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Relationship of HIV status and fatigue, cardiorespiratory fitness, myokines and physical activity

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Abstract

People living with HIV (PLHIV) experience high rates of fatigue which can be improved with physical activity. We examined the relationship between HIV infection, fatigue, cardiorespiratory fitness, physical activity, and myokines. Twenty HIV-positive and 20 HIV-uninfected adults completed an assessment of fatigue, a maximal cardiometabolic exercise test (VO_2 peak, VE/VO_2 , VO_2 at anaerobic threshold), serum measures of myokines (IL-15, IL-7, FGF-21, adiponectin), and wore an accelerometer for seven days. Measures were completed at baseline, 3 and 6 months. At baseline, PLHIV had more fatigue (4.7 ± 2.6 vs 2.8 ± 2.5 , $p=0.01$) and higher peak VE/VCO_2 (33 ± 5.5 vs. 30.2 ± 2.5 , $p=0.06$). Fifty percent of PLHIV engaged in at least one 10-minute bout of physical activity in the past week, compared to 65% of the control group. Over time, HIV

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infection and FGF-21 were associated with fatigue ($p<0.05$). PLHIV have more fatigue and a higher ventilator efficiency and expression of FGF-21 may be a mechanism underpinning this relationship.

Keywords

HIV; Fatigue; Cardiovascular; Muscle Strength

BACKGROUND

With advances in HIV treatment and care, HIV has transitioned from a fatal to a chronic disease.(Wang et al.) Accordingly, people living with HIV (PLHIV) are aging and 25% of all Americans living with HIV are 55 years or older. (Brooks, Buchacz, Gebo, & Mermin, 2012) They are at an increased risk for cardiovascular disease (CVD), are approximately 4.5 fold more likely to die from sudden cardiac death, (Tseng, Moyers, Secemsky, Havlir, & Hsue, 2013) and twice as likely to have an acute myocardial infarction (Paisible et al., 2015) than those who are HIV uninfected. (Zian H. Tseng et al., 2012) Cardiorespiratory fitness (CRF) is a key determinant of CVD and all-cause mortality but is rarely assessed in clinical practice. (Ross et al., 2016)

Low cardiorespiratory fitness is associated with patient-reported fatigue and as PLHIV age, they experience high levels of fatigue. (Solano, Gomes, & Higginson, 2006) Up to 88% of PLHIV are fatigued, (Barroso & Voss, 2013; Pence, Barroso, Leserman, Harmon, & Salahuddin, 2008) which negatively impacts daily functioning, ability to work, and quality of life. Physical activity can reduce CVD (O'Brien, Tynan, Nixon, & Glazier, 2016; Ross et al., 2016) and is associated with reduced fatigue in PLHIV (Webel et al., 2016). PLHIV in the United States have low levels of physical activity (Willig et al., 2018), which can reduce cardiorespiratory fitness and muscle strength and increase fatigue. Yet we lack data examining the relationship between fatigue, cardiorespiratory fitness, and physical activity over time in PLHIV and a well-matched HIV uninfected control group.

PLHIV have reduced cardiorespiratory fitness (Vancampfort et al., 2016) and muscle strength (Schrack et al., 2016) compared with those without HIV. Cardiorespiratory fitness and muscle strength decline with age, but this decline is accelerated in PLHIV.(Schrack et al., 2016) Cardiovascular and muscle function may be measured in terms of endurance (VO_2 peak) and strength (maximum force). The relationship between these parameters and subjective fatigue in PLHIV is unknown. However, data from other populations demonstrates a relationship between cardiorespiratory fitness, fatigue and muscle strength. Sebastião, Hubbard, Klaren, Pilutti & Moti (2017) reported that among adults with multiple sclerosis, reduced cardiorespiratory fitness and muscle strength were significantly associated with fatigue (Sebastião, et al., 2017).

