# Normative values and reference equation for the six-minute step test to evaluate functional exercise capacity: a multicenter study 

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#### Abstract

Objective: To establish normative values and a reference equation for the number of steps climbed during the six-minute step test (6MST) in healthy adults, and to assess the reliability of the test and of the equation. Methods: This was a multicenter crosssectional study involving 468 healthy volunteers (age range: 18-79 years) recruited from the general community in six research laboratories across different regions of Brazil, which is a country with continental dimensions. The 6MST was performed twice (30min interval), and clinical, demographic, and functional variables were evaluated. An independent sample of 24 volunteers was evaluated to test the reference equation a posteriori. Results: The number of steps had excellent test-retest reliability (intraclass correlation coefficient $=0.96$ [ $95 \% \mathrm{Cl}: 0.95-0.97$ ]), and the mean number of steps was $175 \pm 45$, the number being $14 \%$ greater in males than in females. The best performance on the test was correlated with age ( $r=-0.60$ ), sex ( $r=0.28$ ), weight ( $r=0.13$ ), height ( $r$ $=0.41)$, BMI $(r=-0.22)$, waist circumference $(r=-0.22)$, thigh circumference $(r=0.15)$, FVC ( $r=0.54$ ), and physical activity level ( $r=0.17 ; p<0.05$ for all). In the regression analysis, age, sex, height, and weight explained $42 \%$ of the variability of the 6MST. Normative values were established for the 6MST according to age and sex. There was no difference between the 6MST values from the independent sample and its predicted values ( $157 \pm 29$ steps vs. $161 \pm 25$ steps; $p=0.47 ; 97 \%$ of predicted values). Conclusions: The normative values and the reference equation for the 6MST in this study seem adequate to accurately predict the physical functional performance in adults in Brazil.


Keywords: Exercise test; Physical functional performance; Patient outcome assessment; Reference values; Regression analysis.

## INTRODUCTION

With the advent of the COVID-19 pandemic, rehabilitation programs were forced into remote and home delivery models. ${ }^{(1,2)}$ While studies have progressively emerged showing that it is possible to provide physical training, physical activity (PA) counseling, education, and self-management training in settings that differ from traditional rehabilitation centers, ${ }^{(3,4)}$ most exercise tests for initial assessment are still carried out at those centers. With regard to home-based rehabilitation programs, in order to assess exercise capacity, professionals have to make adaptations to create conditions and opportunities for accessibility in different inpatient and outpatient settings. To solve issues such as the need for space (e.g., long corridors) and difficulties in evaluating patients receiving supplemental oxygen, which are limitations that hinder the use of the six-minute walk test, interest has been increasing in the six-minute step test (6MST), a self-paced test in which an individual must go up and down a single step for six minutes. ${ }^{(5-7)}$ The 6MST has the following advantages: it is an easy-to-perform, inexpensive, space-saving test

[^0]that is practical for long-term oxygen therapy users and has been tested and proven to be reliable and valid in different clinical populations. ${ }^{(6,8-12)}$ However, the need for normative values and well-established reference equations makes the interpretability of the test difficult.

One reference equation for the 6MST has been established. ${ }^{(13)}$ However, there were some methodological limitations in that study ${ }^{(13)}$ that may compromise the external validity of that equation. For instance, the study involved a small, single-center sample ( $N=91$ ), the method for selecting the participants was not reported, the study included obese volunteers, and there were few individuals in each age group. In addition, it was not possible to establish normative values, and the authors did not perform analyses with an independent sample to verify the reliability of the equation. ${ }^{(13)}$ Thus, such limitations hamper the interpretation of the test and the identification of individuals with low functional capacity. Therefore, normative values and a reference equation based on a large multicenter sample could improve the interpretability of the 6MST. Normative and/or reference values characterize a defined population in a specific time period, evaluate and compare the performance of an individual within a population, establish comparisons between different clinical conditions, and evaluate the effectiveness of interventions. ${ }^{(14)}$ Thus, the main objectives of the present study were to examine the reliability of the 6MST, to establish the normative values and a reference equation using a large multicenter sample comprising healthy adults from a wide range of ages, and validate the new reference equation for use in Brazil.

