



Original Research

# Association of Step Count with Cardiorespiratory Fitness: Results from the Virtual 2-Minute Step Test



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## KEYWORDS

aging;  
Cardiopulmonary exercise test;  
Exercise test;  
Exercise therapy;  
Frail older adults;  
HIV;  
HIV-1;

**Abstract Objective:** To test the hypothesis that step count based on a virtual 2-minute step test (2MST) predicts cardiorespiratory fitness (CRF).

**Design:** Cross-sectional study.

**Setting:** Veteran Affairs Medical Centers participating in a randomized trial of functional exercise training delivered by videoconferencing.

**Participants:** People with human immunodeficiency virus (HIV) (PWH) who were aged  $\geq 50$  years and clinically stable on antiretroviral therapy were eligible for the trial. Consecutive male participants who were enrolled prior to November 9, 2023 and completed a baseline 2MST and cardiopulmonary exercise test (CPET) were included in the cross-sectional study (N=74).

**List of abbreviations:** 2-MST, 2-minute step test; 6-MWT, 6-minute walk test; ART, antiretroviral therapy; BMI, body mass index; CPET, cardiopulmonary exercise test; CRF, cardiorespiratory fitness; HR, heart rate; HIV, human immunodeficiency virus; PWH, people with HIV; RER, respiratory exchange ratio;  $VO_2$ peak, peak oxygen uptake.

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HIV seropositivity;  
Physical fitness;  
Physical functional  
performance;  
telemedicine;  
telerehabilitation

*Intervention:* None.

*Main Outcome Measures:* Step count was measured by a 2MST performed by live videoconferencing using the Rikli and Jones protocol. CRF was measured by peak oxygen utilization (VO<sub>2</sub>peak) during a modified Bruce cardiopulmonary exercise testing.

*Results:* Most participants (62.2%) were ≥60 years of age. The mean (SD) VO<sub>2</sub>peak was 23.6 (5.7) mL/kg/min, which represented 72.4% (SD, 14.1) of expected VO<sub>2</sub>peak. Step count correlated with VO<sub>2</sub>peak ( $r=0.47$ ,  $P<.001$ ). Multivariable linear regression showed that step count was significantly associated with VO<sub>2</sub>peak independently of age and body mass index. Based on this model, the prediction of VO<sub>2</sub>peak based on step count explained 60% of the variance in VO<sub>2</sub>peak. A Bland-Altman plot showed good agreement between predicted and measured VO<sub>2</sub>peak without heteroscedasticity.

*Conclusions:* The virtual 2MST predicted VO<sub>2</sub>peak independently of age and body mass index in men with well-controlled HIV. In ambulatory populations with known impaired cardiorespiratory fitness, the virtual 2MST may be a feasible and valid estimate of VO<sub>2</sub>peak that can be used in the telerehabilitation setting. Future work is required in more demographically diverse individuals with various chronic conditions.

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Decline in cardiorespiratory fitness (CRF) is a hallmark of aging that independently predicts mortality, disability, and quality of life in diverse populations.<sup>1</sup> Despite prolonged survival with modern therapy, a phenotype of accelerated aging has emerged in people with human immunodeficiency virus (HIV) (PWH) as evidenced by increased risk of geriatric syndromes and disability.<sup>2</sup> CRF is uniquely suited as a physiological marker of accelerated aging in PWH that can be targeted by exercise rehabilitation to attenuate the underlying cellular aging.<sup>3</sup> Aerobic exercise training increases CRF in older adults with a wide range of diseases,<sup>4</sup> including HIV infection.<sup>5</sup> However, individualized exercise strategies may be needed for specific high-risk patients to overcome accelerating aging effects. In order to have a broad impact, this approach will require identification of PWH with low fitness who can easily access an exercise rehabilitation program.

A robust measurement of CRF is peak oxygen utilization (VO<sub>2</sub>peak) at maximum effort during graded exercise; this measurement is commonly referred to as cardiopulmonary exercise testing (CPET).<sup>1</sup> However, the CPET requires specialized equipment and requires the participant to exercise to exhaustion while wearing a face mask. Due to these challenges, various indirect measures of CRF are common. Long-distance walk tests, eg, 6-minute walk test (6MWT), are submaximal exercise tests that are widely used to estimate CRF in ambulatory older individuals, including PWH.<sup>6</sup> Despite its utility as a prognostic indicator, the 6MWT is limited logistically by space and is not feasible in the setting of a telerehabilitation program.

