REFERENCE EQUATION FOR THE TWO-MINUTE WALK TEST
IN ADULTS AND THE ELDERLY

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Running title: 2-min walk test

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Abstract

**Background:** The two-minute walk test (2MWT) has been used in several health conditions, but the interpretation of its results is limited due to lack of reference values.

**Objective:** The aim of this study was to establish a reference equation to predict the distance walked (DW) in the 2MWT for healthy adults and the elderly, and to test its reproducibility.

**Methods:** We evaluated 390 healthy subjects (195 male), aged 18–89 years, with normal spirometry and no history of previous chronic diseases. Two 2MWTs were performed on the same day, 30 minutes apart. To test the reliability of the prediction equation, 70 subjects (35 male) were prospectively included in the study.

**Results:** Males walked farther than females (221 [202–240] m vs. 199 [164–222] m, respectively; p <0.0001). Significant correlations were observed between DW and age (r = -0.50), weight (r = 0.23), height (r = 0.40), and gender (r = 0.35) (p <0.001 for all). Age and gender persisted in the model to predict DW (R²: 0.51). There was no difference between the DW by the subjects (197 [182–216]) and that estimated by the prediction equation (197 [179–222]) (p = 0.68).

**Conclusion:** We established a prediction equation that may be used as a reference to interpret performance on the 2MWT performed by adults and the elderly with different health conditions.

Key words: Walking tests, reference values, exercise testing
Introduction

Walk tests have been widely used for assessing functional capacity in different populations due to the simplicity of its realization, its easy interpretability, and its representativity of daily life activities. The first walk test described in the literature was the 12-minute walk test (12MWT)\(^1\), adapted from the 12-minute running test, more commonly known as Cooper’s test\(^3\).

Based on arguments that the 12MWT might be time-consuming to the evaluator and exhaustive for the patient, Butland et al. tested walking tests of shorter duration, describing for the first time the two- and six-minute walk tests (2MWT and 6MWT, respectively)\(^1\). In this same study, the 2MWT was reproducible and better tolerated by patients with chronic obstructive pulmonary disease (COPD); however, it exhibited less discriminative power for limitations or alterations arising from exercising. Thus, Butland et al. determined that the 6MWT was more informative for assessing patients with COPD\(^4\). Since then, the 6MWT has become the most common method for evaluating functional capacity in different health conditions. Then came the need to establish reference values for the 6MWT. Currently, there are more than ten prediction equations for distance walked (DW) in the 6MWT\(^3\).

Despite the limitations assigned to the 2MWT for patients with COPD, it has been incorporated again for use in this population and has proved to be valid for the assessment of exercise capacity and sensitive for detecting response to interventions in moderate-to-severe patients.\(^4,5\) The 2MWT has also been used in several other conditions, such as lower limb amputations\(^6,7\), the elderly\(^8\), neuromuscular diseases\(^9,10\), and chronic heart diseases\(^11\). However, no reference values have been established for the 2MWT, making it impossible to assess properly whether there is reduced functional capacity when performing this test.
The objective of this study was to establish a reference equation to predict DW in the 2MWT for healthy adults and the elderly, and to test its reproducibility.

**Methods**

**Subjects**

In a cross-sectional study, we evaluated 390 healthy subjects (195 male) from a convenience sample. They were recruited from four campuses of our university, located in four different regions of São Paulo, the community around the university, and relatives of our students and employees. We evaluated 30 men and 30 women in every age decade (18–28 years, 29–39 years, 40–49 years, 50–59 years, 60–69 years, and 70–79 years) and 15 men and 15 women 80–89 years of age. Fewer subjects were evaluated between 80-89 years of age due to the difficulty of finding individuals without musculoskeletal limitations at this age. For inclusion in the study, subjects had to be between the ages of 18 and 89 years, have normal spirometry, body mass index (BMI) ≤30 kg/m², and without a history of neuromuscular, musculoskeletal, or cardiorespiratory diseases. We excluded subjects who smoked or who had stopped smoking for less than two years; those who had uncontrolled hypertension although using regular medication; those who practiced regular physical activity; and those who lacked the capacity to understand how to perform the spirometry and/or the 2MWT. The study was approved by the research ethics committees of the Nove de Julho University – UNINOVE (454934), and all subjects gave written consent before starting the evaluations.

**Assessment**

Body weight was assessed with a beam scale to the nearest 0.1 kg, with subjects
Standing barefoot in light clothing. BMI was calculated as weight/height (kg/m$^2$).

