ABSTRACT | Background: It is important to include large sample sizes and different factors that influence the six-minute walking distance (6MWD) in order to propose reference equations for the six-minute walking test (6MWT).

Objective: To evaluate the influence of anthropometric, demographic, and physiologic variables on the 6MWD of healthy subjects from different regions of Brazil to establish a reference equation for the Brazilian population.

Method: In a multicenter study, 617 healthy subjects performed two 6MWTs and had their weight, height, and body mass index (BMI) measured, as well as their physiologic responses to the test. Delta heart rate (ΔHR), perceived effort, and peripheral oxygen saturation were calculated by the difference between the respective values at the end of the test minus the baseline value.

Results: Walking distance averaged 586±106m, 54m greater in male compared to female subjects (p<0.001). No differences were observed among the 6MWD from different regions. The quadratic regression analysis considering only anthropometric and demographic data explained 46% of the variability in the 6MWT (p<0.001) and derived the equation: $6MWD_{pred}=890.46-(6.11\times age)+(0.0345\times age^2)+(48.87\times gender)-(4.87\times BMI)$. A second model of stepwise multiple regression including $\Delta HR$ explained 62% of the variability (p<0.0001) and derived the equation: $6MWD_{pred}=356.658-(2.303\times age)+(36.648\times gender)+(1.704\times height)+(1.365\times \Delta HR)$.

Conclusion: The equations proposed in this study, especially the second one, seem adequate to accurately predict the 6MWD for Brazilians.

Keywords: walking; exercise test; reference values; regression analysis; rehabilitation.

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Introduction
The ability to walk a distance is an easy and inexpensive way to assess physical capacity in health and illness. Among the field tests proposed in the literature, the 6-minute walk test (6MWT) has been widely used to assess the functional exercise capacity of patients with limiting conditions such as cardiopulmonary diseases. The test is simple, safe and provides a global and integrated response of all systems involved during exercise based on the distance walked in a level corridor during 6 minutes (6MWD). The 6MWT has a submaximal design and, because most activities of daily living are performed at submaximal levels, it is a good reflection of the functional exercise level for daily physical activities. The distance covered during the test has been used to assess response to therapeutic interventions (pharmacological and non-pharmacological) to detect exertional desaturation and need of long-term oxygen therapy and to predict morbimortality in cardiopulmonary diseases.

According to the guidelines of the American Thoracic Society (ATS), the interpretation of the 6MWT should ideally be done by considering age, height, weight, and gender, variables which independently affect the 6MWT in healthy adults. Thus, equations for predicting reference values of the 6MWT are necessary. In fact, some reference equations have been proposed previously for people from different countries, including Brazilian samples. However, aspects that needed to be better...
considered in future studies were identified\textsuperscript{11,12,16,18}. Firstly, it is essential that new studies be performed with larger sample sizes\textsuperscript{11,16,18}. Secondly, since the age range is limited in some of the studies\textsuperscript{2,13,17}, not all equations are applicable to young people. Finally, although the interference of physiologic variables (e.g., heart rate) in the 6MWD has been suggested and considered important\textsuperscript{12,13,18}, few studies have considered these variables in reference equations.

Given that Brazil is a large country with different climates and diverse socioeconomic and cultural conditions, a multicenter study that can provide samples from different regions of the country is vital to establishing a more representative reference equation for the 6MWT\textsuperscript{18}. Therefore, taking into consideration the importance of the 6MWT in clinical and research settings, by circumventing some of the current limitations, we aimed to study the influence of anthropometric, demographic, and physiologic variables on the 6MWT of a large, multicenter sample of healthy Brazilian subjects and to establish an equation for predicting reference values of the 6MWT for the Brazilian population.

● Method

Subjects

In the present multicenter study, although a sample size of 328 subjects was calculated to consider four variables in the regression model, 629 healthy subjects from four centers located in the Northeastern (1), Southeastern (2), and Southern (1) regions of Brazil were included. They were recruited from the local community and among students and employees of four universities, as well as their relatives. All of them had their anthropometric and demographic characteristics assessed and performed two 6MWTs. Data were collected from July 2008 to July 2011. The study was approved by the Research Ethics Committee of Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil (ETIC approval number 390-04). All participants gave written informed consent.

