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Atypical Skeletal Muscle Profiles in Human Immunodeficiency Virus-Infected Asymptomatic Middle-Aged Adults

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Background. Human immunodeficiency virus (HIV)–infected individuals are at increased risk of age-associated functional impairment, even with effective antiretroviral therapy (ART). A concurrent characterization of skeletal muscle, physical function, and immune phenotype in aviremic middle-aged HIV-infected adults represents a knowledge gap in prognostic biomarker discovery.

Methods. We undertook a prospective observational study of 170 middle-aged, HIV-infected ambulatory men and women with $CD4^+$ T-cell counts of at least 350/μL and undetectable plasma viremia while on effective ART, and uninfected control participants. We measured biomarkers for inflammation and immune activation, fatigue, the Veterans Aging Cohort Study mortality index, and physical function. A subset also received a skeletal muscle biopsy and computed tomography scan.

Results. Compared to the uninfected participants, HIV-infected participants displayed increased immune activation ($P < .001$), inflammation ($P = .001$), and fatigue ($P = .010$), and in a regression model adjusting for age and sex displayed deficits in stair-climb power ($P < .001$), gait speed ($P = .036$), and predicted metabolic equivalents ($P = .019$). Skeletal muscle displayed reduced nuclear peroxisome proliferator-activated receptor-γ coactivator 1α–positive myonuclei ($P = .006$), and increased internalized myonuclei ($P < .001$) that correlated with immune activation ($P = .003$) and leukocyte infiltration ($P < .001$). Internalized myonuclei improved a model for HIV discrimination, increasing the C-statistic from 0.84 to 0.90.

Conclusions. Asymptomatic HIV-infected middle-aged adults display atypical skeletal muscle profiles, subclinical deficits in physical function, and persistent inflammation and immune activation. Identifying biomarker profiles for muscle dysregulation and risk for future functional decline in the HIV-infected population will be key to developing and monitoring preventive interventions.

Clinical Trials Registration. NCT03011957.

Keywords. HIV; physical function; skeletal muscle; impairment; internalized myonuclei.

Life expectancy has dramatically increased in patients with human immunodeficiency virus type 1 (HIV-1) infection due to effective antiretroviral therapy (ART) [1]. However, despite effective therapy, inflammation and immune activation remain elevated compared to their uninfected counterparts and, as the HIV-infected population ages, there is an increased risk for functional limitations that impinge on quality of life [2] and are predicted to significantly burden healthcare resources [3].

In the pre-ART era, muscle wasting was a serious complication of HIV-1 infection, characterized by a significant (and rapid) loss in lean body mass and loss in functional capacity. By contrast, with effective ART, functional limitations and impairment can occur, but generally without a dramatic loss in muscle mass [4], suggesting a potential shift in the epidemic from a quantitative loss in muscle mass to a qualitative loss in muscle function that is therefore unlikely to be fully explained by previously observed wasting mechanisms in HIV-1 infection. Recent studies have focused on drug regimens associated with skeletal muscle mitotoxicity [5], but have been limited by the absence of concurrent data on immune status, functional capacity, and simultaneous skeletal muscle phenotyping.

To evaluate skeletal muscle health and the associated risk for future impairment in currently asymptomatic individuals in the context of effective ART, we undertook an observational prospective study in Boston, Massachusetts, of aviremic and asymptomatic HIV-infected, middle-aged (50–65 years) ambulatory men and women and compared them to an uninfected control group of men and women in the same age range. We measured multiple health modalities and herein report their baseline peripheral inflammation and immune activation, physical function, and skeletal muscle quality and composition. We also report levels of fatigue and risk for all-cause mortality, based on self-report and the Veterans Aging Cohort Study (VACS) index, respectively.

METHODS

Study Design and Population

The MATCH (Muscle and Aging in Treated Chronic HIV) study is a prospective observational study conducted at Brigham and

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Women's Hospital in Boston, Massachusetts. The protocol was approved by the Partners Human Research Committee. Written informed consent was obtained from all participants. Men and women were recruited from the Boston metropolitan area, with 170 participants enrolled from April 2015 through October 2016. To be eligible for study entry, participants had to meet the following criteria: 50–65 years of age, live in the Boston metropolitan area, have sufficient lower extremity mobility to undertake functional assessment, be HIV negative (HIV^-) (nonreactive to HIV-1/2 antigen/antibody fourth-generation test, Quest Diagnostics) or HIV-infected (HIV^+) on effective ART with no detectable virus (≤200 copies/mL, confirmed by HIV-1 RNA quantitative real-time polymerase chain reaction, Quest Diagnostics), and have a CD4 count ≥350 copies/μL (Quest Diagnostics). Participants with acute illness in the past 60 days or use of anabolic therapy or corticosteroids within the past 6 months were excluded.

Clinical and Laboratory Evaluations

At study entry, participants completed a baseline assessment that included a questionnaire measuring self-reported fatigue (Functional Assessment of Chronic Illness Therapy–Fatigue [FACIT-F]) [6]. The VACS index was determined for all participants based on a blood chemistry panel (Quest Diagnostics) and lymphocyte subset panel (Quest Diagnostics), including subject age, CD4 cell count, viral load, hemoglobin level, and renal and hepatic biomarkers [7]. Data on the amount of time since HIV diagnosis, current ART regimen use and other medications, and hepatitis C virus and cytomegalovirus (CMV) coinfection were also collected. Peripheral blood samples were used for the measurement of inflammatory biomarkers (C-reactive protein [CRP], interleukin 6 [IL-6], soluble CD14 [sCD14], soluble CD163 [sCD163]) and flow cytometry assessment of immune activation biomarkers (CD8, CD38, human leukocyte antigen – antigen D related [HLA-DR]). A subset of healthy HIV^- and HIV^+ subjects who were enrolled until the target sample size was met received a muscle biopsy from the mid-thigh (vastus lateralis) for histology and immunohistochemistry and/or a computed tomography (CT) scan of the mid-thigh for muscle and fat cross-sectional area (CSA). Baseline physical function assessment included walking distance and gait speed, leg strength and power, and a loaded stair-climb power test.

