related to submaximal HR values. Therefore, we assumed a type I error of 5%, a statistical power of 95%, and an effect size of 0.5, which resulted in a sample size of 45 individuals.

The data collected were initially tabulated in a Microsoft Excel 2016 spreadsheet. Statistical procedures were performed with GraphPad Prism software, version 4.0 (GraphPad Software Inc., San Diego, CA, USA), and SigmaPlot, version 12.0 (Systat Software, San Jose, CA, USA), and the level of statistical significance was set at p < 0.05. The Shapiro-Wilk normality test was used for data distribution analysis. Continuous variables are expressed as mean and standard deviation, as mean difference and 95% CI, or as median and interquartile range. Categorical variables are expressed as absolute and relative frequencies.

The Student’s t-test or the Mann-Whitney test was used in order to compare means between the groups of men and women and between the 6MWT and the 6MST. Categorical variables were compared by using the chi-square test or Fisher’s exact test.

Convergent construct validity was assessed. This type of validity assessment is made against a non-gold standard (the gold standard in this case being CPET), and validity was assessed by calculating Pearson’s correlation coefficient between the number of steps climbed on the 6MST (6SMST)—the test to be validated—and the distance walked on the 6MWT (6MWD)—a test that has been validated for measurement of FEC but is not a gold standard.
The test-retest reproducibility and reliability of the 6MST were analyzed by calculating the intraclass correlation coefficient (ICC) and Pearson’s correlation coefficient. Agreement between the 6MWT and the 6MST was analyzed through Bland-Altman plots with a 95% CI.

RESULTS

The study sample consisted of 48 volunteers. The anthropometric and clinical characteristics of the sample are presented in Table 1.

Regarding performance on the 6MST, 18 (37.5%) and 30 (62.5%) of the individuals performed better on the first and the second 6MST, respectively. As shown in Table 2, there were no significant differences in 6MWD or in S6MST between test and retest, and no learning effect was detected. Table 2 also shows the difference in mean S6MST, as well as in mean 6MWD, between test and retest.

Convergent construct validity showed a moderate positive correlation between 6MWD and S6MST (Figure 1).

Regarding 6MST performance, reproducibility for HR, SBP, and DBP immediately after the test and at one minute of recovery was found to be excellent (ICC > 0.8). Reproducibility for leg fatigue immediately after the test was also found to be excellent, whereas reproducibility for leg fatigue at one minute of recovery was found to be very good (0.6 < ICC < 0.8; Table 3).

Regarding cardiovascular responses, the 6MST produced higher values than did the 6MWT for HR at six minutes, percent predicted maximum HR, and leg fatigue immediately after the test, as well as for SBP immediately after the test and at one minute of recovery. There were no differences in DBP values between the 6MWT and the 6MST (Table 4). The agreement between performance on the test and retest of the 6MST is shown in Figure 2.

DISCUSSION

To our knowledge, this is the first study to validate and verify the reproducibility of the 6MST in individuals with OSA treated with CPAP. We could verify that the 6MST is a valid and reproducible test. In addition, there was no learning effect in the study population, demonstrating that only one 6MST is needed to assess FEC. The effort required to perform the 6MST is comparable to that of a common activity of daily living, dispensing with the need for a retest and saving time in the process of assessing FEC.[10,22,23] In line with the findings of this study, Arcuri et al.[19] also found no learning effect for the 6MST in performance on the 6MWT. The 6MWT is a widespread and widely studied test in the literature, with well-defined criteria for assessing FEC in other populations, and can be safely used as a benchmark against which other tools can be compared and validated.[20,21]

There were no differences in 6MWD or in S6MST between test and retest. Therefore, there was no learning effect, a situation in which there is a need for the individual to get used to the effort to be performed, by means of a proper neuromuscular adaptation to the required task and a reduction of potential psychological factors, such as anxiety.[22]

Thus, in this study population, we suggest that only one 6MST is needed to assess FEC. The effort required to perform the 6MST is comparable to that of a common activity of daily living, dispensing with the need for a retest and saving time in the process of assessing FEC.[10,22,23] In line with the findings of this study, Arcuri et al.[19] also found no learning effect for the 6MST in

