Background: Assessment of heart rate variability (HRV) during the 6-min walk test (6MWT) may be useful for quantifying the effects of interventions on the cardiovascular system.

Objective: To evaluate the reliability of HRV analysis during 6MWT in older adults.

Methods: Thirty-eight participants aged 60 years and older (23 females) performed two 6MWTs at least 30-min apart. The RR intervals of heartbeats were quantified throughout the 6MWTs and the last 2-min were considered for analysis. The square root of the mean square successive difference of RR intervals (RMSSD) and instantaneous beat-to-beat variability (SD1) were the indices used. We compared means, applied intraclass correlations (ICC), and plotted differences in the Bland and Altman approach of these indices.

Results: We found no significant differences in HRV or total distance walked between the first and second tests. The reliability of RMSSD (0.904; 0.815 – 0.950) and SD1 (0.902; 0.812 – 0.949) was excellent considering ICC values and a 95% confidence interval. The agreement of these indices was also acceptable for Bland and Altman plots using logarithms.

Conclusions: The reliability of some HRV indices during the 6MWT was acceptable. This evaluation might be useful in quantifying the effects of various interventions on the autonomic modulation of the heart.
INTRODUCTION

Heart rate variability (HRV) analysis consists of measuring RR intervals of each heartbeat and calculating its variation. During exercise, an increase in intensity results in increased heart rate (HR) and decreased HRV. Heart rate variability is a noninvasive measure that evaluates autonomic nervous system modulation under different physiological and clinical conditions. Currently, portable heart rate monitors are capable of recording RR intervals beat by beat, making HRV assessment easier, practical and inexpensive. Assessing HRV is of extreme importance, since it indicates the autonomic modulation of HR and can therefore provide information on cardiovascular health at rest and during exercise. Certain changes in HRV are important indicators for the early diagnosis of cardiac problems. Thus, HRV assessment has been useful in clinical practice. Previous studies suggest that autonomic modulation of HR is influenced by certain physiological factors such as age and level of physical activity. Studies on HRV have shown a decline in vagal modulation of HR at rest and with advancing age. However, there is little evidence regarding the extent to which these age-related changes occur during submaximal exercise. Several studies have evaluated HRV at rest to detect heart disease in patients and healthy individuals. The chronic effects of physical exercise on resting HRV have been documented in the literature in order to quantify improvements in autonomic modulation (e.g. increases in resting HRV time and frequency parameters) in response to this intervention. These studies clearly show the beneficial effects of exercise in healthy subjects and in patients with chronic diseases. Moreover, vagal control of HR plays an important cardioprotective role during exercise. While the clinical usefulness of HRV at rest has been well characterized, the clinical potential of exercise-induced HRV has not been investigated. Recent evidence indicates that subjects exhibiting higher sympathetic activity during exercise, mainly during the recovery phase, display increased cardiac vagal activity, resulting in marked sympathetic-parasympathetic output. This might partially explain the susceptibility of developing exercise induced cardiovascular events. Therefore, it would be important to assess HRV during exercise in advanced age with the aim of quantifying the effects of various interventions on autonomic modulation of HR. The six-minute walk test (6MWT) was originally developed to assess functional capacity, monitor the effectiveness of various treatments and establish the prognosis of patients with cardiopulmonary disease. However, this test has been validated in a number of clinical populations including asymptomatic older adults. In some clinical settings, it is possible to estimate maximal oxygen uptake ($V_{O2\max}$) using the six-minute walk distance (6MWD). This characteristic makes the 6MWT a simple and inexpensive tool for assessing physical fitness. The reliability and reproducibility of 6MWT has been shown in healthy older adults. During the 6MWT heart rate and $V_{O2}$ show monoexponential kinetics. The steady state is often reached after the third minute of the test. This non-incremental characteristic of the 6MWT enables the proper analysis of HRV, especially if the last minutes of the test are considered for analysis. However, for HRV assessment during the 6MWT to be used in clinical practice, its reliability should be investigated. We hypothesized that the assessment of HRV during the 6MWT is a reliable measurement. Thus, we aimed to evaluate the reliability of HRV assessment obtained during two subsequent 6MWTs in adults older than 60 years. We also assessed the influence of age, gender, hypertension, and body mass index (BMI) on HRV indices during exercise.

