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Inflammation, Immune Activation, Immunosenescence, and Hormonal Biomarkers in the Frailty-Related Phenotype of Men With or at Risk for HIV Infection

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Background. The extent to which inflammation, immune activation/immunosenescence, and hormonal abnormalities are driven by human immunodeficiency virus (HIV) or frailty is not clear.

Methods. HIV-infected frail men (n = 155) were matched to nonfrail, HIV-infected (n = 141) and HIV-uninfected (n = 150) men by age, calendar year, and antiretroviral therapy use (HIV-infected men only). Frailty was defined by ≥3 frailty-related phenotype criteria (weight loss, exhaustion, low activity, slowness) at ≥2 visits, or at 1 visit with ≥1 criteria at ≥2 visits. The following measurements were obtained: interleukin 6, high-sensitivity C-reactive protein, soluble receptors for tumor necrosis factor α 1 and 2, the percentages of $CD4^+CD28^-$, $CD8^+CD28^-$, $CD4^+CD38^+HLA\text{-}DR^+$, and $CD8^+CD38^+HLA\text{-}DR^+$ T cells, dehydroepiandrosterone sulfate, free testosterone, homeostatic model assessment of insulin resistance, and insulin-like growth factor 1. Log-linear regressions were adjusted for a priori selected covariates to determine differences by frailty and HIV status.

Results. In multivariate analyses adjusted for covariates, frailty was associated among HIV-infected men with higher interleukin 6 and high-sensitivity C-reactive protein and lower free testosterone and dehydroepiandrosterone levels. In contrast, HIV infection but not frailty was associated with significantly greater immune senescence (percentage of $CD4^+CD28^-$ or $CD8^+CD28^-$ T cells) and immune activation (percentages of $CD4^+CD38^+HLA\text{-}DR^+$ and $CD8^+CD38^+HLA\text{-}DR^+$ T cells).

Conclusions. Frailty among HIV-infected men was associated with increased inflammation and lower hormone levels, independent of comorbid conditions. Interventions targeting these pathways should be evaluated to determine the impact on prevention or reversal of frailty among HIV-infected men.

Keywords. frailty; HIV; aging; inflammation; hormones.

Frailty is a syndrome characterized by an increased vulnerability to stressors in the face of limited physiologic reserve [1]. A frailty phenotype was defined by Fried et al [2] as impairment in ≥3 of 5 domains: physical slowness, fatigue, low activity, weakness, and physical shrinking. Other authors have used a model of an accumulation of deficits to define frailty, or have modified the original phenotype described by Fried et al, using a combination of objective and subjective measures of health to fit the population or available data [3]. Regardless of the specific definition, frailty has been associated with increased healthcare costs and utilization, poor outcomes after surgical procedures, increased need for skilled nursing care among other outcomes, and ultimately, an increased risk of death [4].

A similarity between the syndrome of frailty and AIDS was recognized early in the human immunodeficiency virus (HIV) era [5]. The occurrence of a frailty-related phenotype was later reported in the Multicenter AIDS Cohort Study (MACS), wherein the highest proportion of the frailty-related phenotype was among persons with low $CD4^+$ T-cell counts or those not receiving antiretroviral therapy (ART) [6, 7]. A subsequent MACS study demonstrated that the frailty-related phenotype was associated with lower AIDS-free and overall survival, even among persons who achieved HIV-1 virologic suppression [8]. A frailty index developed from self-reported data in the Veterans Aging Cohort Study (VACS) was likewise predictive of hospitalization and mortality rates [9]. These phenotypes are different than the original frailty definition offered by Fried et al [2] in that they include subjective measures only and lack extensive validation; however, their association with mortality suggests that both are valuable measures of vulnerability to stressors.

Both frailty and HIV infection are associated with heightened inflammation, hormonal abnormalities, and impairments in the immune system [10, 11]. The extent to which these factors are driven by HIV infection or frailty, particularly among

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virologically suppressed HIV-infected individuals, is less clear. A better understanding of the underlying mechanisms that drive frailty can inform the most appropriate and efficient pathways to target for prevention and treatment of frailty. The goal of the current study was to describe differences in levels of inflammatory, immune, and hormonal markers by frailty status among persons with HIV infection (ie, comparing HIV-infected men with or without frailty) and by HIV serostatus without the influence of frailty (ie, comparing nonfrail HIV-infected and HIV-uninfected men). We hypothesized that both frailty and HIV infection would be associated with abnormalities across all 3 domains.