Statistical Analysis

Baseline characteristics of circulating biomarker levels were compared between groups and were adjusted for age and sex using a *t* test or Mann-Whitney *U* test, when distribution was normal or nonnormal, respectively. Differences in the levels of circulating biomarkers were measured independently and as composite scores. This approach allows for more complex characterization of outcomes in studies with smaller sample sizes [8]. The composite scores were calculated based on quartiles

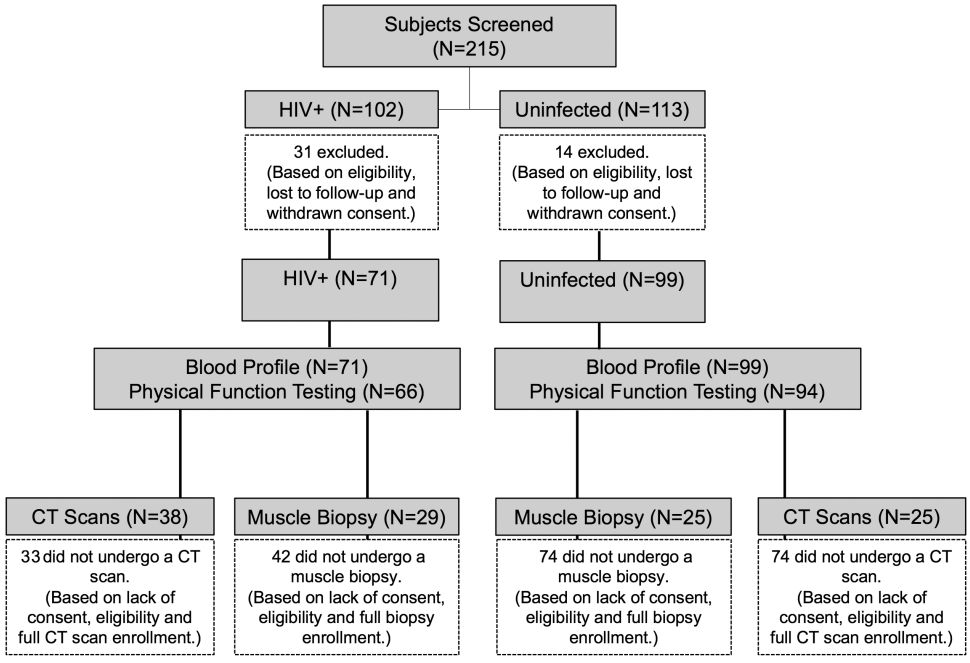

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram of study enrollment and characterization in the Muscle and Aging in Treated Chronic human immunodeficiency virus (HIV) (MATCH) cohort. The baseline characterization period was between April 2015 and August 2016. A total of 215 adult men and women from the Boston metropolitan area, between the ages of 50 and 65 years, consented. A total of 170 participants (71 HIV^+-infected and 99 uninfected) were found eligible and enrolled. Participants were excluded from the analyses if they were outside of the target age range, had detectable viremia (>200 copies/mL), had a CD4 count <350 cells/μL, were nonambulatory, were taking anabolic agents, or had a recent infection. Participants were excluded from the biopsy and computed tomographic (CT) scan procedures based on lack of consent, medical ineligibility, and closure of enrollment.

of expression for each biomarker (Supplementary Methods), similar to prior studies [9]. Physical function assessment and skeletal muscle profiling were evaluated, based on HIV status. Mean and standard deviation or median and interquartile range were provided for normally and nonnormally distributed data, respectively. We considered logarithmic, square root, or Box-Cox transformations for outcomes that were not normally distributed. Additionally, age- and sex-adjusted linear regression models were employed to assess their potential confounding effect. Bivariate associations between HIV status and continuous variables were tested by Pearson or Spearman correlations. A multivariate logistic regression model was used to determine biomarker profiles associated with HIV status. The area under the receiver operating characteristic (ROC) curve was employed to assess the ability of a biomarker's profile to distinguish between groups and to evaluate the relative impact of internalized myonuclei as a discriminating biomarker for HIV status. Validation of our results was performed using a resampling technique [10] that generates unbiased optimism-adjusted bootstrap estimates of the area under the ROC curve.

All hypotheses were tested using a 2-sided α level of .05. Statistical analyses were performed using SPSS version 20 (IBM SPSS, Chicago, Illinois) and SAS version 9.3 (SAS Institute, Cary, North Carolina) software.

RESULTS

Study Participants

Two hundred fifteen adult men and women from the Boston metropolitan area, between the ages of 50 and 65 years, were screened for participation in the MATCH study. A total of 170

Table 1. Characteristics of Study Participants With Comprehensive Profiling of Muscle and Blood Biomarker Subset