## METHODS

## Study design and ethical aspects

This was a multicenter cross-sectional study carried out in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines. ${ }^{(15)}$ Data were collected between March of 2018 and May of 2019. This study was approved by the Research Ethics Committee of the Universidade Federal de Juiz de Fora (Report n. 3.134.323). All volunteers gave written informed consent.

## Procedures

The study prospectively included 476 healthy participants of both sexes (range: 18-79 years of age) who were able to understand and perform all of the procedures proposed. None had any disease that could limit exercise tolerance, such as pulmonary, cardiovascular (except for controlled hypertension without the use of a beta-blocker), and rheumatic diseases. Exclusion criteria were as follows: $18 \mathrm{~kg} /$ $\mathrm{m}^{2}<\mathrm{BMI}<30 \mathrm{~kg} / \mathrm{m}^{2}$; alterations in lung function ( $\mathrm{FVC}<80 \%$ of the predicted value; $\mathrm{FEV}_{1}<80 \%$; and $\mathrm{FEV}_{1} / \mathrm{FVC}$ ratio $<0.7$ ); and significant pain and/ or discomfort at the time of evaluation.

The participants were recruited from all regions in Brazil: north, northeast, south, southeast, and central-west (see supplementary material).

## Measurements

Anthropometric measurements such as weight and height were performed using a stadiometer with a mechanical scale (Welmy, São Paulo, Brazil). A tape measure was used for the determination of abdomen and leg circumference (see supplementary material).

Lung function was assessed before the 6MST in accordance with the Brazilian guidelines for pulmonary function tests. ${ }^{(16)}$ The measurements were then compared with those predicted for the Brazilian population. ${ }^{(17)}$ The PA level (see supplementary material) was determined using the short version of the International Physical Activity Questionnaire. ${ }^{(18,19)}$
Two 6MSTs were performed with a $30-\mathrm{min}$ rest interval. A wooden step ( 20 cm high $\times 40 \mathrm{~cm}$ wide $\times 60 \mathrm{~cm}$ long) with no upper limb support was used. The test speed was not controlled and was determined by the participants themselves. The participants were instructed to go up and down the step for 6 min as many times as possible. ${ }^{(5)}$ They received standardized instructions (see supplementary material) before the start of the test, ${ }^{(20)}$ as well as verbal feedback. ${ }^{(21)}$ $\mathrm{HR}, \mathrm{SpO}_{2}$, and blood pressure, as well as dyspnea and leg fatigue according to the modified Borg Scale, ${ }^{(22)}$ were recorded at rest, immediately after the test, and after the first minute of recovery. The test was interrupted in cases of evidence of oxygen desaturation below $85 \%,{ }^{(23)}$ complaints of chest pain, intolerable dyspnea, leg cramps, staggering, diaphoresis, dizziness, pale or ashen appearance, or any other sign that threatened the safety of the participant. The participant could choose to interrupt the test to rest; however, in any case, the timer was not stopped during the interruption. The best result of the two tests was used for the analysis.

After establishing the normative values and the reference equation, an independent sample of healthy participants from a single center, which was composed of individuals selected using the same eligibility criteria as in the initial sample, performed the 6MST to validate the normative values and the reference equation.

## Sample size

For the calculation of the sample size, the equation by Tabachnick \& Fidell ${ }^{(24)}$ was used, which considers $\mathrm{N}>50+8 K$, where $K$ represents the number of independent variables. Eight independent variables (sex, weight, height, age, abdominal circumference, thigh circumference, calf circumference, and lower limb length) were used, resulting in at least 114 participants. However, we increased the sample size to include a representative number of subjects from each center due to various correlations and multiple regression analysis.

## Statistical analysis

The data were analyzed using the IBM SPSS Statistics software package, version 26.0 (IBM Corp., Armonk, NY, USA). Data distribution was assessed using the Shapiro-Wilk test. Normally distributed data were expressed as means and standard deviations. The 10th percentile was calculated for age range and sex. Sex differences were analyzed with the t-test for independent variables or the Mann-Whitney $U$ test, whereas comparisons among centers were assessed using the one-way ANOVA or the Kruskal-Wallis test, followed by post-hoc tests, when appropriate.