Spirometry (CPX Ultima™ CPX; MedGraphics Corporation, St. Paul, MN) was performed according to recommendations of the American Thoracic Society/European Respiratory Society. The forced vital capacity (FVC) and forced expiratory volume in the first second (FEV$_1$) were compared with those predicted for the Brazilian population.

The 2MWT was performed in a 30m corridor. Two tests were performed, with a rest interval of 30 minutes between them. The subjects were instructed to walk as fast as possible, without running. Based on the recommendations for the 6MWT, encouragement was given after the first minute with the following standardized phrases: “You’re doing well” and “One minute left.” Blood pressure was measured at rest in the standing position, and immediately after the test by sphygmomanometer (DS44 DuraShock, Welch Allyn, Arden, NC). Subjects were instructed to stop the test if they experienced chest pain, dizziness, malaise, or any other symptoms of discomfort. Pulse oxygen saturation was continuously measured (Ohmeda Biox 3740®, Boulder, CO), as was heart rate (HR), by a frequency meter (Polar S810, São Paulo, Brazil). The values recorded were those shown on the display of the equipment at rest, at the end of the first minute and at the end of the second minute. The variation of HR ($\Delta$HR) and SpO$_2$ ($\Delta$SpO$_2$) were considered as the values at the peak of the exercise minus the value at rest. The predicted maximum HR was calculated as 220 – age in years. The peak HR was expressed in absolute and predicted (peak HR/maximum predicted) values. The modified Borg scale was used for evaluation of dyspnea and leg fatigue.

Statistical analysis

The normality of the data was analyzed by the Shapiro–Wilk test. The data showed non-
parametric distribution and were expressed as median (interquartile range 25%–75%). Differences in DW between men and women were analyzed using the Mann–Whitney test. The test with the farthest DW was selected for the next analysis. Spearman’s correlation coefficient was used to correlate the independent variables (age, weight, height) with the dependent variable (DW). For the multiple regression analysis (stepwise), gender was added to the independent variables for definition of the predictive equation. The lower limit of normal (LLN) was calculated as predicted value – (1.64 x residual standard deviation). Intraclass correlation coefficient and Bland–Altman analysis were used to assess the reproducibility of the two 2MWTs. Comparisons of DW among the age groups were performed using the Kruskal–Wallis and Mann–Whitney tests on each pair of groups, with the p value adjusted by the Bonferroni method. The reliability of the prediction equation was tested by comparing the DW performed by the subjects (DW actual) with that estimated by the equation (DW pred) using the Mann–Whitney test and the Bland–Altman analysis.

Results

The baseline characteristics are shown in Table 1.

The comorbidities more prevalent among the subjects were systemic hypertension (18%), diabetes mellitus (3%), and thyroid disorders (3%).

INSERT TABLE 1

The majority of the subjects (n = 295) presented the best performance (i.e., they walked a greater DW in the second 2MWT), 73 subjects walked farther in the first test, and 22 walked the same distance in both tests. There were no significant differences in
the outcomes measured at rest and at the peak of the two 2MWTs (Table 2). The mean difference between the first and second 2MWTs was narrow (Figure 1).

The male subjects walked farther than the female subjects (221 [202–240] m vs. 199 [164–222] m, respectively; p<0.001). The difference did not persist when correcting the DW/height (127 [104–140] vs. 128 [118–139]; p=0.054). Significant correlations were observed between DW and age (r = -0.50), weight (r = 0.23), height (r = 0.40), and gender (r = 0.35) (p<0.001 for all), but not BMI (r = -0.08; p=0.14). There was a reduction in DW in the 2MWT with advancement of age (Figure 2). The reduction in DW from 18–28 years of age to 29–39 years of age was 11.5 m (-4.9%); the reduction was 10.5 m (-4.7%) from 29–39 years to 40–49 years; 6 m (-2.8%) from 40–49 years to 50–59 years; 11.5 m (-5.5%) from 50–59 years to 60–69 years; 9 m (-4.6%) from 60–69 years to 70–79 years; and 13 m (-6.9%) from 70–79 years to 80–89 years. The average decline over the age was 10 m (-4%). Between the extreme ages (18–28 vs. 80–89), the reduction in DW was 61.5 m (-26%).

