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Association between the 6-minute walk test distance and peak cardiorespiratory fitness among people living with HIV varies by fitness level

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People living with HIV (PLWH) develop cardiovascular disease (CVD) earlier and more often than those not infected with HIV (Freiberg et al., 2013). When adjusted for Framingham risk factors, co-morbidities, and substance use, PLWH have a 50% greater risk of acute myocardial infarction (Freiberg et al., 2013). This population also has a higher risk due to HIV-specific factors including antiretroviral medications and chronic immune activation (Duprez et al., 2012).

Cardiorespiratory fitness (CRF) is the ability to transport oxygen to muscles during physical activity; it quantifies an individual's functional capacity and is directly related to the function of critical physiologic systems (e.g., pulmonary ventilation and diffusion, right and left ventricular function, the ability of the vasculature to accommodate and efficiently

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transport blood from the heart to precisely match oxygen requirements, the ability of the muscle cells to receive and use the oxygen and nutrients delivered by the blood, and communicating these metabolic demands to the cardiovascular control center), and is a marker of one's overall health (Ross et al., 2016; Vancampfort et al., 2016). CRF is an important health indicator because it is a stronger predictor of all-cause mortality than traditional risk factors, such as smoking, hypertension, high cholesterol, and type 2 diabetes (Ross et al., 2016). Also, CRF is inversely correlated with the development of different health conditions including CVD (Ross et al., 2016).

The oxygen consumption (VO_2) at peak exercise performance ($\text{VO}_{2\text{peak}}$) is the maximum VO_2 value obtained during an exercise test before VO_2 plateaus, and $\text{VO}_{2\text{peak}}$ quantification is a gold standard for determining CRF; nevertheless, $\text{VO}_{2\text{peak}}$ estimation requires expensive equipment, specially-trained staff, and is time consuming, highlighting the need for a practical fitness assessment that can be routinely used in the clinical setting (Noonan & Dean, 2000; Ross, Murthy, Wollak, & Jackson, 2010). Alternatively, the 6-minute walk test (6MWT) has been used as a substitute for a $\text{VO}_{2\text{peak}}$ measure and provides an estimate of submaximal exercise capacity (Ross et al., 2010). However, only two studies (Beans et al., 2013; Oursler et al., 2009) have analyzed the relationship between $\text{VO}_{2\text{peak}}$ and 6MWT in PLWH; those studies demonstrated moderate correlations between these variables. Moreover, the studies only included men, limiting generalizability of the results.

Vancampfort et al. (2016) recently showed that the CRF levels of PLWH were among the lowest when compared to other vulnerable populations, further highlighting the need for better CRF screening in this population. We investigated whether the 6MWT, as a submaximal measure of CRF, was correlated with $\text{VO}_{2\text{peak}}$ in PLWH. Furthermore, we evaluated the influence of selected anthropometric and physiologic variables on the performance of these tests.

Methods

We analyzed pooled data from participants co-enrolled in two parallel studies (the BOBCAT Study [Webel et al., 2018] and the Active Living and HIV Study [Webel, 2018]), during which the participants completed maximal CRF tests and 6MWTs. All study procedures occurred in an urban setting in northeast Ohio. Our analysis included 27 subjects (19 men, 8 women; ages 52.2 ± 8.3 years; body mass index [BMI] $29.6 \pm 8.2 \text{ kg/m}^2$) living with HIV and taking antiretroviral therapy (ART). The included subjects (a) were at least 18 years of age, (b) had confirmed HIV, and (c) had at least one HIV viral load lower than 400 copies/mL in the past 12 months. Potential subjects were excluded if they: (a) had a medical contraindication for exercise, (b) could not understand spoken English, and (c) were on supplemental oxygen. All subjects signed an informed consent document, and the institutional review board at University Hospitals, Cleveland Medical Center approved the study.