The relationship between cardiorespiratory fitness, muscle strength, and patient-reported fatigue may be moderated by myokines. (Rowe, Safdar, & Arany, 2014) Myokines are molecules produced in the muscle by physical activity, and can have both local and systemic effects. These include both anti-inflammatory and metabolic effects (Fiuza-Luces,

Garatachea, Berger & Lucia, 2013). PLHIV may have diminished skeletal muscle strength perhaps as a result of reduced physical activity. Physical activity, which involves both dynamic activity (e.g. walking, treadmill) and static (weight) components, typically results in improvements in objectively-measured physiological function. Reduced physical activity in PLHIV may disrupt myokine function leading to increased inflammation and impaired metabolic function, (Handschin & Spiegelman, 2008; Russell, 2005) which are associated with fatigue. (Vina, Sanchis-Gomar, Martinez-Bello, & Gomez-Cabrera, 2012)

The overall goal of this study was to longitudinally examine the effect of HIV status on fatigue, cardiorespiratory fitness, muscle strength, physical activity, and circulating myokines over time. Our specific objectives were: (1) To compare levels of fatigue, cardiorespiratory fitness, muscle strength, and myokines between subjects with HIV on antiretroviral therapy and race-, sex-, and age-matched HIV-uninfected controls; and (2) To examine whether HIV status and myokines are independently associated with fatigue, cardiorespiratory fitness, and strength in this population.

METHODS

Design

This prospective longitudinal study was nested within larger two-group randomized clinical trial testing the effect of a systems-based self-management intervention (Boosting health By Changing AcTivity hereafter referred to as BOBCAT) to improve physical activity and cardiometabolic outcomes in sedentary PLHIV ([NCT02553291](#)). The intervention condition has been previously described but, briefly, it was a group-based intervention teaching behavior change techniques and healthy lifestyle education. The intervention was led by a health educator and taught analytical self-management skills (e.g., self-monitoring and tracking) but also contained social features (e.g., social support activities). In the control condition a research assistant provided information on healthy eating and physical activity using an American Heart Association pamphlet (Webel, 2018). Data were collected at baseline, three, and six months. The intervention did not improve physical activity over the six-month study period. (Webel, 2018)

Sample and Recruitment

Twenty community-dwelling PLHIV and 20 well-matched (by race, sex, and age (± 3 years)) HIV-uninfected control subjects were prospectively recruited and were matched to PLHIV already enrolled in the larger randomized clinical trial testing. HIV-uninfected subjects were recruited via ResearchMatch and via flyers posted in primary care clinics. Interested subjects called a research assistant who conducted a telephone screening. To be included in the study, PLHIV had to meet the following eligibility criteria by being: 1) HIV positive, 2) aged 18 years or greater, 3) on anti-retroviral therapy, 4) virally suppressed (HIV Viral Load less than 400 copies/mL in the past year), 5) at high risk for developing CVD (Framingham 30-year CVD risk score $>20\%$ for females and $>30\%$ for males) and 6) if taking a statin medication, have been on it for at least 6 months. Potential subjects were excluded if they 1) had a medical contraindication for exercise, (Gibbons et al., 1997) 2) met recommendations for weekly exercise of 75 minutes of vigorous physical activity or 150 minutes of Moderate to

Vigorous Physical Activity (MVPA) (Webel et al., 2016) (assessed using the 7-day physical activity recall), 3) could not understand spoken English, 4) expected to move out of the immediate area in the next six months, 5) planned surgery in the next six months, 6) were pregnant or planned on becoming pregnant, 7) were diabetic (hgA1c>7%), or 8) enrolled in a formal weight loss program. Additionally, HIV-uninfected subjects had to match the PLHIV subjects on race, sex, and age ($\pm$ 3years).

Subjects meeting the screening criteria were invited to an in-person baseline visit where a research assistant explained the study's purpose, procedures, risk and potential benefits. Those willing to proceed signed an informed consent document and completed blood draw (to check hgA1c) and, if a woman of childbearing age, a urine pregnancy test. All study procedures were approved by the University Hospitals, Cleveland Medical Center's Institutional Review Board.

Measures

The study was conducted over six months with visits at baseline, 3 months, and 6 months. The fatigue assessments and cardiometabolic exercise testing was performed at baseline and 6 months. All other measures were collected at each of the three time points.

Demographics and Health Characteristics—Subjects completed a self-reported demographic survey assessing gender, race, education and monthly income using the secure, web-based data entry application REDCAP.(Harris et al., 2009) Subjects consented to medical chart abstraction where study staff abstracted medical data including years living with HIV, current CD4+ T cell count, CD4+ T cell nadir, current medications, and current comorbid health conditions.

Fatigue—Subjects completed the self-report *HIV-Related Fatigue Scale*, a psychometrically sound scale measuring the intensity of fatigue. (Barroso, Leserman, Harmon, Hammill, & Pence, 2015; Barroso, M., 2002; Pence et al., 2008) As we were mostly interested in the intensity of subject-reported fatigue, and to minimize participant burden, we administered the 7-item HIV-specific fatigue intensity subscale. Five of the items are scored on a 10-point Likert-style scale (fatigue level today, fatigue level most days, degree of fatigue, severity of fatigue, and distress caused by fatigue) and two were scored on a 4-point scale (fatigue during the past week and changes in fatigue during the past week). No items refer specifically to HIV. All items were scored using standard scoring with higher scores indicating greater fatigue intensity.