An alternative test is stepping in place that can be performed in a small space and easily observed by videoconferencing. Various stepping protocols that use a step platform or ergometer have been established to estimate CRF in young, healthy adults but are not well tolerated in older adults or those with chronic comorbidity.<sup>7</sup> The 2-minute step test (2MST) was recognized by Rikli and Jones<sup>8</sup> as a functional performance test for adults ≥60 years of age that estimates physical fitness without heart rate (HR) monitoring or other equipment. The 2MST has been well studied as an alternative to the 6MWT and has shown sensitivity to change after exercise training,<sup>9</sup> but limited information is available on its construct validity to estimate VO<sub>2</sub>peak. Furthermore,

understanding the utility of performing the 2MST virtually could promote telerehabilitation programs.

Our objective was to examine the relationship between performance on the 2MST conducted by live videoconferencing and VO<sub>2</sub>peak in PWH ≥50 years of age, a patient population with accelerated aging. We hypothesized that step count would be positively correlated with VO<sub>2</sub>peak measured by CPET. Our secondary objective was to develop an equation to predict VO<sub>2</sub>peak based on the 2MST after adjusting for key covariates.

## Methods

### Participants

This cross-sectional study included PWH who were recruited from HIV clinics at the Atlanta and Baltimore Veteran Affairs Medical Centers and were enrolled into a multi-site randomized trial of exercise training delivered by videoconferencing, the FITVET Study (NCT04103593).<sup>10</sup> Participants who had completed baseline CPET and functional performance testing prior to November 9, 2023 were included. Eligibility criteria are detailed elsewhere<sup>10</sup> and included PWH age ≥50 years on antiretroviral therapy (ART) with at least 1 HIV-1 viral load <20 c/ml in the prior 6 months, representing clinically stable PWH regarded as older adults.<sup>11</sup> In addition to contraindications to CPET by the American College of Sports Medicine (ACSM),<sup>1</sup> any AIDS-defining illness in the prior 6 months was also an exclusion criterion. Because exercise training required HR monitoring, use of medication in the classes of β-adrenergic blocking agents or nondihydropyridines calcium channel antagonists within the 1 month prior was exclusionary. Written consent was obtained from all participants as approved by the Institutional Review Board and VA Research and Development Committees.

### Procedures

Cardiopulmonary exercise testing was performed using the modified Bruce treadmill protocol following ACSM

guidelines.<sup>1</sup> Participants exercised until voluntary exhaustion or safety criterion was met. Prior to testing, supine resting HR was measured after 2 minutes. Continuous HR was measured during the CPET. Age-predicted maximum HR was calculated by the formula:  $208 - (0.7 \times \text{age})$ .<sup>12</sup> Gas exchange was collected breath by breath and then averaged at 30-second intervals using the Quark CPET<sup>a</sup> or True One<sup>b</sup> metabolic cart. An independent exercise physiologist who was blinded to participant information selected the highest  $\text{O}_2$  mL/kg/minute value within the last minute of exercise as the  $\text{VO}_2\text{peak}$ . Participant effort on the CPET was ascertained for chronically ill adults based on peak respiratory exchange ratio ( $\text{RER}$ )  $\geq 1.05$  or maximum HR  $\geq 90\%$  of APMH.<sup>13</sup> In order to provide a frame of reference to adults without HIV, expected  $\text{VO}_2\text{peak}$  was calculated using an established equation based on sex, age, and BMI.<sup>14</sup> Measured  $\text{VO}_2\text{peak}$  was divided by expected  $\text{VO}_2\text{peak}$  to derive the percent expected  $\text{VO}_2\text{peak}$ . At each site an independent (blinded) physician reviewed electrocardiogram tracings for evidence of cardiac ischemia. Any participants with evidence of cardiac ischemia were excluded from the study and referred for clinical evaluation.

The 2MST was performed following the protocol set by Rikli and Jones<sup>8</sup> which has high test-retest reliability (intra-class correlation coefficient=0.90). Exercise physiologists and participants were located at different Veteran Affairs Medical Centers and viewed each other in real time through the VA intranet system using a high-speed, encrypted, and Health Insurance Portability and Accountability Act (HIPAA)-compliant VA network connection. A research coordinator who was trained in measurement of step height was in the room with the participant. Step height was defined as midway between the participant's patella and iliac crest. This target level was then transferred to the wall with painter's tape. The participant was instructed to step in place for 2 minutes lifting their knees to the height of the mark on the wall as many times as they could. Score on the 2MST was equal to the step count in 2 minutes based on the number of steps in which the right leg reached the target using a hand-held counter. Per the protocol, participants were not coached during the test and were only provided time remaining. Participants were allowed to hold onto the wall or a chair for stability or to rest at any point during the 2 minutes. During the test, all participant instructions and observations were performed by the virtual exercise physiologist without local staff assistance.