Reliability was analyzed using the intraclass correlation coefficient (ICC) of the two-way randoms effect model with $95 \%$ CIs with a single rating, $\operatorname{ICC}_{(2,1)^{\prime}}$ and Bland-Altman analysis. The paired t-test was used in order to compare the performance between the first and second 6MST. The standard error of the mean (SEM) was calculated for the standard error of measurement (SEM = SD' $\sqrt{ }[1-I C C]$ ) and for the minimal detectable change (MDC) at 95\% CI-absolute MDC $=1.96 \times \operatorname{SEM} \times \sqrt{ } 2$; and relative MDC $(\%)=$ (MDC/mean of 1 st and 2 nd tests) $\times 100 .{ }^{(25,26)}$ The test-retest learning effect was calculated as follows: (learning effect (\%) $=$ [2nd test -1 st test $] / 1$ st test $\times 100$ ).
The best of the two test results was considered to establish the normative values. Thus, normative values are presented separately by sex and 10-year age groups (18-28, 29-39, 40-49, 50-59, 60-69, and 70-79 years). The lower limit of normal was obtained from the following equation: mean value - (1.64 $\times$ SEE), where SEE is the standard error of the estimate.

Pearson's or Spearman's correlation coefficients were used, when appropriate, to verify the bivariate correlation between independent variables (age, weight, height, sex, BMI, abdominal circumference, thigh circumference, leg circumference, PA level, FVC, $\mathrm{FEV}_{1}$ [in L and in \% of predicted values for both], and $\mathrm{FEV}_{1} / \mathrm{FVC}$ ) and the dependent variable (number of steps).
To establish the reference equation to estimate the number of steps on the 6MST, the stepwise multiple linear regression analysis was used. Outliers (extremely high or low values) were excluded. Outliers were identified by the box plot: data points $>1.5$ times above the upper quartile or below the lower quartile of the IQR. Only statistically significant variables were kept in the final model ( $p<0.05$ ). The best model was constructed considering the variables with the best independent coefficient of determination ( $\mathrm{R}^{2}$ ), and the SEE was calculated. Independent variables were checked for multicollinearity. There was no evidence for multicollinearity when tolerance values were > 0.1 , the variance inflation factor was < 10 , or correlation coefficients were $<0.7$. ${ }^{(27)}$ The normality of the residual values was tested using the Kolmogorov-Smirnov test with Lilliefors correction.

To verify the reliability of the proposed reference equations, data from an independent sample of 24 individuals recruited according to the same inclusion/ exclusion criteria were used as an additional analysis. The independent sample was recruited from a single center in the southern region. The formula derived from the regression model was applied in this sample, and the predicted values were calculated for the 6MST. In addition, Bland-Altman plots were constructed using this independent sample to visualize the agreement between actual and predicted values for the 6MST. Statistical significance was set at $p<0.05$ for all analyses.

## RESULTS

Of the 570 healthy participants selected, 94 (16\%) were excluded due to $B M I \geq 30 \mathrm{~kg} / \mathrm{m}^{2}(\mathrm{n}=24)$, altered spirometry ( $n=39$ ), and comorbidities ( $n=$ 31). Therefore, 476 were enrolled for initial analysis and, after removing outliers and participants who were unable to perform the second 6MST, 468 participants remained for all analyses (Figure 1). The included participants were divided by age range, in years: 18-28 ( $n=135$ ); 29-39 ( $n=110$ ); 40-49 ( $n=65$ ); 50-59 $(n=65) ; 60-69(n=60)$; and 70-79 $(n=33)$.

## Multicenter sample characteristics

The overall sample comprised healthy individuals only; $58 \%$ were women, $60 \%$ were physically active or very active, and the age range was 18-79 years (Table 1). A more detailed view of all results from each center is available in the supplementary material (Table S1).