METHODS

Subjects

We evaluated 38 volunteers of both sexes, aged 60 years or older, all of whom were active or sedentary but not trained. Volunteers were recruited from among residents of the surrounding community through newspaper advertising. Reported physical activity was collected from the subjects by means of a questionnaire and those with less than 30 min/day of regular physical activity were classified as sedentary. Based on body mass index (BMI), the volunteers were stratified into the following categories: underweight (< 18.5 kg/m²), normal weight (18.5–24.9 kg/m²), overweight (25–29.9 kg/m²) and obese (≥ 30 kg/m²). Due to the high prevalence of overweight and obesity in the Brazilian population, subjects with BMI < 40 kg/m² were included to make the sample more representative. The inclusion criterion was clinical stability, defined as the absence of any acute disease during the 6 weeks before the study. Subjects with
abnormal pre-bronchodilator lung function, i.e. forced expiratory volume in 1 s (FEV1) < 80% of the predicted value or forced vital capacity FEV1 / FVC ratio < 70%, were excluded, as were those with current diagnosis of cardiac/respiratory disease, those with any health problem that might interfere with the ability to engage in physical exercise (e.g. neuronal degenerative diseases) and those with respiratory infections, current smokers, and diabetic patients. However, participants with controlled hypertension and former smokers without tobacco-related diseases were included. Subjects were instructed not to use substances that could alter the autonomic modulation of heart rate (e.g. β-blockers, stimulants, alcohol, etc.). Body weight, height and BMI were measured before the study. Pulmonary function and reversibility tests were performed using a spirometer (Spiropalm, COSMED, Pavona di Albano, Italy), according to criteria established by the American Thoracic Society. Values were presented as percentage of reference values. All subjects gave written informed consent to participate in the study, whose design was approved by the local Ethics in Human Research Committee (CEP UNIFESP n°. 1525/08).

Six-minute walk test
The 6MWT was performed along a straight 27-meter indoor hallway, following in accordance with American Thoracic Society guidelines. Participants were instructed to walk as fast as possible back and forth along the hallway for six minutes. Verbal encouragement was standardized, using the following phrases: “You’re doing well” and “Keep up the good work.” Two tests, at least 30 minutes apart, were conducted. Heart rate was monitored continuously throughout the tests by a portable heart rate monitor (RS800, Polar Electro, Kempele, OY, Finland) to enable HRV analysis. The total distance traveled on both tests was recorded in meters and compared to reference values.

Heart Rate Variability
The RR intervals at rest and during the 6MWT, quantified using a heart rate monitor, were used to assess HRV. The data obtained were recorded during a one-minute rest in the standing position before starting the test and throughout the 6MWT. After the test, data were transferred to a portable computer and stored using Polar ProTrainer 5 software (Polar Electro OY, Kempele, Finland) compatible with the heart rate monitor. The data were visually examined and any inappropriate or premature beat was corrected by interpolated data. Any RR interval with a difference greater than 20% compared to adjacent intervals was automatically and visually filtered. The results were stored as text files and transferred to Kubios HRV software (Biomedical Signal Analysis Group, University of Kuopio, Finland). HRV analysis was carried out during a 1-min rest just before the 6MWT and the last 2-min at the end of the test. In the time domain, the square root of the mean square successive difference of RR intervals (RMSSD) was analyzed. The Poincaré plot was analyzed in a Cartesian scattergram in which each RR interval was correlated with the preceding interval. The SD1 index, which represents the dispersion of points perpendicular to the line of identity and corresponds to the instantaneous beat-to-beat variability, was considered in this method. Heart rate variability examinations were performed between 8:00 and 12:00 AM to avoid diurnal variations. All participants were instructed to have a light meal and to abstain from smoking and drinking beverages containing caffeine or alcohol. They were also asked to avoid strenuous physical activities for at least 2 hours before the recording session.

Statistical analysis
Statistical analysis was carried out using SPSS 15.0 software (Systat Software Inc™, San Jose, CA, USA) and MedCalc 11.1.1 (MedCalc Software™, Mariakerke, Belgium) statistical package. Data are presented as mean ± standard deviation. When necessary (i.e., non-normal distribution), they were presented as median (interquartile). The values of heart rate and its variability obtained in the second walk test were compared between men and women, obese (BMI ≥ 30 kg/m²) and non-obese, and hypertensive and normotensive participants. The relationship between HRV and age during exercise was evaluated by Spearman’s correlation. The reliability of HRV assessment during the 6MWT was assessed by comparing the mean values obtained in the first and second tests (Student’s t test or Mann-Whitney), by calculating the intraclass coefficient of correlation (ICC), and by means of Bland and Altman plots. The ICC was calculated using absolute values of the HRV indices as
well as corrected values in relation to mean RR intervals. Since the differences in HRV indices between two 6MWTs showed non-parametric distribution, we assessed agreement using natural logarithm values. The probability of α error was set at 5%.

RESULTS
We evaluated 38 older adults (23 females, 60.52%) with the characteristics described in Table 1.