Among the independent variables selected from the simple regression analysis (age, weight, height, and gender), age and gender persisted to predict DW in the model, explaining 51% of its variance. The data from the multiple linear regression analysis are
shown in Table 3. The reference equation for DW in the 2MWT was

\[ 2\text{MWT}_{\text{pred}} = 252.583 - (1.165 \times \text{age}) + (19.987 \times \text{gender*}) \]

*male =1 and female = 0

The LLN is DW pred – 44 m

To test the reliability of the prediction equation, 70 subjects (35 male) were prospectively included in the study (age: 53 [34–71]; weight: 68 [59–77]; height: 1.64 [1.59–1.70]; BMI: 25 [22–28]; FVC %pred: 96 [85–103]; FEV₁ %pred: 99 [89–104]). There was no difference between DW by the subjects (DW actual: 197 [182–216] m) and that estimated by the prediction equation (DW pred: 197 [179–222] m) \( (p = 0.68) \). The DW for the prospective sample corresponded to 99% (96–105) of that predicted.

**Discussion**

The present study provides an equation to predict DW in 2MWT. Age and gender explained 51% of the variance in DW. Moreover, men presented better performances than women did, and the 2MWT is highly reproducible in healthy subjects.

There are currently several equations to predict DW in the 6MWT\(^3\), there are two for the incremental Shuttle walk test (ISWT)\(^{17,18}\), but there has been no reference equation for the 2MWT. Age and gender are variables commonly present in prediction equations for walking tests. Age and gender were also the only predictor variables of DW in two previous studies of the 6MWT, and the coefficients of determination were
lower than the observed in the present study ($R^2 = 0.41$ and 0.30)\textsuperscript{19,20}. The strength of association between age and DW in the 2MWT found in our study ($r = -0.50$) is consistent with that observed in studies (from $r = -0.36$ to $r = -0.75$) that have established prediction equation for the 6MWT\textsuperscript{19-25}. The inverse relationship between age and exercise capacity is well known, as normal aging is characterized by reduced functional reserve capacities of the heart, lung, and skeletal muscles, as well as decreased levels of physical activity\textsuperscript{26-28}. Our data showed a substantial reduction in DW (-61.5 m) when comparing the youngest (18–29 years) and oldest (80–89 years) ages. In a previous study of the 6MWT, DW decreased by 16% from 40–49 years (611 ± 85 m) to 70–80 years (514 ± 71 m)\textsuperscript{29}. Considering similar ages in our study (40–49 years and 70–79 years), the reduction observed was about 12%. This confirms that even in short-duration tests, such as the 2MWT, it is possible to observe the impact of aging on the ability to walk. However, future studies evaluating longitudinal decline in DW per decade would confirm whether the cross-sectional decline was equivalent.

On average, in the present study, men walked 12% more than women, which is a percentage similar to that found in previous studies on 6MWT\textsuperscript{19,23,30,31}. The influence of gender on DW has been demonstrated previously in the 6MWT by either of these conditions: prediction equation specific for gender\textsuperscript{30} or gender as a predictor variable in the prediction equation\textsuperscript{19,20, 22,32-34}. Aerobic capacity, represented by maximum oxygen uptake, is around 30% lower in women compared to men\textsuperscript{35,36}. Consequently, women exhibit lower performances in tests of physical capacity, including the 2MWT.

The regression equation of the present study, applied prospectively, was demonstrated to be reliable for estimating DW in the 2MWT. To illustrate the use of our prediction equation, we expressed the absolute values of DW in the 2MWT for people with lower-limb and transtibial amputations\textsuperscript{6,7} and subjects with COPD\textsuperscript{4,5} in percentage
of predicted from our prediction equation. Considering that 95% of the total sample of amputees was made up of male subjects\textsuperscript{6}, we calculated predicted DW for the male gender only, which resulted in 196 m. The best DW for these subjects was 121 m (second 2MWT), which corresponded to 62% of the predicted value. In another study of amputees\textsuperscript{7}, the best DW of inpatient transtibial amputees, 57.5 m, corresponded to 29% and 32% of the predicted value in males and females, respectively; for outpatient transtibial amputees, the best DW was 140.7 m, corresponding to 67% for males and 74% for females. In the study by Leung et al.\textsuperscript{4} (n=45; 37 males; age 71.8 years; VEF\textsubscript{1}: 42 ± 13% of predicted) of COPD patients, we also calculated the predicted values for males, as 82% of the subjects were male. On average, patients walked 130.8m, representing 69% of the predicted values from our prediction equation. Cardiac surgery patients\textsuperscript{11} walked 68% of the predicted distance preoperatively, and 42% postoperatively. Therefore, subjects presented with reduced functional capacity not only because of the low values in relation to the percentage of predicted, but also because the DW was lower than the LNN (121 m vs. 152m for amputees; 140.7 m vs. 165 m for outpatient transtibial amputees; 130.8 m vs. 145 m for COPD patients; and 136 m vs. 155 m, for cardiac surgery patients, respectively). Considering the responsiveness of the 2MWT, a study\textsuperscript{5} has shown that patients with COPD (n=57; 30 males; age 69 years; VEF\textsubscript{1}: 35 ± 12% of predicted) walked 153 m and 162m in the 2MWT, before and after bronchodilator, respectively, corresponding to 79.7% and 84.4% of the predicted values obtained from our prediction equation, considering male subjects. In addition to the improvement in terms of absolute values (9 m), DW reached values greater than 80% of the predicted values after bronchodilator. Following pulmonary rehabilitation, it is an interesting highlight that an improvement in DW in the 2MWT, expressed in percentage of our predicted values, was 11.5%, and quite similar
to that observed in the 6MWT (14%), taking into consideration the predicted values of Enright et al.\textsuperscript{30}