The inclusion criteria were: subjects of both genders aged 18 years or more; ability to understand and perform all procedures proposed; absence of any severe and/or unstable disease which could limit exercise tolerance, such as chronic obstructive pulmonary disease, asthma, cystic fibrosis, interstitial lung disease, angina, myocardial infarction, congestive heart failure, stroke, transient ischemic attack, peripheral vascular disease, and arthritis\textsuperscript{19}. Subjects were excluded if they had a body mass index (BMI) under 18kg.m\textsuperscript{-2} and above 40kg.m\textsuperscript{-2} or if they could not perform two 6MWTs for any reason.

6-minute walk test

In all centers, subjects performed two 6MWTs according to the ATS standardized protocol\textsuperscript{1} with, at least, 30 min of rest between them. The test was performed in a 30-m corridor, and the subjects were instructed to keep walking for 6 minutes. The best walked distance was considered for analysis. The tests were applied by a physical therapist or previously trained physical therapy student, and both 6MWTs were applied by the same assessor. Blood pressure, heart rate (HR), peripheral oxygen saturation (SpO\textsubscript{2}), and perceived dyspnea and leg fatigue (modified Borg scale)\textsuperscript{20} were assessed before and immediately after the tests. During the tests, HR and SpO\textsubscript{2} were continuously verified and standardized phrases of encouragement were spoken to the participants every minute\textsuperscript{1}(ATS, 2002). The predicted maximal HR (HR\textsubscript{max}) was derived from the formula

\[
HR\textsubscript{max} = 220 - \text{age} \quad \text{(ATS, 2002)}
\]

The delta of HR (∆HR), perceived effort (∆Borg), and SpO\textsubscript{2} (∆SpO\textsubscript{2}) were calculated by the difference between the respective value at the end of the test minus the baseline value.

Anthropometric and demographic data and health status

A questionnaire was applied in order to investigate the health status of the participants. Height (cm) and body weight (Kg) were measured, and the BMI was calculated.

Statistical analysis

The statistical analysis was performed using the statistical packages SPSS 17.0 (SPSS Inc., USA) and STATA 12.0 (STATA Corp, USA). The normality of data distribution was evaluated using the Kolmogorov-Smirnov test. The Mann-Whitney test or the independent Student t test was used to compare male and female characteristics. The comparisons between regions were assessed using the Kruskall-Wallis test followed by post-hoc Mann-Whitney, when appropriate. Spearman’s correlation coefficient was used to verify the simple correlation between the walked distance (dependent variable) and age, gender, weight, height, BMI, SpO\textsubscript{2}, HR\textsubscript{max} % predicted, and ∆HR. Two regression models were applied to derive reference equations both considering the association between the variables and the distance walked in the 6MWT. The best models were constructed considering the variables with the

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best independent coefficient of determination ($R^2$) and statistical significance as p<0.05. The first model included only demographic and anthropometric variables as independent variables (age, gender, weight, height, and BMI). In a stepwise multiple linear regression model, the assumptions of normality of the residuals score were not met, so a quadratic regression model (Equation 1) was used. The second model, a stepwise multiple linear regression model, included the same independent variables used in the first model in addition to physiologic variables (SpO$_2$, HRmax % predicted, $\Delta$HR) and the normality of the residuals score was met (Equation 2). Since equation 2 showed a much higher coefficient of determination (see results), further analysis focused on this specific equation. In order to verify the reliability of Equation 2, it was applied a posteriori analysis in a different group of healthy subjects, composed of 58 individuals selected according to the same inclusion criteria in the different centers. In addition, Equation 2 was cross-validated against prediction equations existing in the literature in this group, considering only the subjects with the same age range of each study. The level of statistical significance was considered as p<0.05.

### Results

From the 629 healthy subjects selected, 12 (2%) were excluded and 617 were included in the final analysis. The subjects were aged 19 to 79 years and 52% were women. Table 1 shows descriptive data on characteristics of the studied subjects and outcome parameters of the best 6MWT. The participants were distributed by age range as follows: 19-29 years (n=155); 30-39 years (n=66); 40-49 years (n=77); 50-59 years (n=83); 60-69 years (n=110), and 70-79 years (n=126). According to the BMI classification of body composition (kg/m$^2$), 47.1% were normal (18.5 to 24.9), 37.9% were overweight (25.0 to 29.9), and 14.9% were obese (30.0 to 39.9).