Characteristic	HIV$^+$ (n = 29)	HIV$^-$ (n = 25)	*P* Value
Sex, male, No. (%)	22 (75.9)	14 (56.0)	.123
Age, y	57.0 (54.0–61.0)	56.0 (54.0–60.0)	.869
Race/ethnicity, No. (%)			
White	11 (37.9)	21 (84.0)	<.001
Black	17 (58.6)	3 (12.0)	<.001
Hispanic	1 (3.4)	1 (4.0)	
BMI, kg/m^2	25.0 (23.3–30.1)	27.8 (23.9–30.4)	.461
CD4$^+$ count, cells/μL	695 (523–769)	885 (605–1113)	.037
CD8$^+$ count, cells/μL	755 (623–1188)	345 (274–428)	<.001
Years since HIV diagnosis	19.3 ± 7.9	NA	NA
VACS index	23.0 (22.0–33.0)	22.0 (12.0–28.0)	.083
FACIT-F fatigue score	44.5 (39.0–48.0)	50.0 (48.5–51.0)	.010
HCV infection, No. (%)	9 (31.0)	0	.002
HMG-CoA reductase inhibitor use, No. (%)	14 (48.3)	3 (12.0)	.005
Composite immune activation score[a]	1.34 ± 1.17	0.32 ± 0.63	<.001
CD8$^+$HLA-DR$^+$CD38$^+$, %	1.57 (1.00–2.07)	0.76 (0.57–1.18)	<.001
CD8$^+$CD38$^+$, %	3.38 (2.76–4.31)	2.86 (1.61–3.64)	.034
CD8$^+$HLA-DR$^+$, %	8.04 (6.12–14.3)	4.00 (2.42–5.96)	<.001
Composite inflammatory score[a]	1.21 ± 1.05	0.40 ± 0.76	.001
CRP level, μg/dL	1.08 (0.61–3.67)	1.17 (0.91–1.65)	.952
IL-6 level, pg/mL	0.97 (0.68–1.36)	0.99 (0.56–1.50)	.573
sCD14, ng/mL	2.34 (2.04–3.23)	2.39 (2.13–2.79)	.788
sCD163, ng/mL	0.47 (0.35–0.64)	0.37 (0.28–0.47)	.036
Skeletal muscle profile	(n = 29)	(n = 25)	
Slow type I fiber, %	41.4 (36.5–49.0)	45.6 (36.6–52.9)	.353
CSA of type I fibers, ×1000 μm^2	7.46 (4.63–9.74)	5.44 (4.23–7.24)	.109
Fast type II fiber, %	58.6 (51.0–63.5)	54.4 (47.1–63.4)	.353
CSA of type II fibers ×1000 μm^2	5.66 (4.30–8.40)	4.26 (3.63–6.72)	.105
Collagen index, % of CSA	16.0 (14.1–18.6)	15.4 (12.2–18.1)	.435
Internalized nuclei, % of myofibers	4.38 (2.61–6.96)	2.07 (0.92–3.19)	<.001
Nuclear PGC1α, % of total nuclei	45.1 (27.4–85.4)	80.6 (70.2–92.6)	.006
PAX7$^+$ cells, % of total nuclei	1.68 (0.97–2.22)	1.85 (1.44–2.64)	.185
CD45$^+$ cells, % of total nuclei	0.61 (0.24–1.18)	0.53 (0.28–0.79)	.735

Normally distributed variables are expressed as mean ± standard deviation or median and interquartile range. Continuous variables were compared by *t* test or by Mann-Whitney *U* test, when distribution was normal or not normal, respectively.

Abbreviations: BMI, body mass index; CRP, C-reactive protein; CSA, cross-sectional area; FACIT-F, Functional Assessment of Chronic Illness Therapy–Fatigue; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen – antigen D related; HMG-CoA, hydroxy-methylglutaryl-coenzyme A; IL-6, interleukin 6; NA, not applicable; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α; sCD14, soluble CD14; sCD163, soluble CD163; VACS, Veterans Aging Cohort Study.

[a]Arbitrary units (No. ± standard deviation).

asymptomatic and generally healthy individuals (71 HIV$^+$ and 99 HIV$^-$; 100 men and 70 women) living in the Boston area were determined to be eligible and enrolled. The 54 participants who received a skeletal muscle biopsy for histology (29 HIV$^+$, 25 HIV$^-$) and 63 who received a mid-thigh CT scan (38 HIV$^+$, 25 HIV$^-$) (Figure 1) were enrolled until the sample size of at least 25 participants in each group was met. Self-reported fatigue based on the FACIT-F subscale [6], was significantly higher in HIV$^+$ participants, both in the skeletal muscle biopsy subgroup (P = .010; Table 1) and the total cohort (P < .001; Table 2). ART regimen use reflected current treatment guidelines (Supplementary Table 1).

Immune Profiles

Immune activation was determined based on CD8$^+$ T-cell expression of HLA-DR and CD38 using flow cytometry. Each biomarker was evaluated independently and as part of a composite score. Consistent with prior studies [11–13], HIV$^+$ participants had higher levels of immune activation (Tables 1 and 2; Figure 2). Circulating inflammatory biomarkers were measured in serum and also evaluated independently and as a composite score. Consistent with prior studies [11], HIV$^+$ participants had a higher composite inflammatory score compared with uninfected participants (Tables 1 and 2; Figure 2). Notably, IL-6 levels did not differ based on HIV status in the biopsy subset (P = .573) or total cohort (P = .574), in contrast with prior studies [14, 15]. The VACS index for all-cause mortality [16] was higher in HIV$^+$ participants compared to uninfected participants, both as a trend in the biopsy subgroup (P = .083; Table 1) and significantly in the total cohort (P = .017; Table 2).

Physical Function Assessment

Metrics for physical function were chosen to capture a large range in performance and to minimize potential ceiling effects [17]. When compared to the HIV$^-$ controls, the HIV$^+$ participants displayed significant, albeit subclinical, deficits in physical function (Tables 3 and 4). We assessed HIV infection as a predictor and physical function as an outcome using a linear regression model with adjustment for age and sex and revealed lower median values with significant negative associations for stair-climb power (β = –.18, P < .001), gait speed (β = –.60, P = .036), and predicted metabolic equivalents [18] (β = –.04, P = .019) (Table 3). Leg power and strength were not significantly lower in HIV$^+$ participants, even when analyzed as subgroups based on sex (Table 4).