METHODS

Study Population

The MACS is a prospective study of the natural and treated history of HIV infection among men who have sex with men in the United States, with sites in Baltimore, Maryland–Washington, DC; Chicago, Illinois; Pittsburgh, Pennsylvania; and Los Angeles, California. Enrollment of 6972 participants occurred during 3 time periods: 1984–1985, 1987–1990, and 2001–2003. At each semiannual study visit, physical examinations were performed, questionnaires administered, and blood samples collected for laboratory testing and storage. Self-reported use of antiretroviral medications was summarized at each visit to define prior and current use of ART. Informed consent was obtained from each participant, and approval was provided by each local institutional review board.

Selection of Cases and Controls, by Exposure Status

This analysis employed a matched study design of HIV-infected men without AIDS (defined as no history of an AIDS-defining illness [12]) and HIV-uninfected men in the MACS. The exposure of interest was frailty, defined as follows. First, the frailty-related phenotype was summarized by 4 self-reported subjective measures [6] describing weight loss, exhaustion, low activity levels, and slowness. Weight loss was defined as self-reported unintentional weight loss of ≥10 lb (4.54 kg) since the previous visit. Other criteria were derived from the Short-Form (SF)-36: exhaustion was defined as answering yes to the question: "During the past 4 weeks, as a result of your physical health, have you had difficulty performing your work or other activities (for example, it took extra effort)?" Low activity was defined as feeling "limited a lot" in response to: "Does your health now limit you in vigorous activities, such as running, lifting heavy objects, participating in strenuous sports?" Slowness was defined as feeling "limited a lot" in response to: "Does your health now limit you in walking several blocks?" HIV-infected men were considered frail if they had either (1) ≥2 visits meeting ≥3 frailty-related phenotype criteria or (2) 1 visit meeting ≥3 criteria and 2 subsequent visits meeting 1–2 criteria. The index visit (ie, study entry) for each case was the first visit at which frailty was identified. HIV-infected and HIV-uninfected controls with no history of meeting any frailty-related phenotype criteria were matched to each case patient by age within 3 years and calendar year of visit. Among HIV-infected participants, controls were also matched at the index visit by the reported use of HIV therapy, categorized as highly active ART (HAART), non-HAART therapy, or no ART. The definition of HAART was guided by the Department of Health and Human Services guidelines [13].

Health Status and Clinical Characteristics

Confounders considered for inclusion were taken from data collected before the index visit and included the following: smoking status (never, former, or current), hepatitis C virus (HCV) infection (detectable HCV RNA in serum), depressive symptoms (Center for Epidemiologic Studies Depression score, >16) [14], diabetes, dyslipidemia, and hypertension. HIV-related variables included nadir $CD4^+$ T-cell count (before index visit), $CD4^+$ T-cell count at the index visit, detectable plasma HIV-1 RNA (viral load) at the index visit (based on the limit of detection of the method in use at that time), ART type (no therapy, non-HAART, or HAART), and ART exposure [15]. $CD4^+$ T-cell counts were measured with standardized flow cytometry [16, 17]. In 77% of subjects, viral load was determined with the Roche ultrasensitive second-generation assay (limit of detection, <50 copies/mL); the rest were tested with the Roche Cobas TaqMan assay (limit of detection, <20 copies/mL). Testosterone therapy use was self-reported and available for HIV-infected men only.