### Comparison between regions

Table 2 shows that, despite the differences in anthropometric parameters observed among the 3 different regions, the 6MWD was similar.

### 6MWT determinants and reference equations

There were significant correlations between the walked distance and age ($r=0.55$, p<0.0001), height ($r=0.48$, p<0.0001), BMI ($r=0.40$, p<0.0001), and $\Delta$HR ($r=0.43$, p<0.0001). A model of quadratic regression analysis showed that age, gender, and BMI explained 46% of the variability in the 6MWT ($R^2=0.46$, p<0.0001; Table 3). The derived equation (Equation 1) for the 6MWT based on this analysis considering only anthropometric and demographic data was:

$$6\text{MWD}_{\text{pred}}=890.46-\left(6.11 \times \text{age}\right)+\left(0.0345 \times \text{age}^2\right)+\left(48.87 \times \text{gender}\right)-\left(4.87 \times \text{BMI}\right)$$

(where male gender = 1 and female gender=0).

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Table 1. Characteristics of the subjects and outcome parameters of the best 6MWT.

|                  | Total (n=617) | Male (n=296) | Female (n=321) | p-values* |
|------------------|--------------|--------------|----------------|-----------|
| Age (years)      | 52 (29-67)   | 49 (27-68)   | 53 (31-67)     | 0.509     |
| Height (cm)      | 164 (157-171)| 170 (166-176)| 158 (152-163)  | <0.0001   |
| Weight (kg)      | 68 (59-78)   | 74 (66-81)   | 62 (55-71)     | <0.0001   |
| BMI (kg.m$^{-2}$)| 25 (23-28)   | 25 (23-28)   | 25 (22-28)     | 0.210     |
| 6MWD (m)         | 586±106      | 614±102      | 560±103        | <0.0001   |
| $\text{HR}_{\text{baseline}}$ (bpm)| 82 (73-91) | 80 (71-90) | 83 (75-92) | 0.036    |
| $\Delta$ HR (bpm)| 41 (25-59)  | 41 (25-56)   | 41 (25-58)     | 0.925     |
| %HRmax           | 73 (64-83)   | 73 (64-82)   | 74 (65-84)     | 0.321     |
| SpO$_2_{\text{baseline}}$ (%) | 97 (96-98) | 97 (95-98) | 97 (96-98) | 0.957   |
| $\Delta$ SpO$_2$ (%) | 0 (–2 to –1) | 0 (–1 to 1) | 0 (–2 to –1) | 0.825   |
| Borg F$_{\text{baseline}}$ (pts) | 0 (0-0)     | 0 (0-0)      | 0 (0-0)        | 0.053     |
| $\Delta$ Borg F (pts) | 1.5 (0.5-2.5)| 1 (0.3-2.0) | 1.5 (0.5-3.0) | 0.020     |

Data are expressed as median (interquartile range 25%-75%) except for the 6MWD (mean and SD). BMI: body mass index; 6MWT: 6-minute walk test; HR: heart rate; %HRmax: % of the maximal predicted heart rate; SpO$_2$: peripheral oxygen saturation; Borg F: perceived leg fatigue assessed by the Borg scale. *Comparisons between male and female using the Mann-Whitney test.
The second model of stepwise multiple regression showed that age, gender, height, and ΔHR explained 62% (R²=0.616, p<0.0001) of the variability in the 6MWT (Table 4). The derived equation (Equation 2) for the distance walked in the 6MWT was:

\[
6MWD_{\text{pred}} = 356.658 - (2.303 \times \text{age}) + (36.648 \times \text{gender}) + (1.704 \times \text{height}) + (1.365 \times \Delta HR)
\]

(where male gender=1 and female gender=0).

Reliability of equation 2 and comparison with other reference equations

The characteristics of the other group composed of 58 healthy subjects (23 male and 35 female) included in the \textit{a posteriori} analysis were: age 52±15 years and BMI 26±4 kg/m². When equation 2 was applied in this group, there was no difference between the actual and the predicted 6MWD (565 m vs. 582 m, p=0.11, respectively) with a significant correlation between them (r=0.76, p<0.0001).