Demographics and HIV Characteristics

Demographic characteristics were assessed using a self-reported survey. Participants also consented to extraction of medical data from their medical records including years living

with HIV, current CD4+ T lymphocyte cell count and viral load, and CD4+ T lymphocyte cell nadir.

Anthropometry

Height was measured to the nearest 0.1 cm by asking participants to stand against a stadiometer platform with their shoes off. After removing everything but a light layer of clothing, the participant stepped on a scale, and weight was measured to the nearest kilogram. BMI was calculated by dividing weight in kilograms by height in meters squared. To measure hip and waist circumference, subjects stood with their feet close together, arms at the side and body weight evenly distributed. Waist circumference was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, using a stretch-resistant tape. Hip circumference was measured around the widest part of the buttocks, with the tape parallel to the floor. Waist and hip measurements were repeated three times, and the median was calculated for the final value.

Cardiorespiratory Fitness

Cardiopulmonary exercise tests were performed using a computer-controlled Lodi bicycle ergometer (Groninger, Netherlands) with a MGC Diagnostics Cardiopulmonary Express system (MGC Diagnostics, St. Paul, MN). A trained investigator performed all of the tests using a 20 watt/minute ramp protocol. We measured CRF using a peak oxygen uptake measure, which was defined as the maximal value of oxygen uptake during the final 30 seconds of exercise. The Wasserman-Hansen equation (Hansen, Sue, & Wasserman, 1984) was used to determine the percent of predicted peak oxygen uptake, which was expressed relative to body weight (mL/kg/min).

6-Minute Walk Test

The 6MWT was conducted according to the American Thoracic Society guidelines (Crapo et al., 2002). Before each test, subjects remained seated for 10 minutes. After this rest period, we obtained the baseline measures, which included blood pressure, heart rate, dyspnea, and overall fatigue using the Borg Perceived Exertion Scale (a relative scale of perceived exertion during physical activity where 6 indicates *no exertion* and 20 indicates *maximal exertion*). After that, participants were instructed to walk as far as possible for 6 minutes, back and forth in a 30-meter long corridor, with the distance marked using cones, without running or jogging. Standard phrases of encouragement were given during the test. After each test, a Research Assistant recorded the postwalk heart rate, exertion levels (using the Borg Perceived Exertion Scale), and the distance covered. To calculate the total distance walked, the number of laps and any additional distance in the final partial lap were recorded, using markers on the wall as distance guides. The total distance was rounded to the nearest meter.

Handgrip Strength

The handgrip strength is commonly used to measure overall muscle strength; it was measured with a hand-held dynamometer (Jamar Technologies, Hatfield, PA). The research assistant followed a script and asked the participant about hand surgery or pain that could

have influenced the results. The subject sat in a chair and rested her/his arm on a table with the elbow on top of a mouse pad and arm held at a right angle. The technician adjusted the grip size of the dynamometer to the subject's hand size and explained how the device worked. Subjects squeezed the dynamometer twice as practice trials. After that, two trials were attempted with each hand, and all results were recorded on a form to the nearest 2 kilograms. The values presented were the mean of the best result of each hand.

Serum Laboratory Measures

Serum studies were used to evaluate cardiometabolic health indicators and inflammation. All participants underwent a 12-hour fasting blood draw at the clinical research unit where a trained phlebotomist drew approximately 20 mL of blood. Serum measures of glucose, insulin, high-sensitivity C-Reactive protein, and cholesterol were analyzed from fresh samples using standard clinical procedures and commercially available assays at the hospitals lab. We used the participants' fasting glucose and insulin measures to calculate the Quantitative Insulin-Sensitivity Check Index (QUICKI) for each individual. Interleukin 6 levels were measured in batch from plasma stored at -80 degrees centigrade using the IL-6 Quantikine HS ELISA Kit (R&D Systems, INC, Minneapolis, MN). All assays were conducted according to the manufactures' instructions.

