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**Title:** Reference equation for the two-minute walk test in healthy adults and the elderly

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**Body:** Introduction: The two-minute walk test (2MWT) has been used for evaluating the functional capacity of subjects with cardiopulmonary diseases, but there is no reference equation for predicting the distance walked in this test. Objective: To establish a reference equation for predicting the distance walked in the 2MWT. Methods: This study consisted of two parts. To establish the reference equation, 390 subjects (183 male, aged 18-89 years, FEV1 101±13% pred) performed two 2MWTs, 30 min apart. The greatest distance walked was used for the prediction model. The independent variables included for predicting the distance walked were age, gender, weight, height, and body mass index (BMI). The second part consisted of the prospective application of the reference equation to 70 subjects (35 male, 54±20 yrs, FEV1 99±13% pred) who performed the 2MWT. Results: The walked distance (WD) was quite similar between the two 2MWTs (203±35 m vs 206±36 m), despite being statistically different (p<0.0001). The WD correlated with age (r= -0.65), weight (r= 0.24), and height (r= 0.41) (p<0.001 for all), but not with BMI. The reference equation was:  $2MWT = 252.431 - (1.165 \times age) + (19.992 \times gender)$  (male = 1 and female = 0) (R2= 0.50; p<0.0001). There was no significant difference between the actual WD in the 2MWT and the distance predicted by the reference equation (198 $\pm$ 22 m vs 200 $\pm$ 26 m; p = 0.046) (mean bias = -1.48 m [95% limits of agreement: -31.7m-34.6m]). Conclusion: This reference equation can be used as a reference to determine the percentage of the predicted walked distance in the 2MWT by subjects with various diseases.