Table 2. Characteristics of Total Cohort

Characteristic	HIV$^+$ (n = 71)	HIV$^-$ (n = 99)	*P* Value
Sex, male, No. (%)	49 (69)	51 (51)	.022
Age, y	56.0 (52.0–60.0)	59.0 (54.0–62.0)	.004
Race/ethnicity, No. (%)			
White	33 (46.5)	77 (77.8)	<.001
Black	35 (49.3)	20 (20.2)	<.001
Hispanic	3 (4.2)	2 (2.0)	.444
BMI, kg/m^2	26.4 (23.5–30.9)	27.9 (23.9–32.1)	.257
CD4$^+$ count, cells/μL	695 (553–841)	846 (681–1118)	<.001
CD8$^+$ count, cells/μL	719 (585–1016)	353 (260–467)	<.001
Years since HIV diagnosis	19.5 (12.5–25.0)	NA	NA
FACIT-F score	44.0 (36.5–48.5)	49.4 (45.0–51.0)	<.001
VACS index	22.0 (18.0–34.0)	22.0 (12.0–28.0)	.017
Mean ± SD	25.9 ± 11.6	21.9 ± 9.2	
HCV infection	13 (18.3)	0	< .001
HMG-CoA reductase inhibitor use	26 (39.4)	9 (9.6)	<.001
CD8$^+$HLA-DR$^+$ CD38$^+$, %	1.49 (0.94–2.01)	0.68 (0.51–1·13)	<.001
CD8$^+$CD38$^+$, %	3.21 (2.33–4.26)	2.79 (1.71–3.86)	.038
CD8$^+$HLA-DR$^+$, %	7.38 (4.5–11.9)	3.37 (2.21–5.97)	<.001
Composite immune activation score[a]	1.20 ± 1.08	0.43 ± 0.72	<.001
CRP level, μg/dL	1.71 (0.70–5.77)	1.19 (0.56–2.65)	.019
IL-6 level, pg/mL	1.23 (0.77–1.97)	1.14 (0.72–1.89)	.574
sCD14, ng/mL	2.58 (2.11–3.4)	2.32 (1.95–2.70)	.006
sCD163, ng/mL	0.47 (0.35–0.70)	0.35 (0.27–0.49)	<.001
Composite inflammatory score[a]	1.40 ± 1.12	0.75 ± 0.99	<.001

Normally distributed variables are expressed as mean ± SD or median and interquartile range. Continuous variables were compared by *t* test or Mann-Whitney *U* test, when distribution was normal or not normal, respectively.

Abbreviations: BMI, body mass index; CRP, C-reactive protein; FACIT-F, Functional Assessment of Chronic Illness Therapy–Fatigue; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen – antigen D related; HMG-CoA, hydroxy-methylglutaryl-coenzyme A; IL-6, interleukin 6; NA, not applicable; sCD14, soluble CD14; sCD163, soluble CD163; SD, standard deviation; VACS, Veterans Aging Cohort Study.

[a]Arbitrary units (No. ± SD).

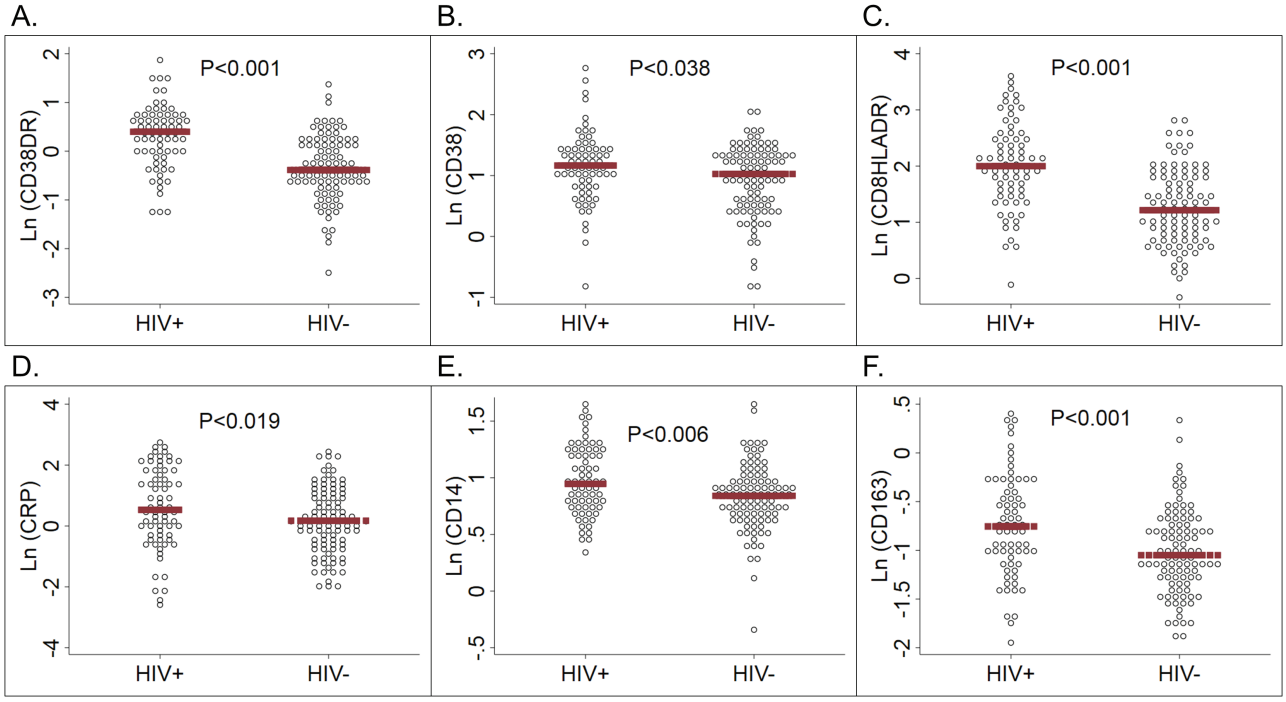

Figure 2. Immune activation and circulating inflammatory biomarkers. Data were plotted as natural log (Ln) vs human immunodeficiency virus (HIV) status. The Mann-Whitney *U* test was used to compare the 2 groups. Bars represent medians. $P < .05$ was considered statistically significant. Immune activation was based on flow cytometric expression of human leukocyte antigen – antigen D related (HLA-DR) and CD38 (*A*), CD38 (*B*), and HLA-DR (*C*) on CD8 T cells. Circulating inflammatory biomarkers were measured in serum including C-reactive protein (CRP; *D*), soluble CD14 (sCD14; *E*), and soluble CD163 (sCD163; *F*).

Skeletal Muscle Characterization

Computed tomographic scans of the mid-thigh were performed in 63 participants (38 HIV^+, 25 HIV^-) and did not differ based on HIV status (Figure 3). Further analysis based on Hounsfield unit (HU) thresholds for fat (–190 to –30 HU), low-density muscle (0–34 HU), high-density muscle (35–100 HU), and total CSA (–190 to 100 HU) also did not differ (Supplementary Table 2), suggesting an absence of quantitative loss in muscle mass. However, consistent with prior studies [19] (Supplementary Figure 1), an overall correlation (independent of HIV status) was observed in anterior quadriceps muscle CSA with leg strength and power, and stair-climb power.