Statistical Analysis

Data normality was verified using the Shapiro Wilk test. To compare the differences between men and women, Student's t tests and Mann-Whitney U-tests were used for continuous variables, and Fisher's and chi-square tests were used for categorical variables. Pearson correlations were used to analyze the relationship between VO_{2peak} and 6MWT for the entire sample, and for different tertiles of VO_{2peak} . To investigate independent predictors of VO_{2peak} and 6MWT, Pearson and Spearman correlations were used to explore the relationship between different variables and both tests. These variables were included in a stepwise multivariate analysis, with forward selection (F probability to enter .05 and probability to remove .1), to generate a predictive model for VO_{2peak} . Significance was set at $p < .05$, and all statistical tests were performed using SPSS 24.0 (IBM, Chicago, IL).

Results

Participants had been living with HIV for an average of 19.1 ± 9.2 years, had been on ART for 14.0 ± 7.2 years, and had an average CD4+ T lymphocyte cell count of 659.1 ± 430.8 cels/mm³, with no differences between men and women ($p > .05$). Men had a VO_{2peak} of 15.9 ± 4.1 ml/kg/min and a 6MWT distance of 415.1 ± 64.6 meters, while women had a VO_{2peak} of 13.8 ± 4.9 ml/kg/min and a 6MWT distance of 366.8 ± 82.5 meters, with no statistical difference between genders ($p > .05$). The waist-to-hip ratio and handgrip strength were higher in men when compared to women ($p < .05$). No differences were found between groups in BMI and serum blood measures ($p > .05$). See Table 1.

The correlation between VO_{2peak} and 6MWT for the entire sample was $r = 0.53$ ($p < .01$), which is shown in Figure 1. We divided the sample based on tertiles of VO_{2peak} , to analyze the correlation between VO_{2peak} and 6MWT based on the subjects' CRF levels. For

subjects with the lowest CRF levels (1st tertile, VO₂peak = 13.3 mL/kg/min), the correlation was $r = 0.68$ ($p < .05$). As the VO₂peak increased, the correlation became weaker and non-significant.

The correlations between anthropometry, blood markers, VO₂peak, and 6MWT were also examined. Weight ($r = -0.47$), BMI ($r = -0.56$), hsCRP ($r = -0.42$), interleukin 6 ($r = -0.43$) and insulin ($r = -0.67$) were inversely correlated ($p < .05$) with VO₂peak. Moreover, QUICKI ($r = 0.67$) and HDL cholesterol ($r = 0.39$) were positively correlated ($p < .05$) to VO₂peak. Only interleukin 6 ($r = -0.45$) was inversely correlated ($p < .05$) with the 6MWT. A regression model including insulin and hsCRP explained 56.3% in VO₂peak variation, which is shown in Table 2.

Discussion

The main finding of our study was that the accuracy of prediction of a VO₂peak through a 6MWT differed based on the subject's CRF levels. Despite a moderate correlation between the VO₂peak and the 6MWT for the entire sample, the correlation was stronger for subjects with the lowest CRF levels and became weaker as the CRF levels increased. Furthermore, among different anthropometric and physiological variables, insulin levels and inflammatory biomarkers exerted significant influence on VO₂peak.

Although CRF is a risk factor for CVD and all-cause mortality, and is a powerful predictor of mortality risk, it is not routinely assessed in the HIV clinical setting (Ross et al., 2016). Because of the costs involved and the time needed to perform the test, the determination of CRF through gold standard methods is not a practical screening assessment; the participation in a maximal fitness test can also be demanding and stressful for PLWH. Among the less expensive tests that estimate submaximal exercise capacity, the 6MWT has been applied to various populations, including young, elderly, and healthy subjects, and subjects with chronic disease; overall, there are moderate-to-strong correlations (0.49–0.73) between CRF and the 6MWT distance (Ross et al., 2010; Uszko-Lencer et al., 2017). Among PLWH, this relationship was previously examined in two studies of male PLWH in the same age range as in our study, and these studies found moderate relationships of $r = 0.60$ (Oursler et al., 2009) and $r = 0.50$ (Beans et al., 2013). The correlation of our entire sample was between the values found in those studies, but there was considerable variation among subjects of different CRF levels, which may have indicated a ceiling effect of the 6MWT.