In the present study, on average, subjects achieved 70\% of the predicted maximum HR, which is similar to what was observed at the peak of the 6MWT by healthy subjects (64\%) with a mean DW of 587 m.\textsuperscript{20} As both 2MWT and 6MWT are self-paced tests, it is possible that the HR achieved during the second minute corresponds to the rest-exercise transition. From this point until the sixth minute, it could remain constant, which would correspond to the steady state. We could not find studies about the behavior of BP during the 2MWT. Higher values of SBP were found at the peak of ISWT\textsuperscript{17} in comparison with that observed in the present study (160 mmHg vs. 140 mmHg). This result was expected, as speed is gradually increased in the ISWT, and the test is not time limited.

The reproducibility of any method must be tested to ensure that its result is reliable, so as to ensure that the differences found are due to interventions or to the patient's evolution, and not a result of oscillations due to variability of the method itself. Reproducibility, therefore, is an indication of the consistency of a measure and is usually evaluated by intraclass correlation coefficient (ICC); coefficients above 0.80 indicate high reliability.\textsuperscript{35} The reproducibility of DW in the 2MWT has been tested in patients with COPD,\textsuperscript{1,5,36} with congestive heart failure (CHF),\textsuperscript{38} idosos,\textsuperscript{8} poliomyelitis,\textsuperscript{9} and amputees\textsuperscript{7}. DW in the 2MWT in these previous studies showed evidence of reliability, with ICC ranging from 0.82\textsuperscript{8} to 0.99\textsuperscript{7}, which was compatible with that observed in our study (0.96 [0.95–0.97]).

Another analysis we used to assess the reproducibility of DW in the 2MWT was the Bland–Altman graphical distribution, which is based on a dispersion diagram, facilitating the interpretation of the magnitude of the disagreement between two
measures (i.e., DW in two 2MWTs). In the present study, the limits of agreement in the Bland–Altman analysis ranged from -27 m to 21 m, which can be considered similar to those found in subjects with poliomyelitis (-21 m to 23 m)\(^9\) and the elderly (-21 m to 21 m)\(^8\). This means that, independent of the health condition, the subject is able to walk a similar distance in two 2MWTs performed at the same day.

**Conclusion**

We established a prediction equation for the 2MWT that may be used as a reference to interpret performance on the 2MWT by adults and the elderly with different health conditions.

**Funding**

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**Conflit of interest Statement**

The authors declare no financial conflicts of interest as well as other forms of conflict of interest, including personal, academic and intellectual issues.
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Figure legends

**Figure 1** - Bland–Altman plot for distance walked (DW) in the first and second 2MWTs. The dark horizontal lines correspond to the mean differences, and the dotted lines correspond to the 95% limits of agreement.

**Figure 2** - Influence of the age on distance walked (DW) in the 2MWT. a: $p < 0.016$ vs. b, and $p < 0.001$ vs. c, d, e, f, and g; b: $p < 0.026$ vs. c, $p = 0.007$ vs. d, and $p < 0.001$ vs. e, f, and g; c: $p = 0.83$ vs. d, $p = 0.026$ vs. e, $p < 0.001$ vs. f, and g; d: $p < 0.03$ vs. e, and $p < 0.001$ vs. f, and g; e: $p = 0.10$ vs. f, and $p = 0.005$ vs. g; f vs. g: $p = 0.21$. Circles correspond to outliers.
Table 1 – Characteristics of the subjects.