### Table 1. General characteristics of the sample.*

| Characteristic                  | Total (N = 48) |
|--------------------------------|---------------|
| Age, years                     | 54.5 (48.0-62.8) |
| Weight, kg                     | 89.7 ± 16.8   |
| Height, m                      | 1.6 ± 0.1     |
| BMI, kg/m²                     | 33.2 ± 5.3    |
| Abdominal circumference, cm    | 108.9 ± 11.5  |
| Neck circumference, cm         | 41.4 ± 4.2    |
| Comorbidities, n (%)           |               |
| Arterial hypertension          | 30 (62.5)     |
| Diabetes mellitus              | 12 (25.0)     |
| Drugs                          |               |
| Antihypertensive agents        | 28 (58.3)     |
| Hypoglycemic agents            | 11 (22.9)     |
| IPAQ                           |               |
| Sedentary                      | 30 (62.5)     |
| Active                         | 18 (37.5)     |
| AHI, events/h                  |               |
| 15 ≥ AHI < 30 events/h         | 31.7 (25.0-46.3) |
| AHI ≥ 30 events/h              | 24 (50)       |
| ESS                            | 8.0 (4.3-14.8) |
| No sleepiness                  | 29 (60.4)     |
| Sleepiness                     | 19 (39.6)     |
| PSQI                           | 4.0 (3.0-6.0) |
| Good sleep quality             | 25 (52.1)     |
| Bad sleep quality              | 23 (47.9)     |
| CPAP                           |               |
| Use ≥ 4 h/night, %             | 70.5 (51.8-87.1) |
| Mean number of hours of use, h | 5.4 (4.3-6.2) |
| Pressure, cmH₂O                | 10.8 (9.0-13.0) |
| Mean AHI, events/h             | 1.8 (1.1-2.8) |
| Difficulty of adaptation       | 26 (54.2)     |

*BMI: body mass index; IPAQ: International Physical Activity Questionnaire; AHI: apnea-hypopnea index; ESS: Epworth Sleepiness Scale; PSQI: Pittsburgh Sleep Quality Index; and CPAP: continuous positive airway pressure. Data expressed as n (%) for categorical variables and as mean ± SD or median (interquartile range) for continuous variables.
Construct validity and reproducibility of the six-minute step test in subjects with obstructive sleep apnea treated with continuous positive airway pressure

Pessoa et al.,(18) when evaluating patients with COPD, found concurrent and predictive validity in the first 6MST, dispensing with the need for a second test.

Comparison of the behavior of physiological variables during the 6MST and during the 6MWT revealed that the 6MST produced greater cardiovascular stress. Similar results were found in a study by da Costa et al.,(24) in which healthy sedentary individuals were evaluated. However, da Silva et al.,(25) when comparing these variables in individuals who had a stroke and underwent the 6MST and the 6MWT, found no differences between the tests. The greater cardiovascular stress produced by the 6MST can be attributed to the fact that the 6MST requires greater leg muscle activity than does the 6MWT. The movement of climbing up and down steps is a vertical activity performed against the force of gravity, which requires greater effort and metabolic demand and, consequently, greater

| Table 3. Reproducibility of the six-minute step test. |
|------------------------------------------------------|
| Variable                                         | ICC (95% CI)      | p       |
| Performance on the test                          | 0.976 (0.957-0.986) | < 0.001 |
| HR at six minutes                                | 0.984 (0.971-0.991) | < 0.001 |
| SBP at six minutes                               | 0.906 (0.832-0.947) | < 0.001 |
| SBP at one minute of recovery                    | 0.826 (0.690-0.902) | < 0.001 |
| SBP at six minutes                               | 0.796 (0.636-0.886) | < 0.001 |
| SBP at one minute of recovery                    | 0.849 (0.730-0.915) | < 0.001 |
| DBP at six minutes                               | 0.927 (0.869-0.959) | < 0.001 |
| DBP at one minute of recovery                    | 0.646 (0.369-0.802) | < 0.001 |

ICC: intraclass correlation coefficient; SBP: systolic blood pressure; and DBP: diastolic blood pressure.
Table 4. Cardiovascular responses to the six-minute walk test (6MWT) and to the six-minute step test (6MST).a

