

# Reliability of the 6-minute walk test and cardiac autonomic markers in active and sedentary older women

## Reprodutibilidade do teste de caminhada de 6 minutos e marcadores autonômicos cardíacos em idosas ativas e sedentárias

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**Abstract** – The aim of this study was to examine the reliability of the 6-minute walk test (6MWT) and post-exercise cardiac autonomic markers in physically active and sedentary older women. Eighteen physically active older women (64.2±3.1 years; 63.0±2.7 kg; 1.52±0.06 m; 26.9±2.7 kg.m-2) who performed Tai Chi Chuan for at least 6 months, and 18 sedentary older women (64.0±3.7 years; 63.8±8.9 kg; 1.49±0.05 m; 28.4±3.5 kg.m-2) were submitted to 6MWT in two separate occasions. Immediately after 6MWT, heart rate recovery at one (HRR1') and two minutes (HRR2'), as well as the heart rate variability (HRV) were recorded. Reliability was verified by intraclass correlation coefficient (ICC) with 95% confidence interval, Bland-Altman plots were used as a measure of agreement, and coefficient of variation (CV) was calculated. High reliability (ICC=0.86) was found for performance in 6MWT (528.8 ± 71.4 m and 473.2 ± 62.4 m; CV=7.9 and VC=8.5%) in both groups. Likewise, high reliability (ICC≥0.84 and ICC≥0.80) was found for HRR 1' (29.0±11.0 bpm and 17.0±8.0 bpm; VC=30.1% and VC =40.2%) and HRR 2' (36.0±10.0 bpm and 24.0±9.0 bpm; VC =23.7% and VC =22.8%) in both groups. Regarding HRV, moderate reliability was found in the active group (CCI≥0.57; VC =35–47%), while moderate-high reliability was found in the sedentary group (CCI=0.65–0.76; VC=34–69%). Agreement was found for all variables analyzed. In conclusion, post-exercise 6MWT, HRR, and HRV are reliable tools to assess functional capacity and cardiac autonomic control in physically active and sedentary older women.

**Key words:** Health in the elderly; Nervous system; Physical Fitness.

**Resumo** – *Objetivou-se verificar a reprodutibilidade do teste de caminhada de 6 minutos (TC6) e de marcadores autonômicos cardíacos pós-esforço em idosas fisicamente ativas e sedentárias. Dezoito idosas ativas (64,2 ± 3,1 anos; 63,0 ± 2,7 kg; 1,52 ± 0,06 m; 26,9 ± 2,7 kg.m-2) praticantes de Tai Chi Chuan (≥6 meses de prática) e 18 idosas sedentárias (64,0 ± 3,7 anos; 63,8 ± 8,9 kg; 1,49 ± 0,05 m; 28,4 ± 3,5 kg.m-2) foram submetidas a dois TC6 com uma semana de intervalo. Foram medidas a frequência cardíaca de recuperação (FCR) de um e dois minutos (FCR1' e FCR2', respectivamente) e variabilidade da frequência cardíaca (VFC) pós-esforço. A reprodutibilidade foi verificada pelos: coeficiente de correlação intraclassa (CCI) com 95% de intervalo de confiança; análise de concordância de Bland-Altman; e coeficiente de variação (CV). O desempenho no TC6 (528,8 ± 71,4 m e 473,2 ± 62,4 m; CV=7,9% e CV=8,5%) apresentou alta reprodutibilidade (CCI=0,86) em idosas ativas e sedentárias, respectivamente. Similarmente, as medidas pós-esforço da FCR1' (29,0 ± 11,0 bpm e 17,0 ± 8,0 bpm; CV=30,1% e CV=40,2%) e FCR2' (36,0 ± 10,0 bpm e 24,0 ± 9,0 bpm; CV=23,7% e CV=22,8%) apresentaram alta reprodutibilidade (CCI≥0,84 e CCI≥0,80) em ambos os grupos. Quanto a VFC verificou-se reprodutibilidade moderada (CCI≥0,57; CV=35–47%) em idosas ativas e moderada-alta nas sedentárias (CCI=0,65–0,76; CV=34–69%). Todas as variáveis apresentaram concordância em ambos os grupos. Conclui-se que o TC6, FCR e VFC pós-esforço são instrumentos reprodutíveis na avaliação da capacidade funcional e controle autonômico cardíaco em idosas ativas e sedentárias.*