Cardiorespiratory Fitness—After completing the surveys, subjects completed a maximal graded exercise test on a bicycle ergometer with continuous monitoring by 12-lead ECG and gas exchange. Tests were performed by the same technician using a computer-controlled Lodi bicycle ergometer (Groninger, Netherlands) with a MCG Diagnostics Cardiopulmonary Express system (MGC Diagnostics, St. Paul, MN). A 20 watt/minute ramp protocol was used with the same trained investigator performing all tests. VO₂ peak was defined as the maximal value of VO₂ during the final 30 seconds of exercise. The Wasserman-Hansen formula (Hansen, Sue, & Wasserman, 1984) was used to determine the

percent of predicted VO_2 peak ml/kg/min. Ventilatory efficiency (VE/VCO_2 slope) was determined by the linear regression slope of the minute ventilation (VE) and VCO_2 . (Sun, Hansen, Garatachea, Storer, & Wasserman, 2002) Anaerobic threshold was manually calculated using the Beaver-Wasserman V-slope method. (Beaver, Wasserman, & Whipp, 1986)

Handgrip Strength—Handgrip strength is a commonly used measure and correlates to overall muscle strength. It was measured using the Jamar hand-held dynamometer. (PhenX Toolkit, 2016) Each subject sat in a chair and rested his or her arm on a table with the arm held at a right angle. A research assistant asked about hand injuries and surgery and then each subject completed two practice trials with each hand. After the practice trials, starting with the right hand, the participant squeezed the dynamometer as hard as possible for three trials. The maximum strength of either hand (all attempts) was recorded as the outcome.

Physical Activity—After completing the study measures, subjects were given an ActiGraph wGT3X-BT triaxial accelerometer (ActiGraph, LLC, Fort Walton Beach, FL) and were taught to wear it during on their hip, all waking hours, except for when showering and swimming, for 7 consecutive days (Anastasopoulou et al., 2014; Berntsen et al., 2010; Strath et al., 2013). Participants were called two days later to ensure they were wearing the accelerometer correctly, address concerns, and to remind them to return it at their follow-up appointment. At that visit, a research assistant checked that the data met the minimum quality standards for valid wear time (at least 4 days and at least 10 hours per day) (Hamilton et al., 2011; Haskell et al., 2012; Hitz, Conway, Palcher, & McCarty, 2014). The accelerometer data were sampled at 30Hz, using 60 second epochs, and the normal filter. (Miguelles et al., 2017) Physical activity was defined as activity ≥ 10 minutes and MVPA was defined as activity ≥ 2690 counts per minute for ≥ 10 minutes. (Caspersen, Powell, & Christenson, 1985) We used the ActiLife software to calculate the amount of time spent in MVPA per valid day using the Sasaki, John, and Freedson (2011) adult cut points for tri-axial accelerometers. (Sasaki, John, & Freedson, 2011)

Myokines—Blood for myokine analysis was collected and stored until it could be analyzed as a batch. We analyzed the following serum myokines based on Fiuza-Luces, et al (2013) review of exercise expressible cytokines including:(Fiuza-Luces, Garatachea, Berger, & Lucia, 2013) inflammatory cytokines (Interleukin 15 and Interleukin 7) and metabolic myokines (FGF-21 and adiponectin). All myokine samples for each time point were done in duplicate, and results were averaged for reporting.

Inflammatory Myokines—We analyzed IL-15 and IL-7 using the Meso Scale Discovery MESO Quickplex SQ 120. The analytes were multiplexed on a custom V-PLEX plate (MSD, K15050D). Serum from each time point and supplied calibrator standards were diluted, incubated, and processed according to the manufacturer's instructions.

Metabolic Myokines—We performed Sandwich ELISAs to measure FGF21 (LSBio, LS-F23399). Serum and the supplied standards were diluted, added to the precoated wells, and incubated according to manufacturer's instructions. Wells were then washed, incubated with TMB substrate, and stopped with supplied stop solution (0.5M HCL) before reading on a

spectrophotometric plate reader at 450nm. We analyzed adiponectin on the MESO Quickplex SQ 120 using a single-PLEX plate (K151BXC-1) using the process described for the inflammatory myokines, above.