Skeletal muscle composition based on fiber type (ie, type I slow-twitch and type II fast-twitch) and size distribution have been previously reported to change with advancing age [20]. To address potential differences in fiber type composition and size distribution based on HIV status, we performed 54 biopsies (29 HIV^+, 25 HIV^-) and utilized immunohistochemistry to assess slow and fast myosin heavy chain expression and size distribution. As shown, there were no apparent differences in men and women when evaluated based on HIV status (Figure 4; Table 1).

Skeletal muscle myonuclei are normally localized on the periphery of the myofiber [21]. Examination of histological sections revealed a significantly increased frequency of mispositioned, internally located myonuclei in HIV^+ participants compared with uninfected participants ($P < .001$; Table 1; Figure 5). The frequency of internalized myonuclei was linearly correlated with immune activation based on expression of HLA-DR on $CD8^+$ T cells ($r = 0.396$, $P = .003$) and with $CD45^+$ cells in skeletal muscle ($r = 0.489$, $P = .0004$). Notably, internalized nuclei did not correlate with any inflammatory biomarkers (Supplementary Table 3). Because the frequency of $CD45^+$ cells in skeletal muscle did not differ based on HIV status ($P = .735$), it is unlikely that the elevated internalized myonuclei reflect an inflammatory myopathy. An association was also observed between internalized myonuclei and years since HIV diagnosis ($P = .007$; Supplementary Table 3).

Table 3. Multivariate Linear Regression Model for Physical Function Based on HIV Status

Functional Outcome	Sample Size. No.	Coefficient (β) for HIV Status (95% CI)	*P* Value
Best loaded SCP, watts	156	–0.18 (–.27 to –.08)	<.001
Gait speed, m/sec	160	–0.60 (–.12 to –.01)	.036
Predicted MET	157	–0.04 (–.08 to –.01)	.019
Leg power, watts	158	–0.07 (–.17 to .03)	.182
Leg press, 1 RM, newtons	158	–0.03 (–.10 to .4)	.420

Multivariate linear regression model for physical function outcomes based on HIV status, adjusted for sex and age.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; m/sec, meters per second; MET, metabolic equivalents; SCP, stair-climb power; RM, repetition maximum.

Table 4. Lower Extremity Physical Function Assessment

Total Sample Set	Male (n = 96)			Female (n = 64)		
	HIV⁻ (n = 49)	HIV⁺ (n = 47)	*P* Value	HIV⁻ (n = 45)	HIV⁺ (n = 19)	*P* Value
Best loaded SCP, watts	470.37 (379.92–522.54)	435.08 (328.81–532.64)	.074	337.89 (292.86–392.57)	263.69 (231.64–380.76)	.005
Gait speed, m/sec	1.51 (1.31–1.80)	1.48 (1.37–1.67)	.235	1.5 (1.37–1.63)	1.32 (1.09–1.58)	.025
Predicted MET	3.02 (2.79–3.33)	2.92 (2.69–3.14)	.026	3.23 (3.00–3.40)	3.14 (2.75–3.36)	.154
Leg power, watts	897.5 (695.5–1035.7)	849.55 (699.2–1028.9)	.341	535.2 (411.1– 655.15)	500 (346.6–625.1)	.230
Leg press, 1 RM, newtons	2253.3 (1881.15–2512.1)	2261.9 (1923.9–2571.9)	.364	1442.9 (1270.15–1700.75)	1412.6 (1256– 1735.6)	.423

Data are shown as median and interquartile range. The *t* test was used to compare natural log-transformed outcomes for the HIV⁺ vs HIV⁻ groups. $P < .05$ was considered statistically significant.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; m/sec, meters per second; MET, metabolic equivalents; SCP, stair-climb power; RM, repetition maximum.

Mitochondrial activity declines with aging [22], and deficits have been identified in association with HIV infection [5]. We therefore chose to evaluate mitochondrial bioenergetics by measuring the frequency of muscle myonuclei that were positive for peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), a master regulator of mitochondrial biogenesis [23]. HIV⁺ participants displayed a significant reduction in the frequency of PGC-1α–positive myonuclei compared with uninfected participants ($P = .006$; Table 1; Figure 5; Supplementary Figure 2), suggesting a deficit in bioenergetic capacity. However, there was no evidence for gross mitochondrial pathology based on histologic staining for ragged-red fibers using a Gömöri trichrome stain (data not shown).

The frequency of internalized myonuclei was not influenced by the use of lipid-lowering medications (statins), the participant's race, or seropositivity for CMV, although CMV seropositivity was significantly more common in the HIV⁺ MATCH participants (data not shown). Additionally, Pax-7 expression and collagen type I expression did not differ, indicating there was no apparent deficit in satellite cells or evidence for muscle fibrosis (Table 1), in contrast to a previous report that assessed subjects with more advanced HIV infection [24].