## Performance and reliability of the test

The reliability analysis included 468 participants. Physiological responses and symptoms induced by the first and second 6MST (6MST-1 and 6MST-2) are reported in Table S2. Regarding the number of steps, although there was a statistical difference between 6MST-1 and 6MST-2 ( $169 \pm 38$ steps vs. $175 \pm 45$ steps; $\mathrm{p}<0.001$ ), this was lower than the absolute and relative MDC ( 21 steps and 12\%, respectively), with excellent agreement (ICC: 0.96; 95\% CI: 0.95-0.97), and SEM was 7.79. The learning effect between 6MST-1 and 6MST-2 was $4.2 \pm 13.7 \%$. The Bland-Altman analysis showed that the agreement between the number of steps climbed during the two tests had a mean difference of -8 steps, with limits of agreement between -39 and 22 steps (Figure S1).

## Normative values

The mean number of steps climbed during the 6 MST was $175 \pm 45$ ( $95 \%$ CI: 171-179). In general, the mean number of steps was $14 \%$ lower for women than for men, and elderly individuals had worse performances on the test. Table 2 shows the mean, standard deviation, $95 \% \mathrm{CI}$, and lower limit of normal for the 6MST in the total sample and also by age group and sex. In the independent sample, all participants

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Figure 1. Flow chart of the participant selection process.
reached more than $80 \%$ of the normative value for the 6MST (Table 2).

## Reference equation

The results of the correlation analysis of anthropometric, demographic, and physiological variables with the best performances on the 6MST are shown in Table 3. The number of steps climbed showed significant positive correlations with, sex, weight, height, thigh circumference, calf circumference, PA level, and FVC (in L and in \% of predicted value), whereas it showed significant negative correlations with age, BMI, and waist circumference.

To establish the reference equation, variables that showed a significant correlation with the number of steps were tested in the regression analysis. There was no multicollinearity between the independent variables. The regression analysis showed that age, sex, height, and weight explained $42 \%$ of the variability in the 6MST: $\mathrm{F}(4,463)=87.117 ; \mathrm{p}<0.001 ; \mathrm{R}^{2}=0.42$. The results revealed the following equation (Table 4):

6MST $=106+(17.02 \times[0:$ woman; $1: m a n])+$ $(-1.24 \times$ age $)+(0.8 \times$ height $)+(-0.39 \times$ weight $)$
where 6MST is expressed in number of steps; age, in years; height, in cm; and weight, in kg .

## Reliability of the reference equation

The independent sample consisted of 24 participants ( 12 men), with a mean age of $48.0 \pm 3.5$ years, a mean BMI of $25 \pm 3 \mathrm{~kg} / \mathrm{m}^{2}$, and a mean $\mathrm{FEV}_{1}$ of 96 $\pm 9 \%$ of the predicted value (Table 1). When the reference equation for 6MST was applied to this group, there was no difference between the number of steps
climbed during the 6MST and the estimated value from the reference equation ( $157 \pm 29$ steps vs. $161 \pm 26$ steps; $p=0.47$ ), with a mean difference of 4 steps and a $95 \% \mathrm{CI}$ of -10 to 2 steps. Bland-Altman plots for this comparison can be seen in the supplementary material (Figure S1).

## DISCUSSION

The present study established normative values for the number of steps climbed during the 6MST in adult participants. Men climbed more steps than did women, and younger participants climbed more steps than older participants, respectively. This study also provided an accurate reference equation for the 6MST. It should be emphasized that this study had three robust methodological features: a large sample for the purpose of establishing 6MST normative values, a multicenter design, and a prospective validation of the normative values.
As expected, elderly individuals had worse performances on the 6MST. Aging is associated with reduced aerobic and anaerobic capacity, resulting from reduced cardiovascular function and from changes in oxidative capacity, and muscle fiber type, as well as in of skeletal muscle structure and function. ${ }^{(28,29)}$ Therefore, this decline seems to be due to both central and peripheral adaptations. Other studies have shown similar results in the relationship between age and exercise capacity using different exercise tests. ${ }^{(13,30)}$

There was no difference between the normative values in the present study and performance in the independent sample. The 6MST results of all participants were higher than $80 \%$ of the predicted value in relation to the normative value. Therefore, we suggest that the normative values in the present