Although both tests involve aerobic activities, the 6MWT is a submaximal measure and demands less effort than the VO₂peak determination. PLWH develop CVD more often than the general population (Freiberg et al., 2013) and may have impaired CRF through cardiovascular dysfunction (Oursler et al., 2009), which may influence the performance on a maximal exercise testing, but not in a submaximal. Also, the use of a maximal exercise testing can be limited in populations with chronic diseases because of pain or fatigue, instead of physical exertion (Noonan & Dean, 2000). PLWH have a high prevalence of fatigue and pain that may have influenced the results. Finally, a meta-analysis including more than 1,000 patients with diverse cardiopulmonary disorders derived an equation to

estimate the VO_2peak from 6MWT values, and found that this equation produced a higher standard error of estimate for subjects with higher CRF levels (Ross et al., 2010). The authors, therefore, recommended that this equation not be used for subjects with VO_2 values above 20 ml/kg/min or 6MWT values above 600 meters, because values exceeding this threshold did not follow a linear pattern, and suggested that the comparisons between both tests produced mixed results for subjects with higher CRF levels. Our data support this recommendation.

Elevated levels of hsCRP, an indicator of systemic inflammation and insulin resistance, are highly prevalent in PLWH and are associated with higher cardiovascular morbidity and mortality in this population (Schouten et al., 2014). Inflammation is also associated with functional impairment in PLWH (Erlandson et al., 2013). Consistent with these studies, levels of insulin and inflammation were stronger predictors of CRF in our sample rather than other variables, such as BMI. Others have also suggested that PLWH are predisposed to lower levels of physical fitness through cardiovascular mechanisms (i.e., presence of hypertension), rather than others (i.e., amount of lean body mass; Oliveira, Wiechmann, Narciso, Webel, & Deminice, 2018; Oursler et al., 2009).

Although providing evidence of an important relationship between VO_2peak and 6MWT in PLWH, our study was limited by its small sample size. Our small sample prevented us from analyzing this relationship based on gender and age range and from further examining the influence of relevant variables on the performance of physical fitness tests. Also, we were unable to determine the exact cutpoint of CRF level where the 6MWT would not predict the VO_2peak values in PLWH, which should be determined in additional studies with larger sample sizes.

Conclusion

Our data demonstrated that 6MWT was associated with VO_2peak in PLWH, and that this association was stronger in subjects with low CRF levels. The data supported the feasibility of the 6MWT to measure CRF in this population, but also suggested that the decision to assess fitness via 6MWT or VO_2peak needs to be individualized to the patient. For example, to determine the CRF of a patient who regularly exercises and is likely to walk further than a certain distance, a clinician or investigator should conduct a maximal cardiorespiratory stress test. Also, the results suggested that co-morbidities related to long-term HIV infection were important predictors of CRF in this population, over traditional factors such as BMI.

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Figure 1.

Correlation between oxygen consumption at peak exercise performance (mL/kg/min) and 6-minute walk test (meters) for people living with HIV of different cardiorespiratory fitness levels. *Note.* R^2 = coefficient of determination.