The 2MST was performed as part of a battery of physical function tests that included the following: timed chair stand, 8-foot up-and-go, 30-second arm curl, and 4-meter walk, which were conducted using a standardized protocol adapted for video conferencing.<sup>10</sup> Gait speed was calculated based on the 4-meter walk. Dominant hand grip strength was measured in person by the on-site research coordinator using a hand dynamometer with 3 trials averaged.

## Statistical analysis

Pearson or Spearman correlation coefficients were calculated to test the relationship between step count and  $\text{VO}_2\text{peak}$  or functional performance measures. Bivariate analyses relying on 2-sample *t* tests and analysis of variance

were used to examine the association between participant characteristics and either step count or  $\text{VO}_2\text{peak}$ . Age was divided into 2 groups,  $\geq 60$  years and  $< 60$  years, based on original validation work in 2MST and available normative data.<sup>8</sup> To evaluate the relationship between step count and  $\text{VO}_2\text{peak}$  with adjustment for potential confounders, linear regression with step count as the independent (predictor) variable and  $\text{VO}_2\text{peak}$  (mL/kg/min) as the dependent variable was performed. In a stepwise manner, participant characteristics found significant at the  $P < .05$  level in bivariate analyses with step count or  $\text{VO}_2\text{peak}$  were entered into the model. Variables improving the proportion of the variation explained by the model adjusted for the number of variables (adjusted model  $R^2$ ) were retained. The model with the highest adjusted  $R^2$  was used to derive a prediction equation for  $\text{VO}_2\text{peak}$  based on step count. Model assumptions were evaluated using residual plots, and multicollinearity was assessed using the variance inflation factor where values equal or close to 1.0 are desirable for each independent variable.<sup>15</sup> A Bland-Altman plot was used to visualize agreement in predicted and measured  $\text{VO}_2\text{peak}$ . Then, heteroscedasticity between predicted and measured  $\text{VO}_2\text{peak}$  was evaluated with a Breusch-Pagan test.<sup>16</sup> Finally, as an exploratory analysis, we tested the relationship of step count with functional performance measures. All data are presented as mean  $\pm$  SD or median (25th, 75th quartile) based on distribution. Analyses were performed with R software version 4.2.2<sup>c</sup> and STATA software version 18.0.<sup>d</sup>

## Results

Among the 89 individuals who met eligibility criteria, 11 did not complete baseline testing because they were lost to follow-up ( $n=6$ ) or participant withdrawal ( $n=5$ ). One participant who completed the CPET on a bicycle ergometer was excluded from this analysis. Three female participants were also excluded given the insufficient number to allow adjustment for established sex-related difference in 2MST<sup>17</sup> and  $\text{VO}_2\text{peak}$ .<sup>1</sup> The final analytical set included 74 consecutive male participants enrolled in FITVET with mean (SD) age of 62.6 (7.0) years. There was no difference in age or race between the analytical set and excluded FITVET participants. Table 1 provides demographic and clinical characteristics. HIV infection was well controlled with the most recent viral load  $< 20$  c/ml in 96% of the participants and clinically insignificant values  $< 100$  c/ml in the remainder. Most participants (76%) had a CD4 cell count within normal limits ( $> 500$  cells/uL). The most common age-related condition was hypertension (61%).

The mean (SD)  $\text{VO}_2\text{peak}$  was 23.6 (5.7) mL/kg/min which represented 72.4% (14.1) of predicted  $\text{VO}_2\text{peak}$ .  $\text{VO}_2\text{peak}$  was associated with age ( $r = -0.61$ ,  $P < .01$ ), BMI, and hypertension (table 1). Most participants (89%) provided a maximum effort on the CPET (table 2). Results of all functional performance tests, including 2MST, are summarized in table 2. Overall, mean (SD) step count was 80.7 (20.2). Step count was significantly associated with smoking, and hypertension (table 1). Older individuals ( $\geq 60$  years) had significantly lower step count than younger individuals ( $< 60$  years) (supplemental fig S1, available online only at <http://www.archives-pmr.org/>).