Table 1. Sample characteristics. ${ }^{\text {a }}$

| Variable | Multicenter sample |  |  |  | Independent sample |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Total $(\mathrm{N}=468)$ | Male $(\mathrm{n}=198)$ | $\begin{aligned} & \text { Female } \\ & (\mathrm{n}=270) \end{aligned}$ | p* | Total $(n=24)$ | p** |
| Age, years | $41 \pm 17$ | $40 \pm 17$ | $42 \pm 18$ | 0.15 | $48 \pm 16$ | 0.05 |
| Height, cm | $165 \pm 10$ | $173 \pm 8$ | $160 \pm 7$ | < 0.01 | $167 \pm 8$ | 0.49 |
| Weight, kg | $68 \pm 13$ | $77 \pm 12$ | $62 \pm 9$ | < 0.01 | $71 \pm 13$ | 0.37 |
| BMI, $\mathrm{kg} / \mathrm{m}^{2}$ | $25 \pm 3$ | $25 \pm 3$ | $24 \pm 3$ | < 0.01 | $25 \pm 3$ | 0.47 |
| WC, cm | $87 \pm 10$ | $91 \pm 10$ | $84 \pm 10$ | < 0.01 | $88 \pm 11$ | 0.45 |
| LRLL, cm | $84 \pm 8$ | $87 \pm 8$ | $83 \pm 8$ | < 0.01 | $86 \pm 7$ | 0.25 |
| TC, cm | $54 \pm 6$ | $53 \pm 6$ | $54 \pm 6$ | 0.09 | $51 \pm 5$ | 0.02 |
| CC, cm | $36 \pm 3$ | $37 \pm 3$ | $35 \pm 3$ | < 0.01 | $37 \pm 3$ | 0.25 |
| Spirometry |  |  |  |  |  |  |
| FVC, L | $3.7 \pm 1.0$ | $4.6 \pm 0.8$ | $3.1 \pm 0.6$ | < 0.01 | $3.8 \pm 1.0$ | 0.78 |
| FVC, \% predicted | $94 \pm 10$ | $96 \pm 11$ | $93 \pm 9$ | 0.01 | $91 \pm 8$ | 0.06 |
| $\mathrm{FEV}_{1}$, L | $3.2 \pm 0.8$ | $3.8 \pm 0.7$ | $2.7 \pm 0.5$ | 0.08 | $3.2 \pm 0.8$ | 0.84 |
| $\mathrm{FEV}_{1}$, \% predicted | $98 \pm 11$ | $99 \pm 12$ | $98 \pm 10$ | 0.32 | $96 \pm 9$ | 0.18 |
| $\mathrm{FEV}_{1} / \mathrm{FVC}$ | $86 \pm 6$ | $85 \pm 6$ | $87 \pm 6$ | 0.01 | $86 \pm 7$ | 0.69 |
| Comorbidities |  |  |  |  |  |  |
| Hypertension | 47 (10) | 19 (10) | 28 (10) | 0.70 | 8 (33) | < 0.01 |
| Diabetes | 8 (2) | 1 (0.5) | 7 (2) | 0.08 | 1 (4) | 0.36 |
| Smoking | 9 (2) | 6 (3) | 3 (1) | 0.08 | 0 (0) | 0.63 |
| BMI classification |  |  |  |  |  |  |
| Underweight | 11 (2) | 4 (2) | 7 (2) | < 0.01 | 2 (8) | 0.40 |
| Normal weight | 242 (51) | 84 (42) | 158 (58) |  | 8 (33) |  |
| Overweight | 215 (46) | 110 (55) | 105 (39) |  | 14 (58) |  |
| Age range, years |  |  |  |  |  |  |
| 18-28 | 135 (29) | 64 (32) | 71 (26) | 0.10 | 4 (17) | 0.04 |
| 29-39 | 110 (23) | 47 (24) | 63 (23) |  | 4 (17) |  |
| 40-49 | 65 (14) | 26 (13) | 39 (14) |  | 4 (17) |  |
| 50-59 | 65 (14) | 26 (13) | 39 (14) |  | 4 (17) |  |
| 60-69 | 60 (13) | 24 (12) | 36 (13) |  | 4 (17) |  |
| 70-79 | 33 (7) | 11 (5) | 22 (8) |  | 4 (17) |  |
| IPAQ |  |  |  |  |  |  |
| Very active | 58 (12) | 30 (15) | 29 (11) | < 0.01 |  |  |
| Active | 226 (48) | 78 (38) | 149 (55) |  |  |  |
| Irregularly active A | 77 (16) | 36 (18) | 41 (15) |  |  |  |
| Irregularly active B | 74 (16) | 39 (19) | 35 (13) |  |  |  |
| Sedentary | 39 (8) | 22 (11) | 17 (6) |  |  |  |