Laboratory Analyses for Outcome Variables

Plasma, serum, and peripheral blood mononuclear cell markers were assessed at the index visit for biomarkers selected within 3 outcome domains (inflammation, hormone, and immune activation/senescence). Inflammation biomarkers were measured from stored serum samples and included high-sensitivity C-reactive protein (hsCRP), interleukin 6 (IL-6), and the soluble receptors for tumor necrosis factor α (sTNFR1 and sTNFR2). Markers were measured in duplicate using commercially available enzyme-labeled immunosorbent sandwich assays (ALPCO Diagnostics), and values were averaged for analysis [18]. Insulin concentrations were measured using radioimmunoassay (Linco Research). Dehydroepiandrosterone sulfate (DHEA-S), free testosterone, and insulin-like growth factor 1 (IGF-1) were measured from cryopreserved samples by means of enzyme immunoassay for DHEA-S (DRG International), immunoenzymometric assay for IGF-1 (Immunodiagnostics Systems), and liquid chromatography–tandem mass spectrometry for total serum testosterone (all measured in Bhasin Laboratory, Boston University). Sex hormone binding globulin was measured using radioimmunoassay and free testosterone was then calculated from total testosterone and sex hormone binding globulin measurement using the Vermeulen

equation [19]. Fasting glucose and insulin have been measured at each semiannual MACS visit since 1999, processed at a central laboratory (Heinz Laboratory), and were used for calculation of the homeostatic model assessment of insulin resistance (HOMA-IR) [20].

For measurement of markers of immune activation and senescence by flow cytometry, frozen peripheral blood mononuclear cells were thawed and stained with anti-CD3 allophycocyanin -H7, anti-CD8 Pacific Blue, anti-CD38 phycoerythrin, anti-HLA-DR fluorescein isothiocyanate, and anti-CD28 allophycocyanin monoclonal antibodies (BD Biosciences) and LIVE/DEAD Fixable Aqua Dead Cell Stain (Life Technologies) for 20 minutes at room temperature. The peripheral blood mononuclear cells were then washed twice with fluorescence-activating cell sorting (FACS) buffer (1% bovine serum albumin in phosphate-buffered saline), resuspended in 500 μL of FACS Lysing Solution (BD Biosciences), and analyzed with a FACS Canto II flow cytometer (BD) using FACSDiva software (BD). Data were collected from ≥10 000 viable $CD8^+$ T cells per tube. The percentages of cells expressing CD38, HLA-DR, and CD28 among $CD8^+$ and $CD8^-$ T cells (the latter reflecting primarily, but not entirely, $CD4^+$ T cells) were determined using fluorescence-minus-one controls.

Statistical Analyses

Fisher exact and the nonparametric Kruskal-Wallis tests were used for univariate comparisons of clinical characteristics and outcomes for categorical and continuous variables, respectively. Linear regression models were used to describe differences by exposure group. To account for leftward skewness of the biomarker distributions, these values were log-transformed. Two models were fit for each biomarker; one was restricted to HIV-infected men, with the exposure being frailty, and the other was restricted to nonfrail men, with the exposure being HIV infection. Models were of the form $\log(\text{biomarker}) = A_0 + A_1 \times \text{Exposure} + A_z \times Z + e$, where e represents residuals that were normally distributed with mean 0 and variance sigma-squared and Z represents a vector of confounders. The difference was described as a percentage and calculated as $[\exp(A_1) - 1] \times 100$. Confounders identified a priori were age, race (black vs nonblack race), body mass index (BMI) in the log scale, obesity (BMI, ≥30 kg/m^2), smoking status, HCV infection, diabetes, dyslipidemia, hypertension, and depressive symptoms for all models. For the models in which the effect of frailty was assessed among HIV-infected men, additional HIV-related variables included low $CD4^+$ T-cell count nadir (<350/μL), low current $CD4^+$ T-cell count (<350/μL), detectable HIV-1 viral load, and type of ART (no ART, non-HAART, or HAART). An α level of 0.05 was considered significant.

To maximize the use of available data, minimize the effect of outlying values, and reduce the amount of missing data, individual variables were summarized from observations collected temporally close to the index visit. Specifically, the summary of 3 visits within 1 year before and including the index visit was used. If 3 visits were not available in this time interval, the first visit ≤6 months after the index visit was used to fill in the missing data. For continuous variables, the mean level from up to 3 visits before and including the visit were used. For categorical variables, the presence of each condition was defined by ≥2 occurrences in the 3 visits before and including the index visit.