|                      | 6MWT         | 6MST         | Δ means          | p       |
|----------------------|--------------|--------------|------------------|---------|
| HR, bpm              |              |              |                  |         |
| At rest              | 74.3 ± 11.9  | 76.0 ± 11.9  | -1.7 (-6.5 to 3.1) | 0.433#  |
| At six minutes       | 112.8 ± 15.5 | 124.8 ± 21.6 | -12.0 (-19.6 to -4.3) | 0.002*  |
| Recoveryb            | 33.6 ± 7.6   | 37.2 ± 11.0  | -3.6 (-7.4 to 0.2)  | 0.064#  |
| HRmax, % predicted   | 68.3 ± 8.9   | 74.2 ± 11.8  | -5.9 (-10.2 to -1.7) | 0.006*  |
| SBP, mmHg            |              |              |                  |         |
| At rest              | 120.8 ± 9.9  | 121.3 ± 9.1  | -0.4 (-4.3 to 3.4)  | 0.744#  |
| At six minutes       | 142.1 ± 16.9 | 151.9 ± 17.7 | -9.8 (-16.8 to -2.8) | 0.010#  |
| At one minute of recovery | 121.9 ± 10.5 | 127.9 ± 13.5 | -5.2 (-10.1 to -0.3) | 0.037*  |
| DBP, mmHg            |              |              |                  |         |
| At rest              | 81.0 ± 5.9   | 81.0 ± 5.6   | 0.0 (-2.3 to 2.3)   | 1.000#  |
| At six minutes       | 84.0 ± 6.4   | 82.7 ± 7.6   | 1.3 (-1.6 to 4.1)   | 0.367#  |
| At one minute of recovery | 81.0 ± 6.9   | 80.2 ± 7.3   | 0.8 (-2.1 to 3.7)   | 0.551*  |
| Leg fatigue, Borg scale |            |              |                  |         |
| At rest              | 2.1 ± 1.9    | 1.7 ± 1.8    | 0.4 (-0.3 to 1.2)   | 0.366#  |
| At six minutes       | 4.1 ± 2.1    | 5.1 ± 1.9    | -1.1 (-1.9 to -0.3)  | 0.017#  |
| At one minute of recovery | 2.5 ± 1.8    | 3.1 ± 1.9    | -0.7 (-1.4 to 0.1)  | 0.084#  |

SBP: systolic blood pressure; and DBP: diastolic blood pressure. aData expressed as mean ± SD or as difference in means (95% CI). bRecovery = HRmax = HR at one minute of recovery. *Student’s t-test: 6MWT vs. 6MST. #Mann-Whitney test: 6MWT vs. 6MST.

Figure 2. Bland-Altman plot for the test and retest agreement between the six-minute step test (6MST), as measured by the number of steps climbed on the 6MST (56MST). Mean error: 1.91 steps.
cardiovascular activity, but that remains within the submaximal effort range. Therefore, the 6MST can be considered more appropriate for assessing FEC, providing data that are more accurate for quantification and prescription of exercise in individuals with OSA treated with CPAP.

Assessment of submaximal exercise capacity in individuals with OSA is found in the literature mainly in studies employing the 6MWT, highlighting potential exercise intolerance accompanied by abnormal cardiovascular responses in this population, even after CPAP treatment, with similar results to those of CPET, as well as a reduction in 6MWD when compared with the optimal 6MWD. Another field test that has been used in this population is the shuttle test, which was able to detect changes in FEC after CPAP treatment, as well as weight reduction and excessive daytime sleepiness. To our knowledge, there have been no studies on the use of the 6MST for assessing individuals with OSA treated with CPAP. In addition to having the same advantages as other field tests, the 6MST does not require a large space for it to be performed and the step can be easily transported to be used in a minimal space. Therefore, better patient monitoring is possible, because the required movement is not large or horizontal.

In this study, the test-retest reproducibility of the 6MST was found to be excellent for the individual’s performance on the test and for the cardiovascular variables analyzed. These data corroborate the results obtained by da Costa et al. and Davi et al. in patients with COPD and in healthy young adults, respectively. In both studies, the reproducibility of the 6MST was found to be excellent for the individual’s performance on the test. Likewise, in their study involving healthy individuals, Arcuri et al. obtained excellent reproducibility for physiological variables and for the individual’s performance on the test. Therefore, the 6MST can be considered an evaluation strategy that uses a simple and easy-to-perform protocol, which positively contributes to its reproducibility and agreement, as well as to lower error rates.

The present study, despite reporting relevant data, has some limitations. The data obtained from the 6MST were not compared with those obtained from CPET, which is the gold standard in the assessment of FEC. The 6MWT, however, is a consolidated tool against which data obtained from other exercise tolerance tests can be compared with and validated. We suggest that larger studies be conducted to develop prediction equations for FEC using the 6MST in patients with OSA treated with CPAP.

We concluded that the 6MST is a valid and reproducible tool to assess FEC in individuals with OSA treated with CPAP. In addition, the 6MST can produce greater cardiovascular stress when compared with the 6MWT, although the 6MST is also characterized as a submaximal test for the assessment of exercise tolerance in this population. In addition to the fact that the 6MST is easy to administer and monitor, is inexpensive, and can be performed in small areas, we found that only one 6MST is needed to assess exercise tolerance reliably in this population, given that there was no learning effect. The convergence of these factors leads to increased feasibility of using the 6MST on a large scale in routine clinical practice at public and private health care facilities.

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