**Palavras-chave:** Aptidão física; Saúde do Idoso; Sistema nervoso.

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## INTRODUCTION

Regular physical exercise is beneficial for virtually all populations. In the elderly, exercise plays a central role in mitigating or even reversing the deleterious effects of the aging process<sup>1</sup>. To confirm the benefits of exercise, many evaluation methods have been presented. In this regard, aging is associated with decreased functional capacity<sup>2</sup>, which directly affects independence and quality of life of this population, as well as changes in the autonomic nervous system, whose dysfunction is related to diseases and mortality<sup>3</sup>, so these components have been target of investigation.

The 6-minute walk test (6MWT) has been used to evaluate the functional capacity and, indirectly, cardiorespiratory fitness<sup>4,5</sup>. Burr et al.<sup>5</sup> directly assessed the maximum oxygen uptake in incremental test and 6MWT and found that this is of moderate to vigorous intensity and may be useful in aerobic fitness classification. The 6MWT has been applied in different populations such as children, adults, seniors, people with disabilities, patients with different diseases, among others<sup>5-9</sup>. The use of the 6MWT has been recommended for the evaluation of older adults due to its good tolerance, acceptance and for better reflecting daily activities<sup>4</sup>.

Agreement and reliability are important factors that determine the choice of a physical fitness test. Agreement concerns the proximity between two measurements in the same individual under the same circumstances, while reliability is the degree of variation of these measures. Reliability and agreement are necessary to ensure the reliability of a method and thus ensure the effectiveness of a particular intervention. The reliability of the 6MWT was confirmed in children<sup>9</sup>, people with disabilities<sup>7</sup>, patients with chronic pain or fibromyalgia<sup>8</sup>, and older adults<sup>6</sup>.

After the end of exercise, due to reduction of cardiovascular demand, there is a reduction in heart rate (HR) and change in the cardiac cycle induced by the parasympathetic reactivation and sympathetic withdrawal<sup>10</sup>. The cardiac autonomic modulation can be noninvasively measured through the *heart rate recovery* (*HRR*) and heart rate variability (*HRV*)<sup>3,11</sup>. *HRR* is the analysis of the amount of heart beats reduced in the first minutes after the end of the maximum or submaximal intensity exercise<sup>4</sup>. The literature has indicated that more trained individuals and or with better health status have higher *HRR*<sup>3,11</sup>. On the other hand, *HRV* corresponds to the temporal oscillations that occur in the cardiac cycle. Studies have shown that the faster or closer *HRV* returns to resting levels, the better the cardiac autonomic control<sup>11</sup>. *HRR* after 6MWT is an important predictor of prognosis of patients with heart failure<sup>12</sup> and pulmonary embolism survivors<sup>13</sup>, health levels in pulmonary arterial hypertension<sup>14</sup>, survival<sup>15</sup> and pulmonary hypertension<sup>16</sup> in patients with idiopathic pulmonary fibrosis. Regarding *HRV*, children with cystic fibrosis have sympathetic predominance after the 6MWT compared with healthy children<sup>17</sup>.

Regarding the reliability of *HRV* and *HRR* after physical exertion, there are few studies in literature investigating athletes<sup>18</sup>, young people

and healthy adults<sup>25-27</sup>. However, although these studies are focused on the application of these methods in various conditions and populations, the assessment of reliability and agreement of the 6MWT, HRR, and HRV after exertion in older adults with different levels of physical activity, still need to be established. Therefore, the aim of this study was to verify the reliability of the 6MWT and autonomic markers (*HRR* and *HRV*) after exercise in physically active and sedentary older women.