RESULTS

Of 4005 potentially eligible participants seen between 1994 and 2010, a total of 155 HIV-infected men met our frailty criteria: 101 (65%) had ≥2 visits with ≥3 frailty-related phenotype criteria; 54 (35%) had 1 visit with ≥3 frailty-related phenotype criteria and ≥2 visits with 1–2 criteria. HIV-infected frail men were matched to 141 nonfrail HIV-infected and 150 nonfrail HIV-uninfected men. The median ages were between 47 and 49 years, and the median year of the index visits was 2006 (Table 1).

Characteristics of the study population are detailed in Table 1. Groups were significantly different in many characteristics; for example, frail HIV-infected men had a much higher prevalence of depressive symptoms by CES-D (54.8%) than nonfrail HIV-infected and HIV-uninfected men (5.0% and 4.7%, respectively, Table 1). Among HIV-infected men, frail men had a similar proportion of low nadir $CD4^+$ T-cell count but a higher proportion of current $CD4^+$ T-cell count <350/μL and detectable HIV-1 viral load than HIV-infected nonfrail men. Similar proportions of frail and nonfrail HIV-infected participants were receiving HAART (74.8% vs 72.3%, respectively). Frail men were more likely to report testosterone therapy use (13.8% vs 5.2%), although 16% (n = 25) and 18% (n = 26) of frail and nonfrail HIV-infected were missing data on self-reported testosterone use (not collected for HIV-uninfected men).

Differences in markers of inflammation, immune activation and senescence, and hormonal dysfunction between HIV-uninfected men, HIV-infected nonfrail, and HIV-infected frail men are shown in Table 2 (unadjusted). Levels of inflammatory markers (IL-6, hsCRP, sTNFR1, and sTNFR2) were highest among frail HIV-infected men and lowest among nonfrail HIV-uninfected men. Markers of immune activation (percentage of $CD38^+HLA\text{-}DR^+$ $CD4^+$ or $CD8^+$ T cells) and senescence (percentage of $CD28^-$ $CD4^+$ or $CD8^+$ T cells) were similar among HIV-infected frail and HIV-infected nonfrail men and significantly lower among HIV-uninfected men (all $P < .001$). Frail HIV-infected men had significantly lower levels of DHEA-S ($P < .001$), free testosterone ($P = .045$), and IGF-1 ($P < .001$) and a trend toward worsening insulin resistance (HOMA-IR; $P = .06$) compared with nonfrail, HIV-infected men. In contrast, only insulin resistance was worse by HIV status, with significantly greater HOMA-IR ($P < .001$)

Table 1. Demographic and Clinical Characteristics of the Study Population at the Index Visit