Statistical Analysis

We first checked the quality of the data by examining the frequencies and descriptive statistics including the distribution of the outcome measures (fatigue, VO₂ peak, VE/VC0₂, VO₂ peak at Anaerobic Threshold, and hand grip strength). We summarized subjects' baseline characteristics by HIV status using descriptive statistics, *t*-tests, and two-sample Wilcoxon rank-sum tests. Using cross-sectional data from the baseline visit, we compared levels of myokines by HIV status using Wilcoxon rank-sum tests and examined the relationship of myokines to the outcome measures separately by HIV status using Spearman rank correlation. Selected biomarkers were then chosen for further analyses based on the Spearman correlations with fatigue. Generalized estimating equations (GEE) with a Gaussian distribution and unstructured correlation structure were used to examine the relationships between the independent variables of interest (HIV status and selected myokines) with the outcome measures over 6 months.

We used a stepwise approach to develop the GEE models. First, we examined the effect of HIV on our outcomes over six months. Then we adjusted the models for selected myokines, and then all models were adjusted for HIV, myokines, BMI, and age. Finally, an HIV*myokine interaction term was added to the model to see if the myokine-outcome relationship was modified by HIV status.

Though our focus was the magnitude and direction of the group differences, an *a priori p*-value 0.10 was set as statistically significant. This decision is consistent with the 2016 *American Statistical Association* Statement on Statistical Significance and p-Values, with an emphasis on cautiously interpreting findings within the overall context. (Wasserstein, RL & Lazar, NA, 2016) All the analyses were performed using Stata 14.0.

RESULTS

Twenty PLHIV and 20 age-, gender-, and race-matched HIV-uninfected subjects were enrolled in the study. At baseline, their average age was 50.5 (47, 55) years, most were male (58%) and African American (95%). PLHIV had been diagnosed with HIV for approximately 13 (6, 20) years, had been on HIV antiretroviral therapy for approximately 12 (6, 18) years, and had a mean recent CD4+T cell count of 614 (469, 774) cells/μL. Though not a matching factor, approximately 30% of participants in both groups currently smoked (*p*=1.0) (Table 1).

At baseline, PLHIV had significantly more fatigue (mean ± SD 4.7 ±2.6 vs 2.8 ±2.5; HIV vs. control, *p*=0.01; Table 2). Cardiorespiratory fitness as measured by VO₂ peak and the VO₂ at anaerobic threshold were equivalent for both groups. The mean VO₂ peak was 16.85 ±5.8 ml/kg/min for the PLHIV and was 16.84 ±5.9 for the control group (*p*=0.99). The VO₂ at anaerobic threshold was 10.63 ±5.5 ml/kg/min for PLHIV and 10.55 ± 2.9 for the control subjects (*p*=0.95). The mean respiratory exchange ratio for both groups was 1.07 ±0.12,

exceeding the 1.0 criterion for a valid and reliable measure of VO_2 peak (Aspenes, 2011). The peak VE/VCO_2 was modestly higher among PLHIV (33 ± 5.5 vs. 30.2 ± 2.5 HIV vs control, $p=0.06$). At baseline, PLHIV had less hand grip strength (69 ± 24.3 lbs vs 83 ± 29.9 lbs, $p=0.11$).

At baseline 50% of PLHIV engaged in some MVPA, compared to 65% of the matched control group ($p=0.26$). Overall, PLHIV engaged in a median of 51 minutes of MVPA per week compared to 41 minutes for the control group ($p=0.99$). When limited to those who engaged in any MVPA, PLHIV ($n=10$) engaged in a median of 92 minutes of MVPA (IQR: 51, 186) compared to 64 minutes (38, 196) among the control group ($n=13$) ($p=0.58$). We found no associations between physical activity and fatigue at baseline, ($p>0.10$) so we did not include physical activity in our subsequent analyses. No significant differences in myokines between the PLHIV and control group at baseline were found, although PLHIV had higher levels of IL-15 (3.07 vs 2.82 , $p=0.15$) and FGF-21 (138 vs 123 , $p=0.18$) (Table 3).