${ }^{\text {a }}$ Values expressed as $n(\%)$ or mean $\pm$ SD. WC: waist circumference; LRLL: length of right lower limb; TC: thigh circumference; CC: calf circumference; and IPAQ: International Physical Activity Questionnaire.
study be used in order to interpret the results of the 6MST. To show the clinical application of our normative values, the number of steps was expressed as a proportion of the normative values in the present study. It is important to mention that, although the limit of $85 \%$ of the maximum HR predicted to stop the test was not adopted in the present study, this may be an important criterion to ensure safety in some clinical populations (e.g., patients with cardiac comorbidities).

The predicted equation accurately estimated the number of steps. Sex, age, weight, and height were the independent variables that remained in the equation. In general, men have less body fat and greater aerobic capacity than do women. ${ }^{(31,32)}$ It
should be noted that if a clinician/researcher wants to verify whether the performance of an individual (for example, a patient with a respiratory problem) on the 6MST can be classified as reduced functional exercise capacity, the lower limit of normal for that individual must be calculated. This can be done using the predicted mean value of the equation ( $1.64 \times$ SEE) ${ }^{(33)}$ or using the 10th percentile. ${ }^{(34)}$ In the present study, men performed better than women by an average of 26 steps. Some physiological differences between the sexes, such as body composition, cardiovascular function, lung function, substrate metabolism, and thermoregulation, may influence exercise performance. ${ }^{(35)}$ As previously mentioned, aging leads to changes in body structure and function.

Table 2. Normative values for the six-minute step test (in number of steps), by sex and age.

| Age range, years | Total ( $\mathrm{N}=468$ ) |  | Male ( $\mathrm{n}=198$ ) |  | Female ( $\mathrm{n}=270$ ) |  | Mean difference (95\%CI) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\begin{gathered} \mathrm{n} \pm \mathrm{SD} \\ (95 \% \mathrm{CI}) \end{gathered}$ | 10th percentile | $\begin{aligned} & \mathrm{n} \pm \mathrm{SD} \\ & (95 \% \mathrm{CI}) \end{aligned}$ | 10th percentile | $\begin{aligned} & \mathrm{n} \pm \mathrm{SD} \\ & (95 \% \mathrm{CI}) \end{aligned}$ | $\begin{gathered} \text { 10th } \\ \text { percentile } \end{gathered}$ |  |
| $\begin{aligned} & 18-28 \\ & (n=135) \end{aligned}$ | $\begin{gathered} 203 \pm 36 \\ (197-209) \end{gathered}$ | 158 | $\begin{gathered} 217 \pm 38 \\ (207-227) \end{gathered}$ | 171 | $\begin{gathered} 190 \pm 28 \\ (183-196) \end{gathered}$ | 150 | $\begin{gathered} 27 \\ (16-38)^{*} \end{gathered}$ |
| $\begin{aligned} & 29-39 \\ & (n=110) \end{aligned}$ | $\begin{gathered} 191 \pm 36 \\ (185-198) \end{gathered}$ | 145 | $\begin{gathered} 200 \pm 34 \\ (190-209) \end{gathered}$ | 159 | $\begin{gathered} 185 \pm 36 \\ (176-194) \end{gathered}$ | 137 | $\begin{gathered} 14 \\ (0.7-27.0)^{*} \end{gathered}$ |
| $\begin{aligned} & 40-49 \\ & (n=66) \end{aligned}$ | $\begin{gathered} 168 \pm 36 \\ (159-177) \end{gathered}$ | 125 | $\begin{gathered} 177 \pm 22 \\ (168-186) \end{gathered}$ | 141 | $\begin{gathered} 163 \pm 42 \\ (149-176) \end{gathered}$ | 113 | $\begin{gathered} 14 \\ (3.8-32.0)^{*} \end{gathered}$ |
| $\begin{aligned} & 50-59 \\ & (n=67) \end{aligned}$ | $\begin{gathered} 163 \pm 33 \\ (154-171) \end{gathered}$ | 123 | $\begin{gathered} 176 \pm 34 \\ (162-190) \end{gathered}$ | 137 | $\begin{gathered} 154 \pm 30 \\ (144-164) \end{gathered}$ | 122 | $\begin{gathered} 21 \\ (5.6-38.0)^{*} \end{gathered}$ |
| $\begin{aligned} & 60-69 \\ & (\mathrm{n}=60) \end{aligned}$ | $\begin{gathered} 137 \pm 42 \\ (126-148) \end{gathered}$ | 99 | $\begin{gathered} 153 \pm 49 \\ (132-174) \end{gathered}$ | 91 | $\begin{gathered} 127 \pm 32 \\ (116-138) \end{gathered}$ | 96 | $\begin{gathered} 26 \\ (5-47)^{*} \end{gathered}$ |
| $\begin{aligned} & 70-79 \\ & (\mathrm{n}=33) \\ & \hline \end{aligned}$ | $\begin{gathered} 118 \pm 43 \\ (103-133) \\ \hline \end{gathered}$ | 68 | $\begin{gathered} 147 \pm 25 \\ (130-164) \\ \hline \end{gathered}$ | 107 | $\begin{aligned} & 104 \pm 43 \\ & (85-123) \\ & \hline \end{aligned}$ | 80 | $\begin{gathered} 43 \\ (14-72)^{*} \\ \hline \end{gathered}$ |