When previous reference equations published in the literature were applied in the \textit{a posteriori} analysis, positive and significant correlations with the walked distance by the 58 subjects were observed (Table 5). The comparison indicated differences in four of them.2,10,11,17

Discussion

This study presented new equations to predict the distance covered during the 6MWT in Brazilian subjects and showed that approximately 62% of the variance was explained by gender, age, height, and the change in heart rate (ΔHR) during the test. When no physiologic variables were entered into the logistic analysis, approximately 47% of the variance was explained by the combination of gender, age, age², and BMI.

The present study has two strong methodological characteristics. Firstly, to the best of our knowledge, this is the study with the largest sample to propose a reference equation for the 6MWT in this population. Secondly, this study had a multicenter design and was therefore the first to have a sample composed of subjects from three regions of the country.

The sample was well distributed according to age, considering the classification of the American College of Sports Medicine22, with 221 (35.8%) young (<39yr), 200 (32.4%) middle-aged (40-64yr), and 196 (31.8%) elderly (65-79yr) subjects. The number of overweight and obese subjects (levels I and II) is similar to the one identified in the Brazilian population.23 Thus, the sample reflects the characteristics and variations as they exist in the real

| Table 2. Anthropometric characteristics and 6MWD values from the three regions. |
|-----------------------------------|-----------------------------------|-----------------------------------|----------------|
| **Southeast (n=194)**             | **South (n=271)**                 | **Northeast (n=152)**             | **p value**^c |
| Age (years)                       | 47.00 (33.0-67.00)                | 58.00* (37.00-69.00)              | 35.50*† (22.00-57.00) | <0.001 |
| Height (cm)                       | 166.00 (158.75-172.00)            | 167.00 (157.00-174.00)            | 160.50*† (154.25-165.75) | <0.001 |
| Weight (kg)                       | 69.95 (62.00-79.28)               | 71.00 (63.00-79.20)               | 59.90*† (55.00-68.81) | <0.001 |
| BMI (kg.m⁻²)                      | 25.48 (23.08-28.13)               | 25.59 (23.39-27.97)               | 23.46*† (21.01-27.23) | <0.001 |
| 6MWD (m)                          | 570.00 (515.75-642.00)            | 581.00 (512.00-658.00)            | 598.00*† (477.13-664.50) | 0.457 |

Data are expressed as median (interquartile range 25%-75%). *Kruskal-Wallis Test. †p<0.01 compared to Southeast; † p<0.01 compared to South. Post hoc Mann-Whitney test. 6MWD= six minute walking distance.

| Table 3. Quadratic regression with the 6MWD as dependent variable considering only demographic variables (Equation 1). |
|-----------------------------------|-----------------------------------|-----------------------------------|----------------|
| **Non-standardized coefficients (B)** | **95% Confidence interval for B** | **p-value** | **Partial R² (%)** |
| Constant                          | 890.46                            | 840.35-940.57                     | <0.001 |
| Age (years)                       | –6.11                             | –8.35–3.88                        | <0.001 |
| Age²(years)                       | 0.0345                            | 0.0117-0.0573                     | 0.003 |
| Gender*                           | 48.87                             | 36.6-61.14                        | <0.001 |
| BMI (kg.m⁻²)                      | –4.87                             | –6.46–3.28                        | <0.001 |

Standard error of the estimate=77.2 m. BMI=body mass index; The derived equation for the 6MWD predicted based on the quadratic regression analysis was: 6MWD_{pred}=890.46–(6.11×age)+(0.0345×age²)+(48.87×gender)–(4.87×BMI); *Male gender=1 and female gender=0. 95% CI =95% confidence interval for the coefficient.
population, increasing the external validity. Previous studies have reported the influence of age\textsuperscript{11,20,24} and BMI on the 6MWD\textsuperscript{11,16,24}.

The results indicated differences between the anthropometric characteristics of subjects from different regions. However, it was not sufficient to produce differences in walking distance when the three regions were compared. Region-to-region difference occurred when comparing the Northeast to the other two regions, although this difference was probably due to the younger age of this specific subgroup. Taking into consideration the absence of differences (Table 4), this indicates the possibility of generalizing the 6MWD reference equations for all Brazilian regions, at least in studies where a large age-range is considered\textsuperscript{15,16}.