## METHODOLOGICAL PROCEDURES

### Sample

The sample was composed of 36 older women, 18 physically active ( $64.1 \pm 4.6$  years,  $63.7 \pm 6.7$  kg;  $1.52 \pm 0.06$  m,  $27.3 \pm 2.7$  kg<sup>-2</sup>), with at least 6 months of Tai Chi Chuan practice (TCC), practicing of only this type of exercise, and 18 sedentary ones ( $64.0 \pm 6.2$  years;  $64.9 \pm 10.5$  kg;  $1.49 \pm 0.05$ m;  $29.0 \pm 3.8$  kg m<sup>-2</sup>), who have not performed systematic exercise in the last six months.

To be included in this study, participants could not make use of cardiac pacemaker, tobacco and beta blockers or calcium inhibitors. Exclusion criteria were presenting resting systolic and diastolic blood pressure on the day of physical tests  $\geq 160/105$  mmHg (according to recommendations of the Brazilian Society of Hypertension), chest pain during the tests, intolerable dyspnea, leg cramps, dizziness or vertigo. All participants were informed about the procedures used, possible benefits and risks linked to study participation, conditioning their participation on a voluntary basis by signing the free and informed consent form. The research protocol was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte (CAAE - 0036.0.051.294-11, Protocol No: 540/11 - CEP / HUOL).

### Experimental design

In this experimental study, participants were evaluated twice with one-week interval between data collections. This design was used for both groups, starting assessments with TCC practitioners and after one month with sedentary individuals. All tests were performed in a covered gym and started in the same period of the day (07:00 am - 09:00 am) to minimize possible circadian effects. Participants were told to keep their activities of the daily living and to avoid any physical activity or exercise other than those performed during the study. In addition, participants were instructed not to consume foods and / or drinks containing alcohol or caffeine 3h previous to tests.

### Walking test protocol

One week before the study, a familiarization protocol was used with the same procedures used in the study. For the 6MWT, procedures followed the recommendations of the American Thoracic Society<sup>4</sup>.

## Analysis of the heart rate recovery and heart rate variability

Heart rate monitor (Polar®, RS800CX model) was used to record HR beat by beat during evaluations. To evaluate the relative intensity achieved during the 6MWT, predicted maximum heart rate ( $HR_{max}$ ) was calculated ( $220 - \text{age}$ ), and the  $HR_{max}$  percentage (HR%) considering the HR value at the end of the 6MWT [ $\%HR_{max} = (HR_{final} \times 100) / HR_{max}$ ]. *HRR* was considered as the absolute difference between HR at the end of the tests HR after one ( $\Delta FCR1$ ) and two minutes ( $\Delta FCR2$ )<sup>4</sup>. To control the factors that influence HR during the recovery phase, individuals remained in the standing position, keeping as still as possible, without any communication with each other or with the evaluator and without the intake of any kind of drink during recovery.

After collecting *HRR*, participants slowly lay on mats arranged next to the starting point of the 6MWT for the collection of post-exercise HRV for 10 minutes. The HRV collection protocol was recommended by the Task Force<sup>22</sup>. For HRV, symbolic analysis was used, which considers the non-linear nature of HRV<sup>23</sup>. The symbolic analysis consists of the distribution of the series of RR intervals, turning them into symbols and from these building standards. All these standards are grouped according to the number and type of variations<sup>23</sup>. Studies with pharmacological blockers and autonomic tests have shown that the index with no variation (0V) and two unlike variations (2ULV) indicate sympathetic and parasympathetic modulation, respectively<sup>24</sup>. Unstable segments were avoided, since data stability is a prerequisite for symbolic analyses<sup>25</sup>. Short-term HRV recordings are conventionally considered stable, as long as the experimental settings are carefully controlled. The records of RR intervals were visually inspected for the removal of outliers, performed by two double-blind evaluators for selection of excerpts. Thereafter, records were automatically filtered (Polar Precision Performance v.3.02.007). For HRV analysis, the symbolic analysis protocol proposed by Porta et al.<sup>26</sup> and the Symbolic Analisi software (Dipartimento di Scienze Precliniche, Universita 'Degli Studi di Milano, Italy) were used. 0V and 2ULV indexes were used for analyses<sup>24,26</sup>.