Table 1. General characteristics of the participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65 ± 4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77 ± 10</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.59 ± 0.09</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>Normal (&lt; 25 kg/m²)</td>
<td>18.42%</td>
</tr>
<tr>
<td>Overweight (25 to 29.9 kg/m²)</td>
<td>31.57%</td>
</tr>
<tr>
<td>Obese (≥ 30 kg/m²)</td>
<td>50%</td>
</tr>
</tbody>
</table>

BMI: body mass index

All the participants exhibited normal spirometric indices in predicted values (FVC = 108 ± 8%; FEV₁ = 105 ± 9%; FEV₁/FVC = 103 ± 11%). Seven participants were normal (18.42%), 12 were overweight (31.57%) and the remainder (50%) were obese according to BMI values. Nineteen participants (50%) were diagnosed with hypertension. There was no statistically significant influence of age, gender, hypertension or BMI on HRV indices obtained during the 6MWT. The results of 6MWT are presented in Table 2.

Table 2. Total distance walked, heart rate, and heart rate variability during the six-minute walk test (6MWT)

<table>
<thead>
<tr>
<th></th>
<th>1st 6MWT</th>
<th></th>
<th>2nd 6MWT</th>
<th></th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance (m)</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>553</td>
<td>83</td>
<td></td>
<td>563</td>
<td>91</td>
<td>0.060</td>
</tr>
<tr>
<td>Distance (%pred.)</td>
<td>104</td>
<td>12</td>
<td>106</td>
<td>13</td>
<td>0.059</td>
</tr>
<tr>
<td>HR final (%pred.)</td>
<td>72</td>
<td>11</td>
<td>73</td>
<td>11</td>
<td>0.065</td>
</tr>
<tr>
<td>Mean RR (ms)</td>
<td>537</td>
<td>79</td>
<td>528</td>
<td>88</td>
<td>0.272</td>
</tr>
<tr>
<td>RMSSD</td>
<td>5.95</td>
<td>2.91</td>
<td>5.83</td>
<td>3.57</td>
<td>0.714</td>
</tr>
<tr>
<td>SD1</td>
<td>4.21</td>
<td>2.06</td>
<td>4.12</td>
<td>2.53</td>
<td>0.718</td>
</tr>
<tr>
<td>SD2</td>
<td>23.06</td>
<td>21.77</td>
<td>18.11</td>
<td>6.59</td>
<td>1.139</td>
</tr>
</tbody>
</table>

RR: RR intervals; HR: heart rate; RMSSD: square root of the mean square differences of successive NN intervals; SD1: instantaneous variability in RR intervals on the Poincaré plot; SD2: long-term variability in RR intervals on Poincaré plot.
The total distance walked on the test was within predicted values for the Brazilian population. Heart rate and its variability as well as total distance walked showed no significant differences between the first and second 6MWT. The reliability of RMSSD and SD1 were excellent considering ICC values (Table 3). The agreement between measurements obtained in the first and second 6MWT was acceptable for both RMSSD and SD1 (Figures 1A and 1B). On the other hand, SD2 exhibited no statistically significant ICC (Table 3).

**DISCUSSION**

The assessment of HRV in response to exercise is a promising field in sports science. Accordingly, its reliability should be acceptable. This study presented a simple and inexpensive assessment of HRV during the 6MWT and showed that some important indices of HRV in time (RMSSD) and nonlinear (SD1) domain parameters are reliable in subjects aged 60 years or older. Despite the tendency of a significant difference in HR and RR intervals observed between walking tests, the HRV indices evaluated remained unchanged and showed high and significant ICCs. RMSSD and SD1 showed excellent reliability (ICC approximately 0.90) and agreement was also acceptable. Our results corroborate those previously described in adults. We found values similar to those observed in a study by Guijt et al.19 for the 6MWT. These authors also obtained two measurements for RMSSD during cycling. Reliability was found to be good to excellent for RMSSD, with mean ICC of 0.84 (95% CI: 0.67 – 0.92). In a study carried out by Tulppo et al2, the confidence interval (95%) of the difference for SD1 normalized for RR intervals was ± 5.3 at rest, decreasing during exercise to ± 3.3 and ± 2.2 for 50 and 75 W of constant exercise, respectively. Some authors consider reproducibility at rest in healthy subjects as well as in patients with myocardial infarction worse than reproducibility during exercise, since assessment at rest may be influenced by several factors (e.g., time of day, food intake, mental state).20, 21. However, Guijt et al.19 observed ICC between 0.75 and 0.98 for RMSSD measured at rest in subject’s aged 34±12 years. Our results during the 6MWT were similar to those found in a previous study 22. Nunanetal22 used a portable heart rate monitor to evaluate HRV at rest (10min) in healthy subjects and observed ICC values of 0.86 (0.71 to 0.93) for RMSSD. In this study, the portable monitor was as reliable as an electrocardiography device. Unfortunately, studies with this objective are quite heterogeneous. A review of the literature showed that the reliability of short-term HRV assessment is controversial.23. The coefficients of variation ranged between 1 and 100% Similar results were reported for ICC and limits of agreement. Thus, it is premature to affirm that HRV assessment at rest is a reliable measure; well-controlled studies are necessary to clarify this topic. 23. Although it has been reported that the reliability of HRV in clinical populations is worse than in healthy subjects,23, the results obtained in the present study during exercise differed since the reliability of the main HRV indices was acceptable even with the large proportion of hypertensive participants enrolled in the present study using their usual medications. In our investigation, several HRV indices in the frequency domain were not evaluated. Unfortunately, studies that assessed the reliability of these indices in response to