**Table 1** Baseline demographics and clinical characteristics of 74 older men with HIV and bivariate associations with peak oxygen utilization and step count from a virtual 2-minute step test

Characteristic	n (%)	Mean $\pm$ SD	
		VO <sub>2</sub> peak (mL/kg/min)	2MST (steps)
Age (y)			
<60	28 (37.8%)	26.9 $\pm$ 4.4 <sup>†</sup>	90.1 $\pm$ 17.1 <sup>†</sup>
$\geq$ 60	46 (62.2%)	21.6 $\pm$ 5.5	74.9 $\pm$ 20.0
Race			
White	8 (10.8%)	24.2 $\pm$ 6.8	73.38 $\pm$ 19.0
Black	64 (86.5%)	23.3 $\pm$ 5.4	81.44 $\pm$ 20.6
Hispanic/Mixed/Other	2 (2.7%)	31.2 $\pm$ 7.4	85.00 $\pm$ 9.9
Heroin or cocaine use			
Current	3 (4.1%)	22.4 $\pm$ 4.6	69.7 $\pm$ 13.4
Prior	36 (48.6%)	24.0 $\pm$ 5.6	78.3 $\pm$ 21.8
Never	35 (47.3%)	23.4 $\pm$ 5.9	84.1 $\pm$ 18.7
Cigarette smoking history			
Current	16 (21.6%)	22.4 $\pm$ 4.3	70.8 $\pm$ 19.0 <sup>†</sup>
Former	28 (37.8%)	22.7 $\pm$ 5.5	78.5 $\pm$ 20.3
Never	30 (40.5%)	25.1 $\pm$ 6.3	87.9 $\pm$ 18.7
Body mass index			
Healthy, 18.5-24.9 kg/m <sup>2</sup>	24 (32.4%)	25.5 $\pm$ 6.0 <sup>†</sup>	78.5 $\pm$ 21.7
Overweight, 25.0-29.9 kg/m <sup>2</sup>	30 (40.5%)	24.0 $\pm$ 5.3	79.4 $\pm$ 21.3
Obese, $\geq$ 30.0 kg/m <sup>2</sup>	20 (27.0%)	20.8 $\pm$ 5.1	85.1 $\pm$ 16.7
Diabetes			
Present	19 (25.7%)	22.4 $\pm$ 5.2	82.3 $\pm$ 20.5
Not present	55 (74.3%)	24.0 $\pm$ 5.8	80.1 $\pm$ 20.3
Hypertension history			
Present	45 (60.8%)	22.4 $\pm$ 5.3*	76.0 $\pm$ 20.3*
Not present	29 (39.2%)	25.4 $\pm$ 5.9	87.8 $\pm$ 18.2
Chronic obstructive pulmonary disease			
Present	4 (5.4%)	20.2 $\pm$ 3.2	76.3 $\pm$ 22.8
Not present	70 (94.6%)	23.8 $\pm$ 5.8	80.9 $\pm$ 20.2
Anemia (hemoglobin<13.0 g/dl)			
Present	11 (14.9%)	21.5 $\pm$ 8.4	76.4 $\pm$ 25.5
Not present	63 (85.1%)	24.0 $\pm$ 5.1	81.4 $\pm$ 19.3
Hepatitis C antibody			
Present	26 (35.1%)	23.6 $\pm$ 5.9	75.1 $\pm$ 21.2
Not present	48 (64.9%)	23.6 $\pm$ 5.6	83.7 $\pm$ 19.2
AIDS-defining illness history			
Present	14 (18.9%)	25.2 $\pm$ 5.4	86.3 $\pm$ 20.7
Not present	60 (81.1%)	23.2 $\pm$ 5.7	79.4 $\pm$ 20.1
CD4 cell count (cell/l)			
<500	18 (24.3%)	22.7 $\pm$ 5.5	75.6 $\pm$ 16.5
$\geq$ 500	56 (75.7%)	23.9 $\pm$ 5.8	82.3 $\pm$ 21.2
HIV copies/mL			
Nondetectable (<20)	71 (95.9%)	23.6 $\pm$ 5.8	80.2 $\pm$ 20.3
Detectable (20-200)	3 (4.1%)	24.4 $\pm$ 3.4	91.7 $\pm$ 17.0
NRTI current use	67 (90.5%)	23.5 $\pm$ 5.8	80.9 $\pm$ 20.7
NNRTI current use	11 (14.9%)	23.7 $\pm$ 4.9	83.4 $\pm$ 19.4
PI current use	6 (8.1%)	23.3 $\pm$ 3.4	91.3 $\pm$ 24.8
INSTI current use	68 (91.9%)	23.7 $\pm$ 5.7	79.8 $\pm$ 20.1

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; 2MST, 2-minute step test.

P values are based on a 1-way analysis of variance or 2-sample t test, as appropriate.

\* P<.05.

† P<.01.