## Statistical analysis

The normal distribution of the data was confirmed by the Shapiro-Wilk test. Data are expressed as mean and standard deviation. The coefficient of variation (CV) of differences for each group was calculated by the ratio between the standard deviation of the differences and the average of all observations multiplied by 100. The Student t test was used to compare the characteristics of groups. Reliability was assessed by intraclass correlation coefficient (ICC; 95% confidence interval). The qualitative ICC classification was  $\geq 0.90$  "very high", 0.70-0.89 "high," and 0.50-0.69 "moderate"<sup>27</sup>. The Bland-Altman<sup>28</sup> test was applied for the agreement analysis between measures. In Bland-Altman plots in the abscissa axis, the averages of the two measurements are shown  $[(M1 + M2) / 2]$ , while in the ordinate axis displays the differences between the two measures ( $M1 - M2$ )

for each participant. A variable has agreement when the line of equality (zero line) is within the 95% confidence interval (95% CI) of the average differences. For all analyses, the significance level was set at  $p < 0.05$  and tests were performed using SPSS 18.0 (SPSS, Chicago, IL, USA).

## RESULTS

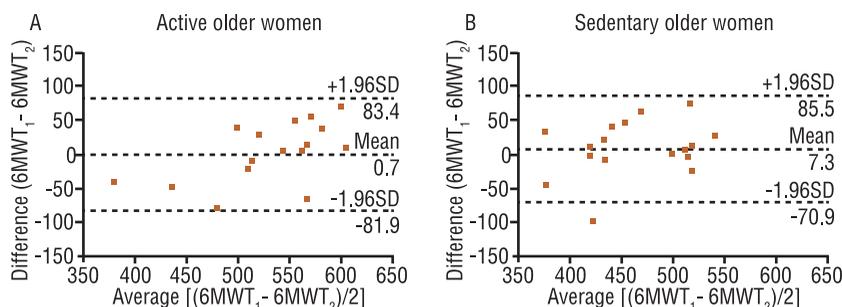
The groups showed no difference in age and anthropometric characteristics ( $P > 0.05$ ). Table 1 shows the results of ICC analysis that indicated high reliability in the 6MWT, final HR, HR% and *HRR* in groups of active and sedentary individuals. The indexes of *OV* and *2ULV* of HRV symbolic analysis showed moderate reliability during the post-exercise recovery for active women while in sedentary participants, the *OV* index showed high reliability and *2ULV* moderate reliability.

Figures 1-3 show the Bland-Altman plots for post-exercise 6MWT (Figure 1), *HRR* (Figure 2) and HRV (Figure 3) for the active and sedentary older women. All variables showed agreement in both groups (zero line is within 95%), in which active individuals showed greater agreement for the 6MWT, *OV* and *2ULV*, while sedentary individuals showed greater agreement in  $\Delta HRR 1$  'and  $\Delta HRR 2$  '. The lowest average differences were found in  $\Delta HRR 1$  'and  $\Delta HRR 2$  ' in sedentary individuals and in 6MWT in active individuals.