<table>
<thead>
<tr>
<th></th>
<th>Total group (n = 390)</th>
<th>Male (n = 195)</th>
<th>Female (n = 195)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>52 [36-68]</td>
<td>52 [36-69]</td>
<td>52 [36-68]</td>
<td>0.83</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.66 [1.58-1.72]</td>
<td>1.72 [1.68-1.76]</td>
<td>1.58 [1.54-1.64]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70 [62-78]</td>
<td>76 [69-83]</td>
<td>64 [57-70]</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>25 [23-28]</td>
<td>26 [23-28]</td>
<td>25 [23-28]</td>
<td>0.71</td>
</tr>
<tr>
<td>FVC (%pred)</td>
<td>97 [89-106]</td>
<td>95 [88-105]</td>
<td>98 [92-108]</td>
<td>0.01</td>
</tr>
<tr>
<td>FEV$_1$ (%pred)</td>
<td>100 [92-109]</td>
<td>99 [92-109]</td>
<td>101 [93-110]</td>
<td>0.18</td>
</tr>
<tr>
<td>FEV$_1$/FVC</td>
<td>84 [79-88]</td>
<td>82 [77-88]</td>
<td>85 [80-88]</td>
<td>0.03</td>
</tr>
<tr>
<td>2MWT (m)</td>
<td>211 [191-234]</td>
<td>221 [202-240]</td>
<td>199 [164-222]</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

p-value for male vs. female, BMI: body mass index, FVC: forced vital capacity, FEV$_1$: forced expiratory volume at 1$^{st}$ second, 2MWT: two minutes walk test.
Table 2 – Data at the peak of the 2MWTs.

<table>
<thead>
<tr>
<th></th>
<th>2MWT First</th>
<th>ICC (IC 95%)*</th>
<th>Best 2MWT</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>119 [102–134]</td>
<td>119 [104–136]</td>
<td>0.94 [0.92–0.95]</td>
</tr>
<tr>
<td>HR (%pred)</td>
<td>70 [62–79]</td>
<td>70 [63–79]</td>
<td>0.92 [0.90–0.93]</td>
</tr>
<tr>
<td>ΔHR (bpm)</td>
<td>38 [24–53]</td>
<td>38 [25–55]</td>
<td>0.91 [0.89–0.93]</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>140 [130–160]</td>
<td>140 [130–160]</td>
<td>0.93 [0.91–0.94]</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>80 [80–90]</td>
<td>80 [80–86]</td>
<td>0.82 [0.78–0.85]</td>
</tr>
<tr>
<td>SpO₂ (%)</td>
<td>97 [95–98]</td>
<td>97 [95–98]</td>
<td>0.80 [0.75–0.84]</td>
</tr>
<tr>
<td>ΔSpO₂ (%)</td>
<td>0 [-1–0]</td>
<td>0 [-2–0]</td>
<td>0.82 [0.78–0.89]</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>0.5 [0–2]</td>
<td>0.5 [0–2]</td>
<td>0.87 [0.84–0.89]</td>
</tr>
<tr>
<td>Leg fatigue</td>
<td>0.5 [0–2]</td>
<td>0.5 [0–2]</td>
<td>0.88 [0.85–0.90]</td>
</tr>
<tr>
<td>DW (m)</td>
<td>211 [189–231]</td>
<td>211 [191–234]</td>
<td>0.97 [0.96–0.97]</td>
</tr>
</tbody>
</table>

* P < 0.001 for all ICC (first vs. second 2MWT). HR: heart rate, Δ: peak at exercise – resting; SBP: systolic blood pressure, DBP: diastolic blood pressure, DW: distance walked.
Table 3 - Predictor variables for distance walked (DW) in the 2MWT obtained from multiple linear regression analysis

<table>
<thead>
<tr>
<th></th>
<th>Unstandardized coefficients (B)</th>
<th>Standard error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>243.262</td>
<td>4.15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Age</td>
<td>-0.954</td>
<td>0.07</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Gender</td>
<td>26.955</td>
<td>2.73</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Bland–Altman plot for distance walked (DW) in the first and second 2MWTs. The dark horizontal lines correspond to the mean differences, and the dotted lines correspond to the 95% limits of agreement.