**Table 2** Cardiorespiratory fitness and functional performance of 74 older men with HIV

Characteristic (mean [SD] unless otherwise indicated)

Cardiopulmonary exercise test	
VO <sub>2</sub> peak, mL/kg/min	23.6 (5.7)
VO <sub>2</sub> peak, L/min	2.04 (0.51)
% expected VO <sub>2</sub> peak, mL/kg/min	72.4 (14.1)
Maximum RER, median (IQR)	1.11 (1.05-1.17)
% age-predicted HRmax	94.5 (8.4)
Maximum effort CPET, n (%)	66 (89.2%)
Time on treadmill, min*	13.71 (3.54)
Functional performance testing	
2-minute step test, steps	80.7 (20.2)
Time to complete 10 chair stands, sec, median (IQR)	21.7 (17.9-25.5)
8-foot up-and-go, sec, median (IQR)	5.46 (4.58-6.30)
30-s arm curl, reps	21.2 (6.4)
Gait speed, m/sec	1.14 (0.23)
Grip strength, kg, median (IQR)	40.7 (34.0-47.3)

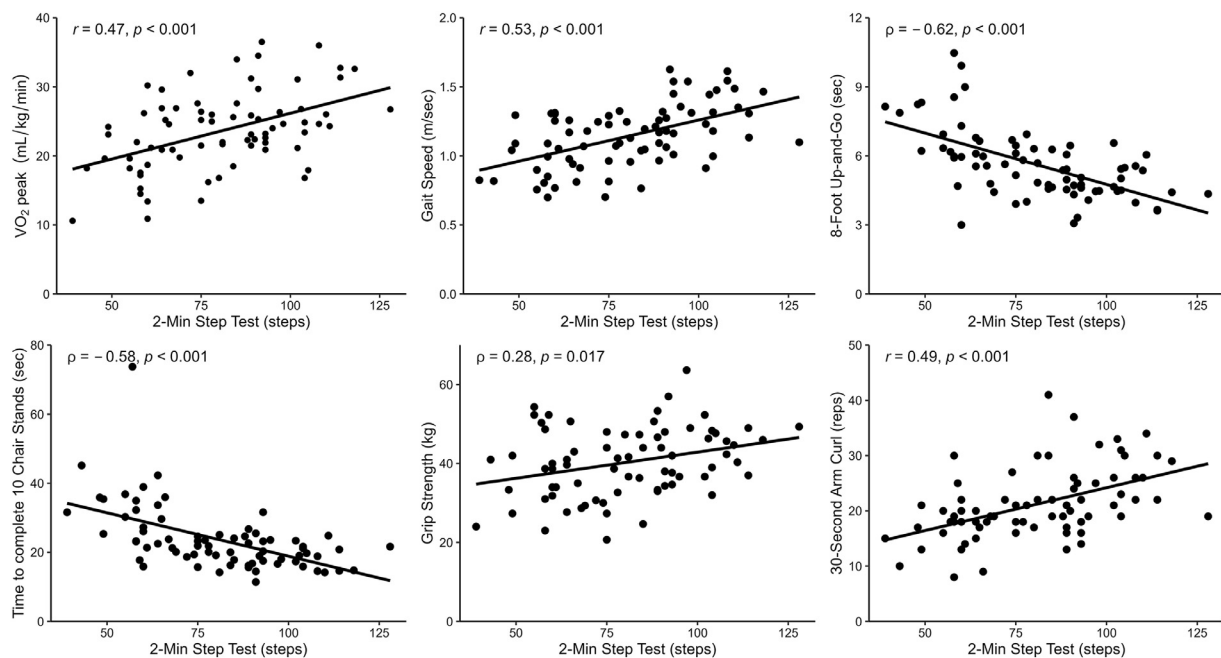
NOTE: Age-predicted HRmax:  $208 - 0.7 \times \text{age}$ ; Expected VO<sub>2</sub>Peak:  $77.96 - (0.92 \times \text{BMI}) - (0.32 \times \text{age})$ .Maximum effort CPET defined as Maximum RER  $\geq 1.05$  or % age-predicted HRmax  $\geq 90\%$ Abbreviations: BMI, body mass index; CPET, cardiopulmonary exercise test; IQR: interquartile range; HR, heart rate; HRmax, maximum heart rate; RER, respiratory exchange ratio; VO<sub>2</sub>peak, peak oxygen utilization.

\* Treadmill duration not included for 1 CPET due to technical issue.