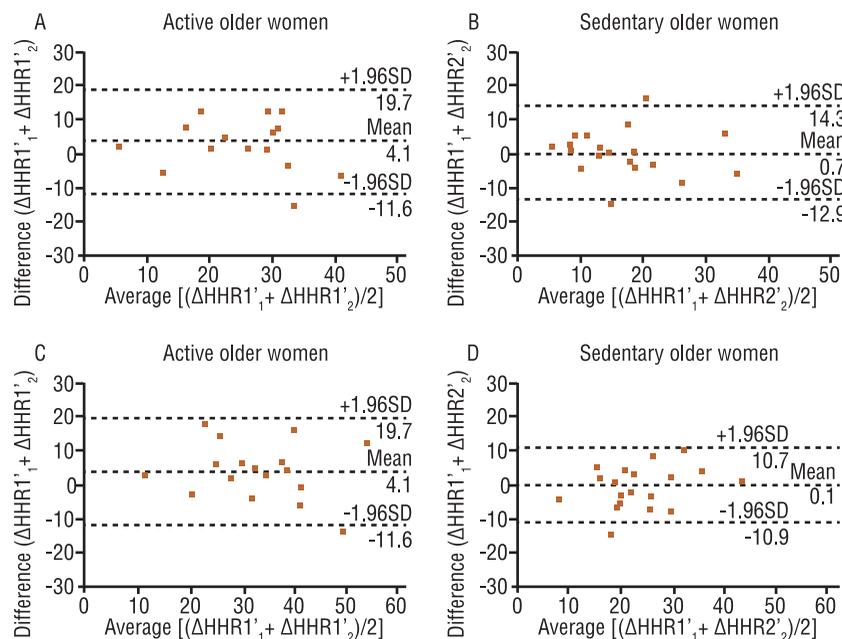
**Table 1** - Performance reliability in the 6-minute walk test and post-exercise cardiac autonomic control variables in active and sedentary individuals.

	Active (n=18)					Sedentary (n=18)				
	1 <sup>st</sup> 6MWT	2 <sup>nd</sup> 6MWT	CV	ICC	CI 95%	1 <sup>st</sup> 6MWT	2 <sup>nd</sup> 6MWT	CV	ICC	CI 95%
Distance (m)	528.8 ± 71.4	528.1 ± 48.3	7.9%	0.86***	0.63–0.94	473.2 ± 62.4	466.0 ± 53.2	8.5%	0.86***	0.64–0.94
HR <sub>final</sub> (bpm)	123.0 ± 12.0	123.0 ± 11.0	6.5%	0.88***	0.68–0.95	112.0 ± 12.0	111.0 ± 13.0	5.9%	0.93***	0.81–0.97
%HR <sub>max</sub>	79.0 ± 8.8	79.0 ± 7.7	6.5%	0.89***	0.72–0.96	71.7 ± 7.2	71.1 ± 7.5	5.5%	0.92***	0.79–0.97
$\Delta FCR 1'$ (bpm)	29.0 ± 11.0	24.0 ± 10.0	30.1%	0.84***	0.57–0.94	17.0 ± 8.0	16.0 ± 8.0	40.2%	0.80***	0.47–0.96
$\Delta FCR 2'$ (bpm)	36.0 ± 10.0	32.0 ± 11.0	23.7%	0.85***	0.61–0.94	24.0 ± 9.0	24.0 ± 8.0	22.8%	0.88***	0.70–0.95
<i>OV</i>	39.2 ± 12.9	40.2 ± 12.6	35.3%	0.57*	0.14–0.84	35.2 ± 17.0	43.6 ± 13.4	34.1%	0.76***	0.36–0.91
<i>2ULV</i>	11.9 ± 5.4	9.5 ± 3.5	47.2%	0.57*	0.14–0.83	14.3 ± 6.2	14.2 ± 3.5	69.0%	0.65*	0.08–0.87