In our initial analysis of myokines, FGF-21 was negatively correlated with fatigue among both HIV and HIV-uninfected subjects, whereas the associations of FGF-21 with VO_2 peak and VO_2 at anaerobic threshold were in divergent directions among HIV compared to HIV negative subjects (Figure 1). For IL-15, similarly divergent associations were seen for fatigue and, to a lesser extent, VE/VO_2 . Other associations between myokines and outcomes are shown in Figure 1. Given the associations of FGF-21 and IL-15 with fatigue and cardiorespiratory fitness outcomes, as well as the possibly significant HIV interactions, we selected these myokines for further investigation in longitudinal analyses.

In our repeated measures analysis over 6 months, having HIV was positively associated with, and FGF-21 was negatively associated with, subject-reported fatigue in fully adjusted models (p 's <0.10) (Table 4). Furthermore, having HIV was associated with decreased ventilatory efficiency and reduced hand grip strength over time. IL-15 was positively associated with fatigue independent of HIV status in the minimally adjusted model; however, further adjustment for age and BMI attenuated this relationship. There was a trend towards a significant HIV interaction between: (1) fatigue and IL-15, p for interaction= 0.08 , and (2) VE/VO_2 and FGF-21, p for interaction= 0.09 .

DISCUSSION

This is among the first studies to longitudinally examine the relationship between subject-reported fatigue, cardiorespiratory fitness, hand-grip strength and physical activity in PLHIV and well-matched HIV-uninfected control subjects. Compared to the control participants, over six months PLHIV experience more patient-reported fatigue, have a higher ventilator efficiency, and reduced hand grip strength. In contrast to recent systematic reviews, we found that PLHIV and the control group had equally diminished cardiorespiratory fitness.

Over the six-month study period, subject-reported fatigue was significantly higher among PLHIV than among the controls. This was expected, but these data are novel in that this is the first prospective, longitudinal comparison of fatigue between PLHIV and a well-matched

control group. It is notable that fatigue did not increase over time. Among PLHIV, patient-reported fatigue was not associated with cardiorespiratory fitness over time ($p>0.10$) but among control subjects fatigue was associated with VO_2 peak ($p=0.10$) and VO_2 peak at anaerobic threshold ($p=0.01$). Among the control participants, objectively-measured cardiorespiratory fitness levels were associated with subject-reported fatigue levels. However, this relationship was altered in PLHIV suggesting that their fatigue may not be cardiorespiratory in nature. Further, only one myokine, fibroblast growth factor (FGF-21) was associated with reduced fatigue over time. FGF-21 is secreted from skeletal muscle in response to physical activity (Mathai, Bonen, Benton, Robinson, & Graham, 2008) and is a marker of adipocyte function and regulator of glucose metabolism in muscle. (Jung & Kim, 2014) Srinivasa et al (2015) found that PLHIV have elevated levels of FGF-21 compared with HIV uninfected controls. (Srinivasa et al., 2015) Further, Lindgaard et al (2013) found that plasma FGF-21 was elevated in PLHIV compared to HIV-uninfected controls, but that this relationship was more pronounced (8-fold increase) in skeletal muscle FGF-21 mRNA. (Lindgaard et al., 2013) They conclude that FGF-21 may act more potently in a local manner. While no studies could be located that have examined the direct relationship between FGF-21 and subject-reported fatigue, our results indicate that FGF-21 is associated with reduced fatigue in PLHIV. Taken together, these data suggest that further in-depth mechanistic investigations of skeletal muscle expression of FGF-21 on subject-reported fatigue may help identify novel mechanisms underpinning the increased fatigue in this population.

We found poor cardiorespiratory fitness levels among both the PLHIV and HIV-uninfected controls with peak aerobic capacity substantially less than previously published studies. In his systematic review, Vancampfort found that among PLHIV, the average VO_2 peak was 26.4 ml/kg/min and this is among the lowest levels of those living with chronic disease. (Vancampfort et al., 2016) We found substantially lower levels among PLHIV (16.0 ml/kg/ml; IQR 13, 21.3) but that the control group (15.6 ml/kg/ml; IQR 14.1, 17.9) was equally low and the values of both groups were constant over 6 months. Several reasons for this exist. First, we prospectively matched subjects on age, gender and race. Previous investigations in PLHIV have matched on age and gender, but not race. Using race as a matching criteria, we may have controlled for both biological and sociodemographic variables that associate with cardiorespiratory fitness (e.g., stress, poverty, educational attainment). (Pandey et al., 2016; Shishehbor, Gordon-Larsen, Kiefe, & Litaker, 2008) These sociodemographic variables are of particular interest since approximately half of PLHIV in the U.S. are unemployed, significantly higher than the general population. (Worthington, O'Brien, Zack, Mckee, & Oliver, 2012) Unemployment may lead to a more sedentary lifestyle, resulting in cardiorespiratory deconditioning, further impairing their health and daily functioning. We also purposefully enrolled subjects who were at higher risk for CVD: our subjects were older and had elevated BMIs -- variables which reduce the VO_2 peak. Studies in Vancampfort's review tended to have younger, less obese, and therefore likely healthier subjects. However, since both the PLHIV and the controls had low fitness levels these results must be replicated in a larger sample.