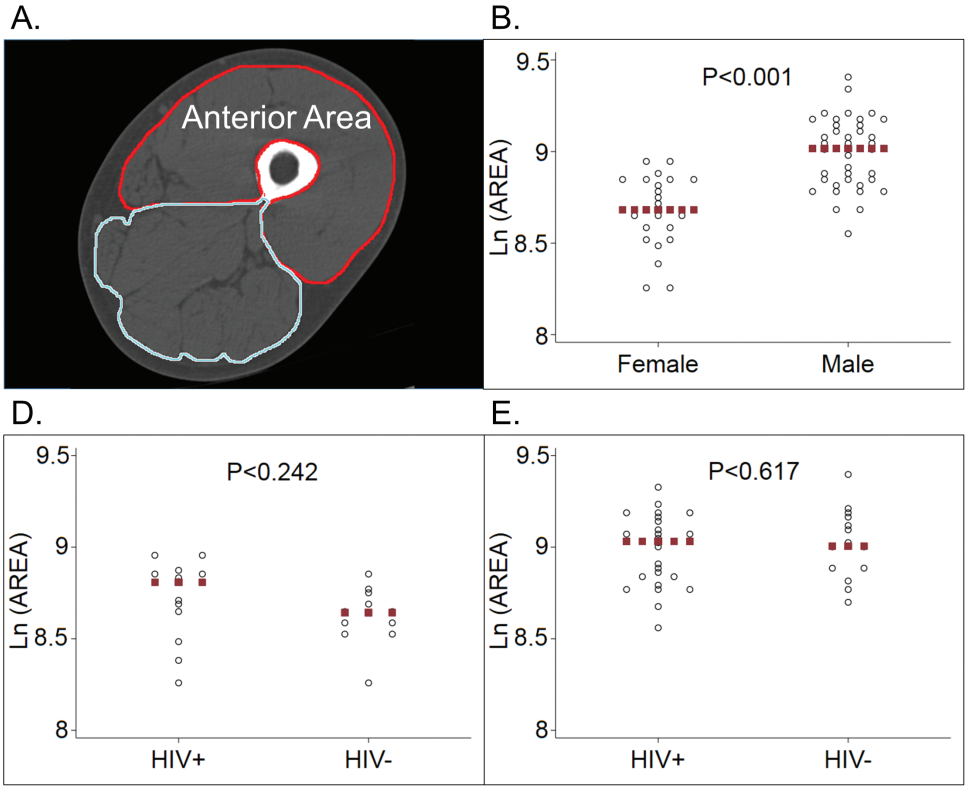

Figure 3. Cross-sectional area (CSA) of mid-thigh muscle. Data were plotted as natural log (Ln) vs human immunodeficiency virus (HIV) status. Bars represent medians. The Mann-Whitney *U* test was used for between-group comparisons. Computed tomographic images of mid-thigh muscle were analyzed using ImageJ software. *A*, Anterior CSA measurements (Hounsfield units [HU] from –190 to 100) based on sex (*B*) and HIV status of women (*C*) and men (*D*) are shown.

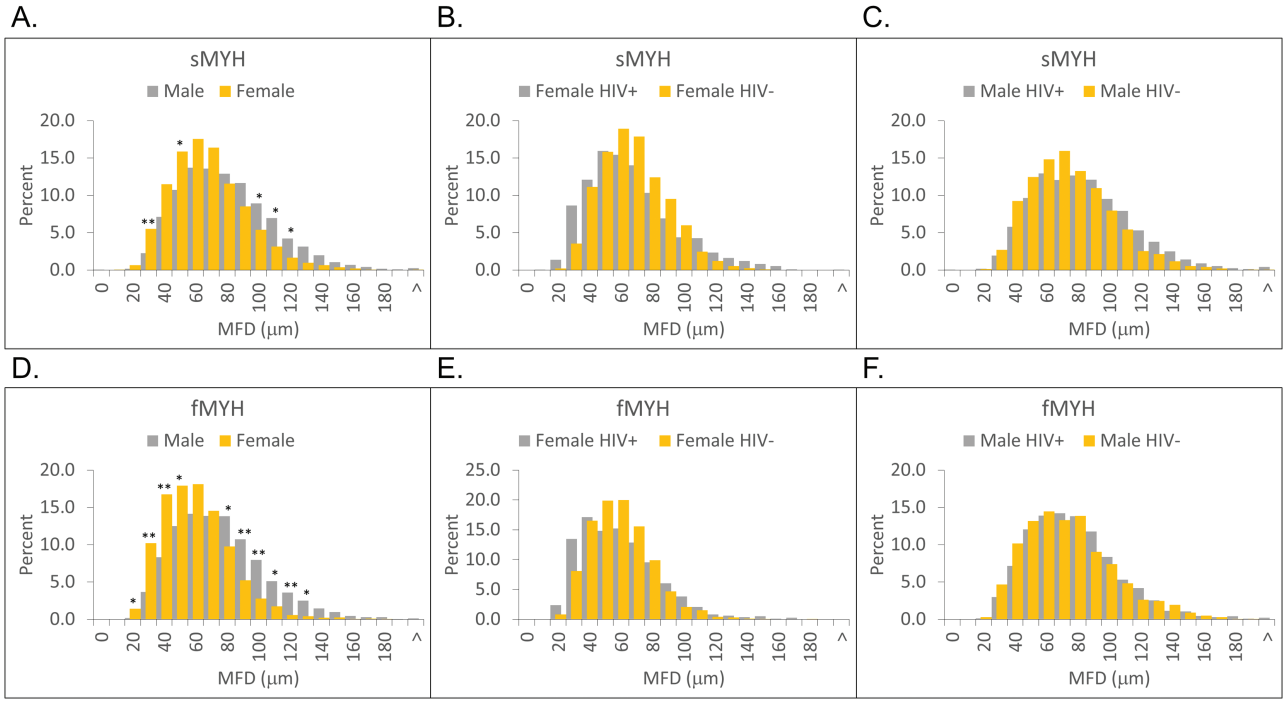

Figure 4. Muscle fiber type and size distribution based on sex and human immunodeficiency virus (HIV) status. The unpaired *t* test was used to compare the 2 groups within each bin. **P* < .05; ***P* < .01. Muscle sections were stained with slow-twitch type I myosin heavy chain (MYH) and laminin. Images were taken with the ×20 objective lens in a grid of 15 × 10. Myofibers were analyzed with the image analysis software Cell Profiler. Laminin signals were used to outline myofibers. Fibers with a MYH median intensity <0.3 and those ≥0.3 were assigned as type II fast-twitch (fMYH) and type I slow-twitch (sMYH) fibers, respectively. The distribution of myofiber size (minimum Feret diameter [MFD], defined as the minimum distance of parallel tangents at opposing borders of the muscle fiber [60]) is shown based on sex (*A* and *D*; 36 male and 18 female) and HIV status of female (*B* and *E*; 7 HIV^+ and 11 HIV^-) and male (*C* and *F*; 22 HIV^+ and 14 HIV^-).