Step count correlated with VO<sub>2</sub>peak ( $r=0.47$ ,  $P<.001$ ) and all functional performance measures (fig 1). When stratified by age group, the correlation was markedly attenuated for age  $<60$  years ( $r=0.002$ ,  $P=.993$ ) and improved for  $\geq 60$  years ( $r=0.52$ ,  $P<.001$ ). There was a significant increase in mean VO<sub>2</sub>peak by step count grouped in tertiles (fig 2). In multivariable linear regression models, step count remained significantly associated with VO<sub>2</sub>peak when adjusted for age and other participant characteristics which were significant at the bivariate level (table 3). In the final model including age and BMI, step count explained 61.8% of the variance in VO<sub>2</sub>peak (table 3) and was used to derive the following equation to estimate VO<sub>2</sub>peak:  $0.082$  (step count)  $-0.426$  (age [years])  $-0.568$  (BMI [kg/m<sup>2</sup>])  $+ 59.309$  with an estimated residual SE of 3.591. Residual plots showed good model fit, with no evidence of multicollinearity (variance inflation factor  $< 1.4$  for all variables). The Bland-Altman plot showed good agreement between the predicted VO<sub>2</sub>peak and the measured VO<sub>2</sub>peak (supplemental fig S2). Heteroscedasticity was not observed using a Breusch-Pagan test ( $P=.19$ ).

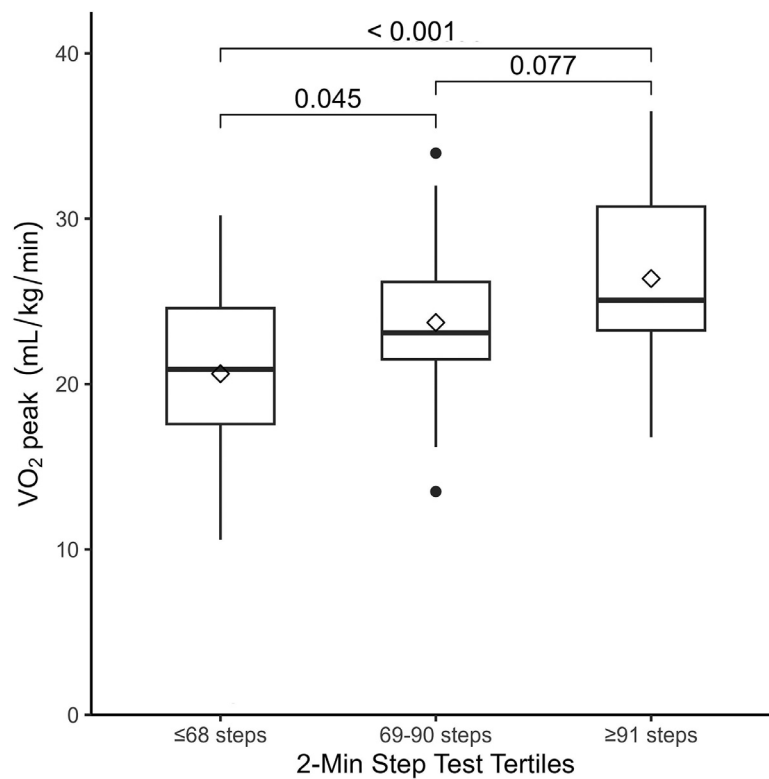
## Discussion

Our findings advance our understanding of the virtual 2MST as a submaximum test to estimate CRF that can be used in telerehabilitation programs with older adults. Our results specifically apply to men  $\geq 50$  years of age with well-controlled HIV, who represent a target patient population for exercise interventions to attenuate accelerated aging. These preliminary data in the 2MST contribute novel



**Fig 1** Association of step count and measures of physical function and cardiorespiratory fitness in 74 older men with HIV. In each scatterplot, step count from the 2-minute step test (2MST) is the independent variable of interest (x-axis) and measures of physical function and cardiorespiratory fitness (VO<sub>2</sub>peak) are included as the dependent variables of interest (y-axis). Bolded lines in each scatterplot represent the fitted line of a bivariate (unadjusted) linear regression model. The Pearson ( $r$ ) or Spearman ( $\rho$ ) correlation coefficients also are reported with  $P$  values, as appropriate. Abbreviation: VO<sub>2</sub>peak, peak oxygen utilization.