*p < 0.05 for the difference between male and female.

Table 3. Correlation between the outcome variable (total number of steps) on the better of the two six-minute step tests) and dependent variables.

| Independent variable | Best test result |  |
| :---: | :---: | :---: |
|  | R | p |
| Age, years | -0.60 | < 0.01 |
| Sex | 0.28 | < 0.01 |
| Weight, kg | 0.13 | 0.01 |
| Height, cm | 0.41 | < 0.01 |
| BMI, $\mathrm{kg} / \mathrm{m}^{2}$ | -0.22 | < 0.01 |
| WC, cm | -0.22 | < 0.01 |
| LRLL, cm | 0.04 | 0.37 |
| TC, cm | 0.15 | 0.01 |
| CC, cm | 0.14 | 0.01 |
| FVC, L | 0.54 | < 0.01 |
| FVC, \% predicted | 0.23 | < 0.01 |
| $\mathrm{FEV}_{1}$, L | 0.01 | 0.95 |
| $\mathrm{FEV}_{1}$, \% predicted | 0.03 | 0.53 |
| $\mathrm{FEV}_{1} / \mathrm{FVC}$ | 0.01 | 0.95 |
| Physical activity level | 0.17 | < 0.01 |

WC: waist circumference; LRLL: length of right lower limb; TC: thigh circumference; and CC: calf circumference.

Weight influences the performance on the 6MST, as it increases the workload due to horizontal and vertical displacements against gravity that occur when climbing the step. ${ }^{(36)}$ Height was probably considered a factor because the taller the person is, the longer his/her legs are, allowing them to favor the climb mechanically and contributing to the execution of a greater number of steps during the test. ${ }^{(37)}$ The fact that the coefficient of determination was moderate may suggest that other independent variables, such as muscle mass and motivation, may also contribute to determining the performance on the 6MST. However, the reference equation obtained in the present study is attractive, because the independent variables are easily available in clinical practice. In this context, Arcuri et al. ${ }^{(13)}$ proposed the first reference equation for the 6MST, showing that age and sex accounted
for $48 \%$ of the variation in test performance and that the increase in waist circumference accounted for $2 \%$ of the test variance. However, that study had some limitations that may restrict its external validity, ${ }^{(13)}$ including the fact that the study was conducted in a small single-center sample (91 participants) and that the equation was not tested prospectively in an independent sample. Although it was not an aim of the present study, we tested the reliability of the equations proposed by Arcuri et al. ${ }^{(13)}$ using the performance of our independent sample, and those equations produced a larger standard deviation of the differences and showed a tendency toward underestimating the prediction of performance on the 6MST. However, our proposed equation was considered valid, as there was no significant difference between the actual and predicted number of steps climbed by the independent sample, and the mean of the differences between both was lower than the MDC.