Association of Internalized Nuclei With HIV Status

Whether internalized myonuclei was a useful discriminator for HIV infection was assessed by first using univariate (Supplementary Table 4), and then multivariate (Supplementary Tables 5 and 6) logistic regression modeling. Logistic regression using the best-performing inflammatory and immune activation biomarkers (Supplementary Table 5) or composite scores (Supplementary Table 6) supported a relatively good discrimination of HIV as an outcome (C-statistic = 0.85 or 0.84, respectively). Notably, composite scores were significantly associated with HIV status (odds ratio [OR], 2.83 [95% confidence interval {CI}, 1.20–6.65] and OR, 3.35 [95% CI, 1.48–7.61], for inflammation and immune activation scores, respectively; Supplementary Table 6). After inclusion of internalized myonuclei as a variable, the performance of the model based on the C-statistic increased from 0.84 to 0.90 (Figure 6). Internalized myonuclei as a variable was statistically significant independent of composite immune activation and inflammatory scores (OR, 3.14 [95% CI, 1.25–7.85]; *P* = .015).

DISCUSSION

This observational study describes a cohort of asymptomatic middle-aged HIV-infected men and women with no evidence for loss in skeletal muscle mass, but that nevertheless have elevated inflammation and immune activation, elevated self-reported fatigue, emerging deficits in physical function, and on muscle biopsy display atypical decreases in skeletal muscle nuclear PGC-1α and elevated internalized myonuclei that are more often seen in elderly uninfected subjects. Previous studies have reported that HIV^+ adults experience functional decline more rapidly than expected for their age, with frailty phenotypes occurring at earlier ages [2]. Although these studies have been informative, they have been limited by the absence of concurrent data on skeletal muscle, immune profiles, self-reported fatigue, and physical function assessment.

Aging is associated with a seemingly inexorable decline in muscle mass [25], characterized by a notable decline in leg muscle CSA, muscle fiber size, and number [26]. While none of these features were evident in our MATCH cohort, we did, however, observe atypical skeletal muscle cellular profiles suggesting that HIV infection is associated with asynchronous muscle aging. When present, internalized myonuclei can indicate a response to acute injury [27] and accumulate with natural aging, based on reports in healthy adults ≥70 years old [28] and in aged mice [29]. The increased frequency of internalized myonuclei and the reduction in nuclear PGC-1α observed in these middle-aged, currently asymptomatic HIV^+ adults may reflect premature features of skeletal muscle aging.

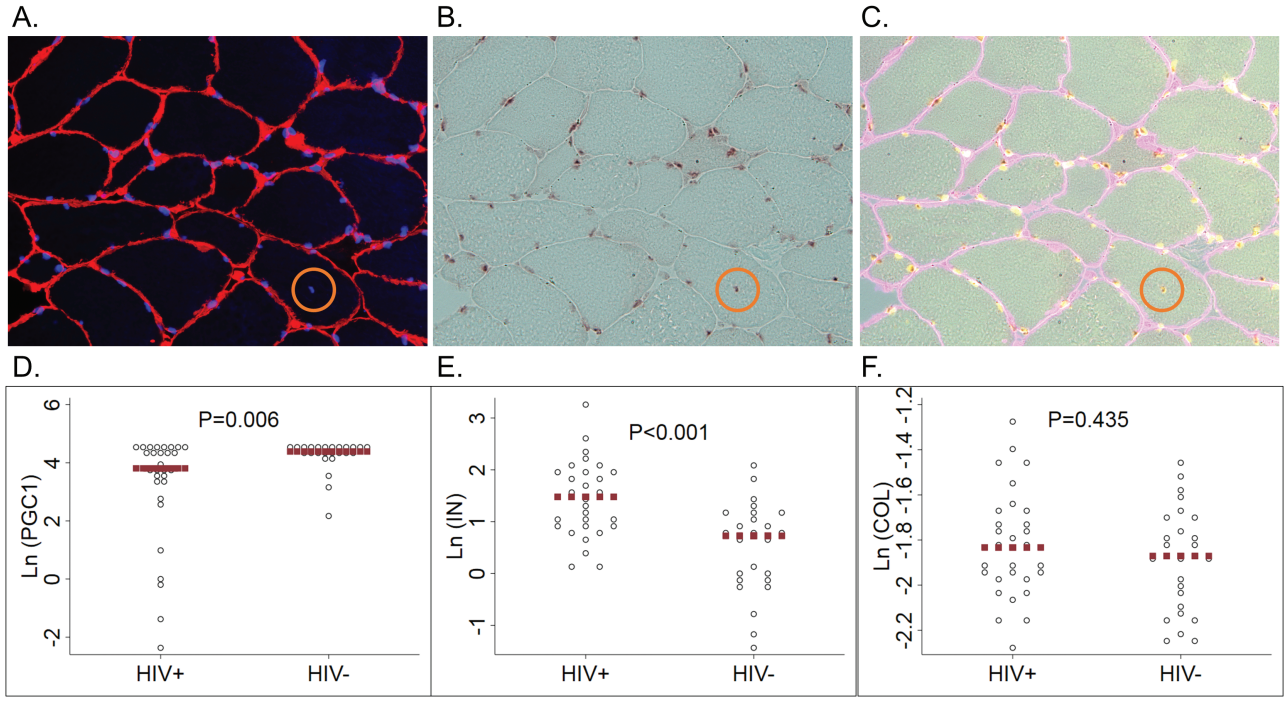

Figure 5. Skeletal muscle profiles based on peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) localization, internalized nuclei, and collagen. The Mann-Whitney *U* test was used to compare the 2 groups. $P < .05$ was considered statistically significant. *A–C*, All muscle fibers were fluorescently stained (*A*) with type I collagen (red), Hoechst (blue), and PGC-1α (reddish-brown) stained with peroxidase (*B*). *C*, Images were merged showing collagen (magenta), Hoechst (yellow), and PGC-1α (reddish-brown) with evident of internalized myonuclei in skeletal muscle of human immunodeficiency virus–infected (HIV^+) and –uninfected (HIV^-) Muscle and Aging in Treated Chronic HIV (MATCH) participants (orange circles). Measurements of nuclear PGC-1α (*D*), internalized myonuclei (*E*), and collagen (*F*) are shown. Bars represent medians; 29 HIV^+ and 25 HIV^-. Representative muscle fibers from HIV^+ participants are shown.