**Fig 2** Association of step count and  $VO_2$ peak (mL/kg/min) in 74 older men with HIV. Step count from the 2-minute step test (2MST) was grouped by tertile and presented as a boxplot to compare differences in  $VO_2$ peak. Means are shown as diamonds and medians are depicted as bold lines. The 1st and 3rd quartiles represent the box edges and range is shown by whiskers. Outliers are presented as filled black dots.  $P$  values from pairwise mean comparisons are based on 2-sample  $t$  tests. The  $P$  value for the trend across tertiles is based on an analysis of variance. Abbreviation:  $VO_2$ peak, peak oxygen utilization.

information on use of this functional performance measure among PWH and support further evaluation in larger cohorts including women. Applications in other older adults with chronic disease should be explored as well.

We found a moderate correlation between step count and  $VO_2$ peak in sedentary but clinically stable PWH  $\geq 50$  years of age. Although the correlation was strongest among those  $\geq 60$  years of age, step count remained associated with

$VO_2$ peak independently of age. A systematic review by Bohannon and Crouch<sup>9</sup> of 30 studies which used the Rikli and Jones 2MST protocol concluded that the 2MST was a valid measure of aerobic capacity, based primarily on the 6MWT. We are aware of only 1 study that compares the Rikli and Jones 2MST against CPET results.<sup>18</sup> Węgrzynowska-Teodorczyk et al<sup>18</sup> found that step count was significantly associated with  $VO_2$ peak ( $r=0.33$ ) based on a modified Bruce CPET among 168

**Table 3** Models from multivariable linear regression predicting  $VO_2$ peak (N=74)

Variable	Model 1	Model 2	Model 3	Model 4	Model 5
Step count	0.132 <sup>†</sup>	0.067*	0.082 <sup>†</sup>	0.082 <sup>†</sup>	0.081 <sup>†</sup>
Age (y)	–	–0.401 <sup>†</sup>	–0.426 <sup>†</sup>	–0.426 <sup>†</sup>	–0.420 <sup>†</sup>
Body mass index (kg/m <sup>2</sup> )	–	–	–0.568 <sup>†</sup>	–0.568 <sup>†</sup>	–0.571 <sup>†</sup>
Hypertension history (yes vs. no)	–	–	–	0.017	–
Cigarette smoking history (current/former vs never)	–	–	–	–	–0.374
Constant	12.933	43.291	59.309	59.307	59.369
Model $R^2$	0.222	0.412	0.618	0.618	0.619
Model adjusted $R^2$	0.211	0.395	0.602	0.596	0.597

NOTE: Parameter estimates from linear regression with  $VO_2$ peak (mL/kg/min) as the dependent variable are shown. Multivariable models include step count as the primary independent (predictor) variable. Participant characteristics found significant at  $P < .05$  in bivariate analyses with either  $VO_2$ peak or step count (table 1) were entered stepwise into the model. Variables improving the proportion of the variation explained by the model adjusted for the number of independent variables (adjusted model  $R^2$ ) were retained. Model 3 was used to derive a prediction equation for  $VO_2$ peak given that it produced the highest model adjusted  $R^2$ .

Abbreviation:  $VO_2$ peak, peak oxygen utilization.

\*  $P < .05$ .

<sup>†</sup>  $P < .01$ .

men with heart failure and mean (SD) age of 59 (12) years. Although RER data were not provided, the low mean  $\text{VO}_2\text{peak}$  suggests poor effort on the CPET that could have attenuated the correlation between step count and  $\text{VO}_2\text{peak}$ . However, step count strongly correlated with isometric knee extension strength ( $r=0.61$ ), similar to our finding for timed chair stands and highlighting lower extremity strength as a component of the 2MST. Ricci et al<sup>19</sup> also directly measured  $\text{VO}_2\text{peak}$  but determined step count using a different 2MST protocol, specifically a fixed-height step platform and metronome. Among these 31 young adults with obesity, step count significantly correlated with  $\text{VO}_2\text{peak}$  that was measured by a portable ergospirometry system worn by participants while stepping on the platform for 2 minutes ( $r=0.55$ ).

Our predictive formula for  $\text{VO}_2\text{peak}$  based on modeling of step count includes age and BMI. Although only 60% of the variance in  $\text{VO}_2\text{peak}$  is explained, this represents a small improvement from the equation derived by Ricci et al<sup>19</sup> in younger adults which only included BMI and explained 53% of the variance. Both studies lacked sufficient number of both men and women to include sex as a covariate. Importantly, our results contribute to the body of literature specifically in the Rikli and Jones 2MST protocol for older adults. Further work is needed to determine other factors which minimize the variance in the predictive formula, such as leg strength, and to assess generalizability across a wider range of CRF. Nevertheless, our results support use of the 2MST to estimate  $\text{VO}_2\text{peak}$ .