Table 1Demographic, Clinic, and Health Indicators of the Sample ($n = 27$)

	Total ($n = 27$)	Men ($n = 19$)	Women ($n = 8$)
	$M \pm SD$, or n (%)	$M \pm SD$, or n (%)	$M \pm SD$, or n (%)
Age (years)	52.2 $\pm$ 8.3	51.5 $\pm$ 8.7	53.9 $\pm$ 7.4
Body mass index (kg/m ²)	29.6 $\pm$ 8.2	29.1 $\pm$ 7.3	30.7 $\pm$ 10.4
Race (African American/Black)	24 (88.9%)	16 (84.2%)	8 (100.0%)
Years living with HIV	19.1 $\pm$ 9.2	20.2 $\pm$ 9.8	16.6 $\pm$ 7.5
Years on ART	14.0 $\pm$ 7.2	13.6 $\pm$ 7.6	15.0 $\pm$ 6.6
CD4+ T lymphocyte count (cells/mm ³)	659.1 $\pm$ 430.8	549.9 $\pm$ 306.6	918.3 $\pm$ 581.8
CD4+ T lymphocyte nadir (cells/mm ³)	205.6 $\pm$ 153.2	210.1 $\pm$ 168.3	195.1 $\pm$ 119.2
Viral load (undetectable < 20 copies/ml)	17 (63.0%)	11 (57.9%)	6 (75.5%)
Handgrip strength (kg)	66.9 $\pm$ 18.2	74.4 $\pm$ 14.5	49.3 $\pm$ 13.5**
Waist-to-hip ratio	0.96 $\pm$ 0.08	0.98 $\pm$ 0.07	0.91 $\pm$ 0.08*
VO ₂ peak (ml/kg/min)	15.4 $\pm$ 4.4	15.9 $\pm$ 4.1	13.8 $\pm$ 4.9
6-minute walk test distance (meters)	400.7 $\pm$ 72.3	415.1 $\pm$ 64.6	366.8 $\pm$ 82.5
Glucose (mg/dl)	98.2 $\pm$ 24.9	98.8 $\pm$ 28.9	96.9 $\pm$ 12.7
Insulin (ml)	16.9 $\pm$ 11.2	15.7 $\pm$ 8.5	19.8 $\pm$ 16.2
hsCRP (mg/l)	2.2 $\pm$ 1.8	2.3 $\pm$ 1.9	2.1 $\pm$ 1.5
Total cholesterol (mg/dl)	170.1 $\pm$ 42.4	161.1 $\pm$ 40.9	191.4 $\pm$ 40.3
HDL cholesterol (mg/dl)	50.5 $\pm$ 16.2	49.8 $\pm$ 16.9	52.1 $\pm$ 15.2
LDL cholesterol (mg/dl)	93.4 $\pm$ 35.2	86.3 $\pm$ 33.9	110.5 $\pm$ 34.2
Tryglicerides (mg/dl)	130.8 $\pm$ 80.4	125.3 $\pm$ 88.2	143.9 $\pm$ 61.2
QUICKI	0.32 $\pm$ 0.04	0.32 $\pm$ 0.03	0.32 $\pm$ 0.04
Interleukin-6 (pg/ml)	3.6 $\pm$ 3.1	3.9 $\pm$ 3.5	2.5 $\pm$ 0.9

Note.

*
 $p < .05$ for the difference between men and women;**
 $p < .01$ for the difference between men and women;

Data about race and viral load are expressed as absolute (relative) values and other data are expressed as mean $\pm$ standard deviation; VO₂peak = Oxygen consumption at peak exercise performance; ART = antiretroviral therapy; hsCRP = High-sensitivity C-reactive Protein; HDL = High-density lipoprotein; LDL = Low-density lipoprotein; QUICKI = Quantitative Insulin-Sensitivity Check Index.

Table 2

Unstandardized Coefficients From Multiple Linear Regression Predicting Oxygen Consumption at Peak Exercise Performance (ml/kg/min)

Variables	Model 1	Model 2
Insulin (ml)	-0.274 *	-0.282 *
hsCRP (mg/l)	-	-0.987 *
Constant	20.210	22.401
Model R ²	0.426	0.601
Model adjusted R ²	0.400	0.563

Note.

* $p < 0.01$;

hsCRP = High-sensitivity C-reactive Protein; R² = coefficient of determination.