PLHIV demonstrated a clinically significant elevation in the ventilator efficiency slope (VE/VCO_2) at peak exercise vs controls over time. Elevations in the VE/VCO_2 slope implies

impairment of CO₂ removal during exercise and is associated with increased mortality in heart failure.(Arena, Myers, Aslam, Varughese, & Peberdy, 2004) Mechanisms responsible for this impairment can include reduced pulmonary perfusion, pulmonary ventilation/perfusion mismatch, decreased cardiac output, elevations of pulmonary artery pressure and uncoupling of the right ventricle-pulmonary artery circuit. (Balady et al., 2010; Hussain et al., 2016) We did not measure hemodynamic parameters that would allow us to assess the etiology of this finding. Yet, this suggests that PLHIV may harbor a burden of subclinical cardiovascular impairment helping to explain their greater CVD and fatigue burden. To our knowledge, reference values of VE/VCO₂ among PLHIV have not been published. Elevated VE/VCO₂ in PLHIV requires further validation in larger longitudinal cohorts to adjudicate its prognostic value.

Muscle strength is important for the health and the ability of adults to function well at home, leisure and at a job; decreased muscle strength is also associated to a higher risk of mortality and hospitalization in older adults. (Cawthon et al., 2009; Newman et al., 2006) We found sustained differences in muscle strength by HIV status over time ($\beta=-14.35$ lbs for PLHIV, $p=0.07$). Terzian et al., (2009) analyzed hand grip in the Women's Interagency HIV Study cohort and found that HIV-infected women had an average hand grip of 63.9 lbs compared with HIV uninfected controls (66.1 lbs).(Terzian et al., 2009) However, these participants were younger (PLHIV, median 41 years; control group, median 38 years). Schrack et al., (2016) found an average hand grip strength among 50 year-old HIV-infected men was 83.5 lbs vs 84.2 lbs among their control group. (Schrack et al., 2016) Taken together, data suggest that HIV-infected women need to engage in more strength exercises to maintain muscle strength.

This study has several important strengths including prospective, longitudinal data collection, use of objective measures of physical activity and maximal cardiorespiratory stress tests, and enrolling a well-matched control group. We also enrolled a largely African-American sample, allowing us to examine a population that is increasingly living, and aging, with HIV. However, we have several limitations. First, this is a small, single site study. Though we found novel, statistically significant differences between groups, it would be prudent to examine our results in a larger, and perhaps multi-site, sample before implementing clinical changes based on our findings. Also, while the BMIs between the groups were not statistically significant, the PLHIV had an average BMI of 26.6 and the control group 30.7, suggesting an important difference. This difference was the reason for including BMI in our GEE models. Our inclusion criteria required that subjects could not meet current U.S. physical activity guidelines. We applied this criterion because we wanted to know more about PLHIV who were at the greatest risk for developing CVD, and would most likely benefit from increased physical activity. However, while this criterion may blunt the overall amount of physical activity participants engage in, it would not affect the differences found between the two groups. Nonetheless, these findings can only be extrapolated to those PLHIV who are not currently meeting exercise guidelines. Finally, our sample size was small for the analyses conducted and along with an a priori p -value <0.10 may lead to incorrect conclusions. This limitation, again, suggests that these novel relationships should be examined in a larger study, longitudinal to verify these results.

CONCLUSION

Using rigorous, objective methods and a longitudinal design, we found that PLHIV have more patient-reported fatigue and that they may have a higher ventilator efficiency reduced hand grip strength, compared with well-matched HIV-uninfected adults. A mechanism underpinning this relationship may include expression of FGF-21. Despite our study strengths, our small sample size coupled with our findings, suggest that further confirmatory research is warranted and likely to help us better understand the drivers of elevated fatigue and cardiovascular risk among PLHIV.