The influence of the relative intensity of the test represented by the ∆HR was identified as an important variable with 41% of partial correlation in Equation 2. The study by Chetta et al.\textsuperscript{13} mentioned

### Table 4. Multiple linear stepwise regression analysis with the 6MWD as dependent variable considering demographic variables and delta heart rate (Equation 2).

|                         | Non-standardized coefficients (B) | 95% Confidence interval for B | p-value | Partial R\textsuperscript{2} (%) |
|-------------------------|-----------------------------------|-------------------------------|---------|-------------------------------|
| Constant                | 356.658                           | 209.36-503.96                | <0.0001 |                               |
| Age (years)             | -2.303                            | 21.07-52.23                  | <0.0001 | 47.90                         |
| Gender\textsuperscript{a} | 36.648                           | 1.11-1.63                  | <0.0001 | 19.50                         |
| Height (cm)             | 1.704                             | -2.66- -1.95                | <0.0001 | 16.50                         |
| ∆ HR                    | 1.365                             | 0.84-2.57                 | <0.0001 | 40.60                         |

Standard error of the estimate =64.3 m. The derived equation for the 6MWD based on the regression analysis was: 6MWD\textsubscript{pred}=356.658−(2.303×age)+(36.648×gender)+(1.704×height)+(1.365×∆HR). *Male gender=1 and female gender=0.

### Table 5. Comparison and correlation between the actual walked distance in the \textit{a posteriori} group (n=58) (median 565 meters) and the predicted distance by other previously published equations.

| Equation/age range (years) | Number of pairs with similar age | Predicted distance (m) | Spearman Correlation (rho; p-value) |
|----------------------------|---------------------------------|------------------------|-----------------------------------|
| Enright and Sherrill\textsuperscript{9} 40-80 | Male (n=117) | 555 (411-654) | 0.63; 0.005 |
|                             | Female (n=173) | 518 (367-642) | 0.39; 0.028 |
| Troosters et al.\textsuperscript{2} | 50-85 (n=31) | 606 (431-747)* | 0.57; <0.0001 |
| Gibbons et al.\textsuperscript{10} | 20-80 (n=79) | 667 (558-803)* | 0.63; <0.0001 |
| Chetta et al.\textsuperscript{13} | 20-50 (n=102) | 597 (530-680) | 0.53; 0.006 |
| Camarri et al.\textsuperscript{11} | 55-75 (n=22) | 661 (601-746)* | 0.58; 0.006 |
| Iwama et al.\textsuperscript{16} | 13-84 (n=134) | 550 (477-643) | 0.59; <0.0001 |
| Dourado et al.\textsuperscript{17} | ≥40 (n=90) | 598 (464-688)* | 0.48; <0.0001 |
| Soares and Pereira\textsuperscript{15} | 20-80 (n=132) | 550 (398-661) | 0.71; <0.0001 |

Data expressed as median (minimum-maximum). *p<0.0001 compared with the walked distance of the 58 individuals=565 m (352-870). Mann-Whitney Test.
the importance of considering the heart rate during the 6MWT, however this variable was not included in the reference equation proposed by those authors. Poh et al.\textsuperscript{12} considered the % of predicted HRmax in the equation as well as age, height, and weight and explained 78% of the variance of the 6MWD, despite the small sample size (n=35). The authors considered that the use of the % of predicted HRmax in the equation may be limited when measuring the 6MWD in subjects with diseases or medications which have an influence on HRmax. However, the use of the change in HR during the test (\(\Delta HR\)) could, at least in part, counteract this limitation. This may happen since these diseases and medications interfere not only in the HR at the end of the test but also in the resting HR, and therefore their influence on this specific outcome (\(\Delta HR\)) may be counterbalanced and reduced.