Although the 6MST is reliable, at present, there is no clear indication that one test is sufficient; therefore, two tests are recommended to account for any potential learning effect. Furthermore, the learning effect of the 6MST was considered small in healthy subjects but should be investigated in clinical conditions. An SEM of 7.79 and an MDC of 21 steps were also identified. Thus, in order to consider that there is an improvement in 6MST performance, the number of steps should increase by more than 21 steps. Our error rate and MDC were lower than the MDC of 27.26 steps and the SEM of 11.75 reported by Arcuri et al. ${ }^{(13)}$ We believe that this difference is due to the methodological characteristics of the present study, in which a stratified sample enabled by a larger sample size was included and age groups were better distributed.

Research on the 6MST has been desirable and timely. It is expected that the 6MST will be more frequently used with advances in new intervention strategies, such as telerehabilitation and home-based rehabilitation. In the current scenario, only $5 \%$ of individuals with an indication for pulmonary rehabilitation have access to

Table 4. Stepwise multiple linear regression model for the six-minute step test.

| Variable | Unstandardized <br> coefficient (B) | $95 \% \mathrm{CI}$ | SEE | p | Standardized <br> coefficient (Beta) |
| :--- | :---: | :---: | :---: | :---: | :---: |
| Constant | 106 | 20.45 to 191.85 | 43.611 | 0.015 |  |
| Sex | 17.02 | 8.30 to 25.73 | 0.108 | $<0.001$ | 0.185 |
| Age | -1.24 | -1.58 to -1.16 | 4.435 | $<0.001$ | -0.522 |
| Height, cm | 0.8 | 0.29 to 1.46 | 0.299 | $<0.001$ | 0.194 |
| Weight, kg | -0.39 | -0.77 to -0.005 | 0.196 | 0.04 | -0.110 |

SEE: standard error of the estimate. Sex code: [0: woman; 1: man].
the 6MST and complete it with good adherence. ${ }^{(38)}$ The reasons behind this fact are multifactorial, including logistical issues, socioeconomic barriers, and family dependency. However, traditional assessment tests such as the six-minute walk test and the shuttle walk test are difficult to apply at home because of the need for physical space. In this context, the 6MST is a potential alternative method for the evaluation of this population.

This study has some limitations. There were fewer participants in the older age group. However, a statistical difference could still be observed in the performance of this group when compared with other age groups. More recent data from the United Nations have shown that the world population still has a higher proportion of young people (> 15 years of age), estimated at $65.3 \%$ of the total population, than that of older people ( $>65$ years of age), who account for $9.1 \%$ of the total population. ${ }^{(39)}$ The proportions of participants by age group in our study corresponded with the proportions in the real world. Although participants were provided with a structured questionnaire about their health condition at the time of screening (the exception being spirometry), they did not undergo physical examinations, and medical records were not reviewed. It is therefore possible that some of the self-reported "healthy" participants had a disqualifying medical condition. Regarding PA, our results show estimates of participants categorized as more physically active than the general population. This may have happened for two reasons: i) the assessment of active/inactive physical behavior was performed using a questionnaire, which is known not to be as reliable as activity monitors; and ii) bias associated with voluntarism, which tends to attract more people who are physically active in studies involving exercise/PA.

There were few variable adjustments in the models. The small size of the independent sample, recruited from a single center, was not calculated a priori to test the accuracy of the equation, which is also a limitation. However, the reliability of the equation must be confirmed in future studies involving other populations.
In conclusion, this study has provided accurate normative values and a reference equation for the 6MST based on a large sample of healthy individuals within an age range between 18 and 79 years in Brazil. These findings might facilitate the identification, quantification, and interpretation of functional impairments with a quick, easy-to-perform test for use in clinical practice and research.

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## AUTHOR CONTRIBUTIONS

VSA and CM: guarantors of the data, the data analysis, and the study. SDC, DPA, TMDO, GFS, AKM, PDL, MLRD, IFC, AJ, GFBC, FM, and RRB: study design; data analysis and interpretation; drafting and review of the manuscript. All authors have read and approved the final version of the manuscript.

## CONFLICTS OF INTEREST

None declared.

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