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Figure 1: Spearman Correlations Between Myokines and Outcomes at Baseline

a: *p*-value 0.10; b: *p*-value 0.05; MVPA: Moderate to Vigorous Physical Activity

Table 1:

Subject Characteristics by HIV Status

Variable	HIV+ Adults (n=20)	HIV- Matched ² Adults (n=20) ³	Reference range
	Total	Total	
Age (years) (IQR)	50 (46.5, 54.8)	50.5 (46.3, 54.8)	
Male (%)	11 (55)	11 (55)	
African-American (%)	19 (95)	19 (91)	
Education			
High School Diploma or less (%)	10 (50)	9 (43)	
Some college or technical training (%)	6 (30)	5 (24)	
College Degree (Bachelor's or higher) (%)	4 (20)	6 (29)	
Currently Working (%)	0 (0)	7 (33)	
Perceived Stress Categories			
Low Perceived Stress (%)	7 (35)	7 (33)	
Moderate Perceived Stress (%)	12 (60)	12 (57)	
High Perceived Stress (%)	1 (5)	0 (0)	
Depression Categories			
Minimal Depression (%)	16 (80)	17 (81)	
Mild Depression (%)	2 (10)	2 (10)	
Moderate Depression (%)	1 (5)	1 (5)	
Severe Depression (%)	1 (5)	0 (0)	
Hand Grip lbs	66 (52.3, 91.5)	80 (76, 106.5)	
HIV Characteristics			
Time living with HIV (years)	13 (6-, 1)	n/a	
Time on Antiretroviral Therapy (years)	12.5 (5.8, 18)	n/a	
Current CD4 Count (cells/μL)	676.5 (462, 779.3)	n/a	500–1,500
CD4 Nadir (cells/μL)	107.5 (19, 301)	n/a	Higher is better
Cardiometabolic Characteristics			
Current Smoker (%)	6 (30)	6 (27)	

Variable	HIV+ Adults (n=20) Total	HIV- Matched ² Adults (n=20) ³ Total	Reference range
Body Mass Index (kg/m ²)	26.6 (23.2, 34.6)	30.7 (26.7, 38.3)	18.5–24.9
HgA1C (IQR)	5.4 (5.2, 5.6)	5.8 (3.4, 6.1)	<5.7
HOMA-IR (IQR)	3.0 (1.9, 8.1)	3.8 (1.0, 5.6)	<2.0
Hip Circumference (cm)	106.6 (92.3, 122.3)	111.1 (103, 122)	
Waist/Hip ratio	0.92 (0.88, 0.94)	0.94 (0.86, 1.0)	<0.9 in Men, <0.85 in Women

¹: All variables are reported as medians and IQR unless otherwise noted;

²: Participants were matched on age ($\pm$ 3 years), sex, and race

³: There were no statistically significant differences between the two groups ($p>0.10$)

Table 2:

Fitness and Exercise Parameters by HIV Status¹

Time point	Fatigue Level	VO ₂ peak ml/kg/min	VE/VCO ₂	VO ₂ at anaerobic threshold ml/kg/min	Hand grip (lbs)	Moderate-to-Vigorous Physical Activity (minutes per week)	Steps per day
HIV+ Subjects (n=20)							
Baseline	4 (2.3, 7)	16 (13, 21.3)	32 (30, 35)	9.6 (7.3, 12.8)	66 (52.3, 91.5)	51.0 (0, 146.1)	6094.4 (3509.9, 9580.3)
3 months	n/a	n/a	n/a	n/a	67 (54.3, 82)	27.1 (0, 62.3)	4620.1 (3594.1, 6032.9)
6 months	3.5 (1, 6)	15.7 (14.5, 19.2)	31.5 (29, 34.8)	10.7 (7.3, 13.1)	66.5 (53.8, 89.5)	14.2 (0, 118.1)	5219 (3397.4, 7096.4)
HIV-Subjects (n=20)							
Baseline	1.5 (1.0, 5.3)	15.6 (14.1, 17.9)	30 (28, 32)	10.2 (9.0, 12.4)	80 (70, 106.5)	40.6 (0, 133)	7080.6 (4742.4, 8305.4)
3 months	n/a	n/a	n/a		83.5 (74.5, 105.3)	50 (10.6, 87)	6599 (5015, 9740.6)
6 months	3 (1, 4.8)	16.2 (11.7, 18.0)	30 (28, 33)	10.1 (8.3, 13.0)	83 (45.8, 95.5)	19 (0, 106.0)	7511.8 (5257.3, 9199.3)

¹: All variables are reported as medians and IQR

Table 3:

Baseline Myokines HIV Status¹

Pathway	Variable ¹	Mechanisms	HIV+ Adults (n=20)	HIV- Matched Adults (n=20)	Difference ²
Inflammatory	IL-15	Secreted by skeletal muscle and is upregulated in exercise and inflammation. Stimulates expression of contractile proteins and subsequent muscle cell hypertrophy (Guo et al. 2016). Animal models suggest IL-15 promotes myogenesis and may protect against the inflammation-mediated skeletal muscle atrophy which occurs in sarcopenia and cachexia (O'Leary et al. 2017). It can improve glucose homeostasis in skeletal muscle and liver, and improve myofibre phenotypes towards a more oxidative pattern (Duan et al. 2017).	Total 3.07 (2.83, 3.32)	Total 2.82 (2.32, 3.25)	0.15
	IL-7	It plays a central and critical role in the homeostasis of the immune system; IL-7 participates in human T cell development and homeostatic proliferation, generation of CD4 ⁺ and CD8 ⁺ and is essential for mouse B cell development (Nguyen et al. 2017).	20.5 (13.1, 25.4)	17.99 (14.2, 19.8)	0.66
Metabolic	FGF-21	Increases glucose uptake, modulates lipid metabolism, and sensitizes insulin response in metabolically active organs. It is synthesized mainly in the liver, but it is also expressed in adipose tissue, skeletal muscle, and many other organs. Its concentration in plasma is increased in patients with obesity, insulin resistance, and metabolic syndrome, to protect the tissues from an increased oxidative stress environment. It is considered a stress-responsive hormone in humans (Gómez-Sámano et al. 2017).	138.0 (94.7, 194.7)	123.0 (94.7, 157.4)	0.18
	Adiponectin	An adipokine with anti-inflammatory, antioxidant, antiatherogenic, pro-angiogenic, vasoprotective and insulin-sensitizing properties (Staiger et al. 2017). It is positively associated with lower extremity fat and negatively correlated with truncal fat. Regulates mitochondrial biogenesis and improves lipid metabolism in skeletal muscle (Li et al. 2016).	1.17 × 10 ⁷ (7581998, 1.64 × 10 ⁷)	1.21 × 10 ⁷ (1.3 × 10 ⁷ , 1.37 × 10 ⁷)	0.39

¹: Data presented as median and Interquartile Ranges;

²: Add differences between the HIV-infected group and the HIV-uninfected group were conducted using a Wilcoxon rank-sum test

Table 4:

The Effect of HIV on Fatigue, Fitness and Hand Grip Strength Over Time using Generalized Estimating Equations ($n=37$)

	HIV Status		Myokines and HIV		Fully adjusted	
	β	p-value	β	p-value	β	p-value
Fatigue (0 “no fatigue” to 10 “high fatigue”)						
HIV+	1.34	0.023	1.79	0.004	1.48	0.018*
FGF-21			-0.003	0.001	-0.002	0.002*
IL-15			0.025	0.047	0.020	0.160
Age (years)					-0.056	0.308
BMI (kg/m ²)					-0.081	0.044*
VO ₂ peak (ml/kg/min)						
HIV+	-0.309	0.844	-0.216	0.900	-1.77	0.228
FGF-21			-0.002	0.275	-0.002	0.327
IL-15			-0.031	0.205	-0.040	0.110
Age (years)					-0.114	0.374
BMI (kg/m ²)					-0.426	<0.001*
VE/VCO ₂ ratio						
HIV+	1.833	0.102	2.640	0.013	2.651	0.015*
FGF-21			0.001	0.626	0.001	0.622
IL-15			0.006	0.786	0.006	0.787
Age (years)					0.014	0.885
BMI (kg/m ²)					0.001	0.985
VO ₂ at Anaerobic Threshold (ml/kg/min)						
HIV+	-0.162	0.883	-0.259	0.817	-0.893	0.413
FGF-21			-0.002	0.293	-0.001	0.359
IL-15			-0.009	0.708	-0.018	0.472
Age (years)					-0.033	0.722
BMI (kg/m ²)					-0.159	0.023*
Hand Grip Strength (lbs)						

	HIV Status		Myokines and HIV		Fully adjusted	
	β	p-value	β	p-value	β	p-value
HIV+	-0.393	0.959	-10.64	0.174	-14.35	0.069*
FGF-21			0.002	0.749	0.00	0.814
IL-15			0.096	0.440	0.079	0.493
Age (years)					-0.413	0.551
BMI (kg/m ²)					-0.939	0.044*