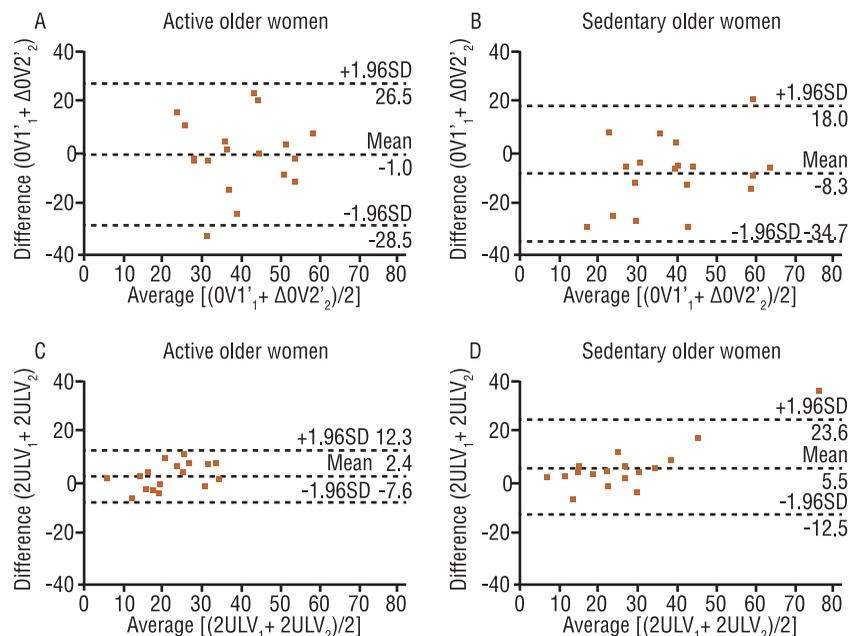
Note: 6MWT = 6-minute walk test; CV = coefficient of variation; ICC = intraclass correlation coefficient; CI = confidence interval; HR = heart rate; % HR<sub>max</sub> = percentage of maximum heart rate;  $\Delta HRR 1'$  = delta of 1 min *heart rate recovery*;  $\Delta HRR 2'$  = delta of 2 min *heart rate recovery*; *OV* = no change; *2ULV* = two unlike variations; \*  $P < 0.05$ ; \*\*\*  $P < 0.001$ .



**Figure 1.** Bland-Altman plots showing the performance agreement in the 6-minute walk test (6MWT) in active (n = 18) and sedentary women (n = 18). SD = standard deviation.



**Figure 2.** Bland-Altman plots showing the agreement of the heart rate recovery of one (A and B) and two minutes (C and D) after exercise in active (n = 18) and sedentary older women (n = 18), respectively.  $\Delta HRR1'$  = heart rate recovery of one minute;  $\Delta HRR2'$  = heart rate recovery of two minutes; SD = standard deviation.



**Figure 3.** Agreement (Bland-Altman) among heart rate variability indexes of no variation (A and B) and two unlike variations (C and D) after exercise in active (n = 18) and sedentary older women (n = 18) respectively. OV = no change; 2ULV = two unlike variations; SD = standard deviation.

## DISCUSSION

This study aimed to verify the reliability of the 6MWT performance and autonomic markers (*HRR* and *HRV*) after exercise in physically active and sedentary older women. The performance in the 6MWT and *HRR* at one and two minutes showed high reliability in both groups, while in the

sedentary group, 0V and 2ULV indexes had moderate and high reliability, respectively, and moderate for both indexes for the active group. In addition to reliability, all these variables showed agreement.

The performance in the 6MWT showed high reliability in active and sedentary groups, with variation of approximately 8 and 9%, respectively. Additionally, performance showed agreement in both groups, with average difference between test and retest of 0.7 m in active women and 7.3 m in sedentary women. These results are similar to studies with other populations<sup>6-9</sup>. Goemans et al.<sup>9</sup> applied the 6MWT in a sample of children aged 5-12 years and found very high reliability ( $ICC > 0.95$ ) when considering the entire sample and high reliability ( $ICC > 0.80$ ), when considering age subcategories every two years. Similar results were found for people with intellectual disabilities ( $ICC = 0.96$ )<sup>7</sup> and a sample aged 3-85 years ( $ICC = 0.92$ )<sup>6</sup>. However, this was the first study that evaluated and confirmed the reliability of the 6MWT in elderly subjects with different levels of physical activity. It is noteworthy that to avoid factors that may affect reliability, individuals need familiarization and receiving standardized verbal encouragement. Therefore, the 6MWT is a reproducible and consistent measure of functional capacity of both active and sedentary older subjects.