A recent international multicenter study evaluated the geographic variations of the 6MWD in a cohort of healthy adults and suggested that the resulting distance could be influenced by factors such as the speed of habitual walking and other aspects related to lifestyle such as the motivation of the subject and/or the assessor\textsuperscript{25}. These aspects were not evaluated in the present study, however they could influence the \(\Delta HR\). As the 6MWT is self-paced, the use of the \(\Delta HR\) considers the freedom of the subjects to choose the speed, despite the standardized instructions and encouragement. Furthermore, the influence of encouragement on the subject’s performance has been discussed since 1984 by Guyatt et al.\textsuperscript{26}, and the ATS Guidelines (2002)\textsuperscript{3} consider it an important determinant of the distance covered in the 6MWT. Nevertheless, motivation involves two aspects: extrinsic and intrinsic motivation\textsuperscript{27}. Standardizing motivation phrases is a way to control the extrinsic factor, which produces different responses in subjects depending on their intrinsic motivation. The \(\Delta HR\) could indicate how the subjects respond to the extrinsic motivation during the 6MWT. This self-determination theory has been used to evaluate the response of changes in exercise training behavior\textsuperscript{26} but has not been evaluated in self-regulated exercise tests. All of these factors help to explain, at least in part, the positive role of including \(\Delta HR\) in the prediction of the 6MWD’s normal values. Recently, the use of rest HR was considered to predict maximal oxygen consumption based on the 6MWT\textsuperscript{28}.

Equation 1, which considered only anthropometric and demographic variables, explained almost half of the variance in the 6MWT. This showed that age, gender, and BMI are important to predict the 6MWD in agreement with the vast majority of the studies designed to propose reference equations for this test. It is, therefore, an option to be used in case it is not possible to obtain the \(\Delta HR\) and use Equation 2.

The comparison with other studies allowed us to identify that the equations proposed by Troosters et al.\textsuperscript{2}, Gibbons et al.\textsuperscript{10}, Camarri et al.\textsuperscript{11}, and Dourado et al.\textsuperscript{17} predicted 6MWD with a statistical difference in comparison to the distance observed in the present study. These discrepancies could be related to differences in the test protocol, encouragement, and different aspects of motivation, as previously discussed. Sample size\textsuperscript{2,3,10,17}, combined with different characteristics of each population\textsuperscript{2,3,10}, could also contribute to this discrepancy. The equation proposed by Iwama et al.\textsuperscript{16} for the Brazilian population, based on the same age range and a sample of 134 subjects, explained only 30% of the variation in the 6MWD. Dourado et al.\textsuperscript{17} also found similar results, explaining 54.3% of the 6MWD variance in a model based on the age, weight, height, and gender of 90 healthy adults, not including young subjects. Based on 132 volunteers (also 20-80 years of age) better distributed using the adjusted quadratic model, Soares and Pereira\textsuperscript{19} proposed a model based on height, age, and BMI and explained 55% of the variance in the 6MWD.

Despite a larger sample, our first equation, based only on anthropometric and demographic data, explained 46% of the variance. This relatively low coefficient of determination can be partially explained by the difference in anthropometric variables between different regions included in the study, without difference between 6MWD values. On the other hand, Equation 2 (including HR) explained 62% of the variance in the 6MWD, well above the previous equations. Despite the fact that this physiological variable is a result of the work performed in the test, it can contribute to the understanding of the expected performance for individuals with different anthropometric characteristics and, particularly, in different age ranges. Thus, at present, Equation 2 seems to stand out as superior (i.e. has fewer limitations) for accurately predicting the 6MWD for Brazilians.

The 6MWT is frequently used in physical therapy routine. The ATS recommends the assessment of HR during the test. In this way, the use of Equation 2 could help determine whether changes in the 6MWD are also related to intrinsic motivation, which is an open field of investigation in regard to self-paced tests.

Given that 38.4% (Equation 2) and 53.5% (Equation 1) of the variance could not be explained...
by these models, future studies could explore the contribution of other behavioral and/or physiologic factors. The repeatability of the 6MWT with the same subject in a sequence of days could contribute to evaluate the influence of HR and intrinsic motivation behavior on 6MWD variation. It would be also interesting to assess the subjects’ level of physical activity. This information could be evaluated as an independent variable and/or used to better identify the subjects’ HR response.

Limitation
Although this was a multicenter study, the sample was not randomized. However, we evaluated volunteers from different centers, which can, at least in part, guarantee the diversification of the sample.

Conclusion
In summary, the present study showed that the distance covered during six minutes can be better explained and predicted when considering the heart rate changes during the test in addition to the anthropometric parameters. Furthermore, we have proposed two new reference equations, one of them including the heart rate changes during the test. These equations, especially the second, seem to be adequate to accurately predict the 6MWD for Brazilians.

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