### Table 3. Intraclass correlation coefficients (95% confidence intervals) for heart rate variability measures between 1st and 2nd six-minute walking tests

<table>
<thead>
<tr>
<th>Measure</th>
<th>ICC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMSSD (ms)</td>
<td>0.904*</td>
<td>0.815 – 0.950</td>
</tr>
<tr>
<td>RMSSD/RR</td>
<td>0.902*</td>
<td>0.811 – 0.949</td>
</tr>
<tr>
<td>SD1/RR</td>
<td>0.902*</td>
<td>0.812 – 0.949</td>
</tr>
<tr>
<td>SD2/RR</td>
<td>0.353</td>
<td>-0.245 to 0.664</td>
</tr>
<tr>
<td>SD2</td>
<td>0.214</td>
<td>-0.109 – 0.497</td>
</tr>
</tbody>
</table>

*p< 0.05; ICC: intra-class correlation coefficient; CI: confidence interval; RR: RR intervals; RMSSD: square root of the mean square differences of successive NN intervals; SD1: instantaneous variability in RR intervals on the Poincaré plot; SD2: long-term variability in RR intervals on the Poincaré plot.
physical exercise are scarce, especially in adults. Reliability has been poor in research evaluating the effects of various interventions able to increase sympathetic activity\textsuperscript{24, 25}. Lord et al.\textsuperscript{26} found poor test-retest reproducibility of LF power during controlled respiration in chronic heart failure patients. By analyzing HRV reliability under resting conditions and two types of sympathetic activation in chronic heart failure patients, Piepoli et al.\textsuperscript{27} generated coefficient of variation values between 4–30%. They concluded that poor reliability existed under conditions of sympathetic activation (e.g. exercise) rather than at rest in this population. Indeed, frequency domain measures require stationary RR time series\textsuperscript{1}. This is unlikely during self-pace walking, where speeds in each section of the test are not controlled. This largely invalidates the use of spectral measures during 6MWT. In the present study we were unable to find a relationship between age and HRV indices during exercise. On the other hand, Tulppo et al.\textsuperscript{2} observed an influence of age and physical activity level on SD1 values. During exercise performed at constant load (100 W) on a cycle-ergometer, SD1 values were significantly higher in younger individuals and those who engaged in high levels of physical activity. These differences might be attributed to the age groups assessed by Tulppo et al.\textsuperscript{3}, including participants aged 24-34, 35-46 and 47-64 years, compared to our age group (60 years or older). Moreover, the workload was constant (75 or 100 W) in the Tulppo et al.\textsuperscript{2} study.\textsuperscript{2} The six-minute walk test is self-paced and walking speed is determined by the examinee, producing wide variability in exercise intensity. Our study has practical implications. For example, de la Cruz Torres et al.\textsuperscript{28} observed that patients with heart disease, as expected, had the worst HRV results at rest; however, they also showed abnormal HRV responses (using Poincaré plots) during constant-load submaximal exercise. On the Poincaré plot, controls showed a similar reduction in both the transverse (SD1) and longitudinal axis (SD2) in response to submaximal exercise. Similar results were reported for adults with Down syndrome. Although there was no significant difference between patients with Down syndrome and controls at rest, patients showed higher values for the low frequency/high frequency ratio of Fast Fourier transformation as well as higher low frequency values during exercise. Some studies have found that assessment of HRV during walking was responsive and able to quantify the improvement in autonomic modulation of HR in patients with chronic obstructive pulmonary disease and in those with heart failure\textsuperscript{29,30}. These results suggest the clinical usefulness of assessing HRV during walking. Accordingly, the present study contributes to scientific knowledge, describing the most reliable HRV indexes that might be assessed during 6MWT. We conclude that the reliability of HRV measures during the 6MWT is good for some indices in the time domain as well as in non-linear methods (RMSSD and SD1). These indices could be useful in quantifying the effects of various interventions on the autonomic modulation of heart rate, especially during exercise. On the other hand, researchers and clinicians should be cautious when using other indices not evaluated in the present study, mainly frequency domain indices. The reliability of these indices, with tighter control of potentially confounding variables, warrants further investigation.

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