*HRR* of one and two minutes after 6MWT also showed high reliability and Bland-Altman analysis confirmed the agreement of these variables in both groups. Interestingly, sedentary women showed lower mean differences between test and retest compared to active women in both in *HRR* of one (0.7 vs. 4.1 bpm) and two minutes (-0.1 vs. 4, 0). This result may be due to increased variability among individuals induced by regular exercise practice, where adherence and the level of adaptability of participants can result in higher average difference. In addition, regardless of group, *HRR* showed high CV, ranging from 30% to 24% in the active group and from 40% to 23% in the sedentary group, for *HRR* of one and two minutes, respectively. Studies with healthy and physically active men found low to moderate *HRR* reliability after maximum and submaximal effort<sup>20,29</sup>. This difference in results can be explained by the fact that these studies used a sample of young people, while in the present study, the sample consisted of older women and aging affects post-exercise parasympathetic reactivation. Therefore, despite the high *HRR* reliability and agreement in active and sedentary women, large variation in these indexes might be expected.

The HRV indexes of 0V and 2ULV in the symbolic analysis showed moderate and high reliability in the sedentary group, respectively, and moderate for both indexes in active group. The mean difference between test and retest was lower in the active group compared to sedentary group for 0V (-1.0 vs. -8.3) and 2ULV (2.4 vs. 5.5). These results indicate that active women show a greater agreement for these variables. However, high variation was observed in both groups, where the active group had CV of approximately 35% and 47%, and the sedentary group 34% and 64% for 0V and 2ULV, respectively. This wide variation corroborates with the findings of Al Haddad et al.<sup>19</sup>, who found large discrepancy in the reliability mea-

surements of HRV indexes of the time and frequency domains after exercise in moderately active men. In contrast, Boulosa et al.<sup>18</sup> found moderate to high reliability for HRV indexes of the time domain in endurance athletes. One possible explanation for the discrepancy in results between this and other studies<sup>18,19</sup> may be the exercise intensity, since lower intensities generate faster parasympathetic reactivation<sup>30</sup>. In this study, 38.9% of active women reached  $\geq 85\%$  HR<sub>max</sub> in the 6MWT and 61.1% reached 67-84%, while among sedentary women, only 5.6% (one participant) reached  $\geq 85\%$  HR<sub>max</sub> and 94.4% reached 59-79% HR<sub>max</sub>. In the aforementioned studies, exercise was carried out at maximum or supramaximal intensities<sup>18,19</sup>. Therefore, despite reliability and agreement, the application of the HRV measure after exercise should consider a wide range of variation related to interindividual variability and exercise intensity.

Limitations of this study include lack in control of environmental variables such as temperature and relative humidity, lack of sleep, nutrition and physical activity control, as well as the sample size in each group. On the other hand, some strengths of this study includes the fact that evaluations were carried out under conditions similar to those found in a professional practice environment, and there was a process of familiarization with the tests and trial procedures, which eliminates learning effect. Moreover, the evaluation of physically active and sedentary older women has good applicability both in the professional practice environment and for evaluating the effect of intervention on individuals with different levels of physical activity.

## CONCLUSIONS

The 6MWT is a measure of high reliability and agreement in active and sedentary older individuals. On the other hand, although *HRR* and HRV have moderate to high reliability and agreement, there was a large variation in both groups. These measures can be used in the assessment of the functional capacity and post-exercise cardiac autonomic control in physically active and sedentary older women.

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