Various psychometric properties of the 2MST in older adults have been studied. Large studies of younger<sup>20</sup> and older healthy individuals show excellent intrarater and interrater reliability of the 2MST.<sup>8,20,21</sup> Concurrent validity has been shown by comparing performance in 2MST with previously validated tests in healthy older individuals<sup>8,22,23</sup> and different patients populations, predominantly cardiovascular disease<sup>18,21,24-27</sup> but also in patients with diabetes,<sup>17</sup> chronic kidney disease,<sup>28</sup> and joint disease.<sup>29</sup> We present results for the first time in individuals with HIV. A robust effect size for the 2MST has been demonstrated in several center-based exercise interventions, supporting its responsiveness.<sup>9</sup> An important question for further study is whether the percent change in step count from exercise training is comparable to percent change in  $\text{VO}_2\text{peak}$  when increasing CRF is the primary objective.

The 2MST is associated with other measures of functional performance in addition to the chair stand test which we noted earlier and others have reported previously<sup>17,24,25</sup> Our results support prior work showing association of step count with grip strength<sup>24</sup> and timed-up-and-go.<sup>25,27</sup> Our comprehensive functional panel also found that step count is associated the 30-second arm curl and gait speed. These results underscore the interplay between functional measures of fitness, strength, and balance that are captured by the 2MST.

The wide range of mean step counts in the 2MST across different studies is not surprising given the impact of comorbidity<sup>18,28</sup> and increased age.<sup>17,18,23,25</sup> The mean step count ranges from 29.1 among stroke patients to 110.8 among women with osteoporosis.<sup>9</sup> In our study, among participants  $\geq 60$  years of age, the mean (SD) step count of 74.9 (20.0) is similar to men aged 85-89 years (75 [24]).<sup>30</sup> None of the HIV-related characteristics were associated with step count, supporting the 2MST as a benchmark of accelerated aging in

individuals with well-controlled HIV. Among men 60-94 years of age without HIV, cut-off values for the 2MST are available by 5-year age groups to predict independent living, and demonstrate a 38.1% decline in step count over the 30 year age span.<sup>22</sup> Determination of cut-off values for PWH may provide similar benefits as well as identify individuals who would benefit from exercise rehabilitation but otherwise would not qualify based on traditional age-related comorbidity.

With advances in telerehabilitation, there is growing evidence to support the virtual conduct of the 2MST. Results from Berlanga et al<sup>23</sup> in healthy adults  $\geq 65$  years of age showed robust agreement between the 6MWT distance and the step count from a videorecording of the 2MST. Our study further supports validity of the 2MST using live videoconferencing in a center-based setting to estimate  $\text{VO}_2\text{peak}$ . Gero-fit, a clinical exercise rehabilitation program in veterans  $\geq 65$  years of age, supports the feasibility of the virtual 2MST in the telerehabilitation setting.<sup>31,32</sup> A similar telerehabilitation program found that increase in step count was comparable regardless of the modality of the 2MST, virtual versus in-person.<sup>33</sup> However, larger studies are needed to compare testing modalities and to determine if there is a significant difference between center-based and home-based settings.<sup>23,34,35</sup> Notably, our results are based on a real-time virtual 2MST that was conducted in a supervised setting. Accordingly, our results cannot be generalized to the home setting.

## Study limitations

The primary study limitation is the homogeneity of our participants with HIV that limits the generalizability of our findings. Yet, our participants represent a target population for telerehabilitation programs that focus on increasing CRF toward the goal of healthy aging. With this in mind, we chose not to limit the analysis to participants  $\geq 60$  years of age, the age group in most 2MST validation research. Further work is needed to replicate and validate our findings in cohorts with and without HIV that includes women and have a wider age range. Finally, additional clinical and functional factors needed to be identified in order to improve the capacity of step counts to predict  $\text{VO}_2\text{peak}$ . This future research may support inclusion of the 2MST in interdisciplinary medical rehabilitation programs or telemedicine.

## Conclusions

In summary, this study demonstrates the feasibility of the virtual 2MST among older PWH using live videoconferencing in a center-based setting. Results supports the utility of the 2MST as a surrogate measure for  $\text{VO}_2\text{peak}$  in men. Future research is needed in larger, more demographically and clinically diverse cohorts to refine and validate use of the 2MST, virtual and in-person, to predict CRF and to identify step count thresholds which predict clinical outcomes.

## Suppliers

- a. Quark CPET, COSMED

- b. True One, Parvo Medics
- c. R version 4.2.2, R Foundation for Statistical Computing
- d. STATA version 18.0, StataCorp LLC

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## Disclosures

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