In contrast with the pre-ART era, an association between an elevated frequency of internalized myonuclei and asymptomatic HIV infection in the current era of effective ART has not been described and quantified [30]. Internalized myonuclei can be a nonspecific feature of muscle disorders, as well as natural aging, making the etiology of the internalized myonuclei currently unclear. Because internalized myonuclei may be an outcome associated with chronic exposure to antiviral drugs, future studies defining ART regimen may be informative. The observed reduction in nuclear PGC-1α may suggest compromised aerobic capacity despite a lack of gross mitochondrial or muscle pathology (based on the absence of ragged-red fibers and fiber type and size distribution).

An increased risk for reduced exercise capacity has been linked to elevated internalized myonuclei and to loss in PGC-1α –dependent gene expression [31]. Mitochondrial DNA mutations have been previously described in skeletal muscle of HIV^+ adults on early regimens; although those studies did not include physical function assessment [5]. Future studies comprehensively evaluating mitochondrial bioenergetics, exercise capacity, and intolerance should be informative. As PGC-1α affects neuromuscular junction (NMJ) activity [32], future studies will be needed to determine potential associations between NMJ activity and the atypical skeletal muscle phenotype we are observing in chronic HIV infection.

Cross-talk between immune cell subsets and skeletal muscle tissue is essential for normal muscle maintenance, including for example, muscle cross-talk with monocytes [33], B cells [34], and CD8 T cells [35]. Therefore, a more comprehensive

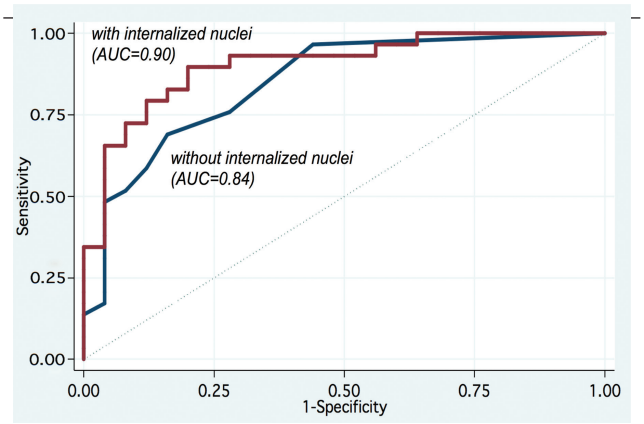

Figure 6. Receiver operating characteristic (ROC) curves associated with human immunodeficiency virus (HIV) infection. Curves are based on multivariate logistic regression models of the prediction of risk with the use of composite scores for inflammation and immune activation with or without internalized myonuclei. The area under the ROC curve (AUC) was used to assess internalized myonuclei as a discriminating biomarker for HIV status. As a primary analysis, we considered models with composite immune activation score and inflammation score as biomarkers with and without internalized myonuclei.

evaluation of this cross-talk will be needed to better define mechanisms underlying dysregulation of immunomyogenic homeostasis in chronic HIV infection.

Prior studies have reported that HIV$^+$ adults experience functional decline that is atypical for their age [2, 36] and, although not a universal finding [13], may be associated with inflammation [12, 15]. In our MATCH cohort, the HIV$^+$ participants displayed elevated CRP, sCD14, and sCD163, but not IL-6, suggesting that this cohort is less advanced in the disablement process, especially given our observation of gait speeds >1.3 meters per second. The complex interaction(s) between comorbid conditions (eg, inflammation, immune activation, myopathy, fatigue) underscore the need for risk indices based on multisystem dysfunction, analogous to existing tools for mortality (ie, VACS index) and frailty [36, 37].

This study extends prior reports of elevated fatigue observed in symptomatic adults with detectable viremia [6] to now include asymptomatic HIV$^+$ middle-aged individuals with undetectable viremia. This raises the concern that, despite the absence of significant differences in muscle mass or CSA, there may be an ongoing accumulation of subclinical deficits.

Persistent inflammation and immune activation are established features of chronic HIV infection [38] and effectively discriminate HIV status and comorbid risk [39]. When we considered a cross-platform biomarker profile consisting of inflammation and immune activation, self-reported fatigue (FACIT scores), and all-cause mortality (VACS index), we observed that inclusion of internalized myonuclei increased HIV discrimination (Figure 6; Supplementary Tables 5 and 6).

This study has several limitations. First, the cohort in this study is small (N = 170) and may not be generalizable to all middle-aged HIV$^+$ adults. In addition, although we identified a clear correlation between internalized myonuclei in skeletal muscle and immune activation, we could not detect associations with physical function. This may reflect the healthy status of the participants and currently low penetrance of the muscle phenotype at this time. Longitudinal follow-up with expanded functional assessment may be informative. The muscles sampled (eg, vastus lateralis) may also not fully reflect the functional tests. Additional physical function assessments and biopsy sampling from other muscle groups may be informative. Factors such as current and prior ART regimen use—in particular exposure to early-generation, more toxic therapies—were not fully considered, although in a recent study that included a comparison of nonfrail HIV$^+$ adults with uninfected adults, there were no associations between physical function and differing ART drug use or in total testosterone levels [15]. An ongoing follow-up study assessing prior ART regimen use in MATCH and potential effects on skeletal muscle may be informative.

Prior studies in uninfected adults have reported that age-dependent loss in strength can decline disproportionately to loss in muscle mass [40], indicating that change in muscle mass and strength are not linearly related. Follow-up will be needed to determine the timing and magnitude of loss of muscle mass and its relation to loss in function with aging HIV$^+$ adults. Identifying biomarkers for future impairment that can guide strategies for preventing functional decline in this vulnerable population will likely assume greater importance over time.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. M. M. formulated the research question. M. M., T. T., V. G., T. S., A. A. A., and P. E. S. contributed to study design, interpretation of analysis results, and writing of the article. T. T., V. G., T. S., B. B., B. M., E. H., J. M., and E. W. generated the data. K. M. P. and Z. L. did the statistical analyses. All authors contributed to the content and revisions of the manuscript.

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