Variable[a]	HIV-Uninfected Men, No. (%)[b] (n = 150)	HIV-Infected Men, No. (%)[b] or Median (IQR)		*P* Value
		Nonfrail (n = 141)	Frail (n = 155)	
Age, median (IQR), y	48.5 (41.5–53.1)	47.7 (40.4–53.0)	49.1 (41.4–53.9)	.71[c]
Date, median (IQR)	2006 (2002–2008)	2006 (2001–2008)	2006 (2001–2008)	.76[c]
Black race	25 (16.7)	40 (28.4)	51 (32.9)	.003[d]
Hispanic	8 (5.3)	14 (9.9)	17 (11)	.18[d]
Metabolic health				
BMI, median (IQR), kg/m^2	25.0 (23.5–27.2)	25.7 (23.6–27.0)	23.9 (21.8–27.5)	.02[c]
Obesity	16 (10.7)	5 (3.7)	23 (14.9)	.004[d]
Fasting glucose >100 mg/dL	37 (30.8)	45 (41.7)	65 (54.2)	.001[d]
Diabetes	7 (4.7)	22 (15.6)	41 (26.5)	<.001[d]
Dyslipidemia	94 (75.2)	106 (89.8)	114 (84.4)	.01[d]
Metabolic syndrome	34 (22.7)	57 (40.4)	75 (48.4)	<.001[d]
Cardiovascular and renal health				
Hypertension	26 (17.3)	40 (28.4)	65 (41.9)	<.001[d]
eGFR (by CKD-EPI), median (IQR), mL/min/1.73 m^2	84 (75–99)	97 (83–108)	94 (78–106)	<.001[c]
Behavioral and psychological health				
Never smoker	45 (30)	40 (28.4)	26 (17.1)	<.001[d]
Former smoker	85 (56.7)	71 (50.4)	56 (36.8)	
Current smoker	20 (13.3)	30 (21.3)	70 (46.1)	
≥14 alcoholic drinks per week	18 (12)	11 (7.8)	6 (3.9)	.03[d]
Illicit drug use	95 (63.3)	76 (53.9)	94 (61.8)	.21[d]
HCV infection	2 (1.3)	6 (4.3)	27 (17.5)	<.001[d]
Depressive symptoms (CES-D score >16)	7 (4.7)	7 (5.0)	85 (54.8)	<.001[d]
HIV-related health and therapy				
Testosterone therapy	NA	6 (5.2)	18 (13.8)	.03[d]
Incident AIDS	0 (0)	13 (9.2)	30 (19.4)	<.001[d]
Nadir CD4$^+$ T-cell count				
Median (IQR), cells/μL	NA	287 (184–400)	272 (164–438)	.78[c]
<200 cells/μL	NA	44 (31.2)	54 (34.8)	.54[d]
<350 cells/μL	NA	95 (67.4)	96 (61.9)	.33[d]
Current CD4$^+$ T-cell count				
Median (IQR), cells/μL	NA	559 (401–694)	476 (291–636)	.01[c]
<350 cells/μL	NA	23 (16.3)	49 (31.6)	.003[d]
Detectable viral load	NA	58 (41.4)	78 (50.3)	.13[d]
ART type				
Non–highly active	NA	24 (17)	27 (17.4)	.10[d]
Highly active	NA	102 (72.3)	116 (74.8)	
None	NA	15 (10.6)	12 (7.7)	
Any exposure to ART agent				
NRTI	NA	127 (90.1)	142 (93.4)	.39[d]
NNRTI	NA	75 (53.2)	97 (63.8)	.08[d]
INSTI	NA	6 (4.3)	8 (5.3)	.79[d]
Entry inhibitor	NA	0 (0)	1 (0.7)	>.99[d]
Stavudine	NA	70 (49.6)	89 (58.6)	.13[d]
Zidovudine	NA	96 (68.1)	108 (71.1)	.61[d]
Indinavir	NA	38 (27)	45 (29.6)	.70[d]
Nelfinavir	NA	26 (18.4)	37 (24.3)	.26[d]
Cumulative exposure to ART agent, median (IQR), y				
NRTI	NA	5.97 (1.84–10.3)	5.08 (2.12–9.33)	.52[c]
NNRTI	NA	0.13 (0–2.78)	1.2 (0–3.73)	.06[c]
INSTI	NA	0 (0–0)	0 (0–0)	.69[c]
Stavudine	NA	0 (0–3.43)	0.59 (0–2.94)	.74[c]
Zidovudine	NA	2.26 (0–5.3)	1.39 (0–4.35)	.44[c]
Indinavir	NA	0 (0–0.14)	0 (0–0.68)	.78[c]
Nelfinavir	NA	0 (0–0)	0 (0–0)	.33[c]

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; CES-D, Center for Epidemiologic Studies–Depression; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; HAART, highly active ART; HCV, hepatitis C virus; HIV, human immunodeficiency virus; INSTI, integrase strand transferase inhibitor; IQR, interquartile range; NA, not available; NNRTI, nonnucleoside reverse-transcriptase inhibitors; NRTI, nucleoside/tide analogue reverse-transcriptase inhibitors.

[a] Illicit drug use included crack/cocaine, heroin/opioid, amphetamines, speedballs, or injection drug use. Hypertension, as systolic BP ≥140 mm Hg, diastolic BP ≥90 mm Hg and/or a diagnosis of hypertension with use of antihypertensive medications. Obesity was defined as a BMI ≥30 kg/m^2; diabetes, as hemoglobin A1c ≥6.5% and fasting glucose >126 mg/dL or diagnosis based on use of medications; dyslipidemia, as fasting total cholesterol ≥200 mg/dL, low-density lipoprotein ≥130 mg/dL, high-density lipoprotein <40 mg/dL, and triglycerides ≥150 mg/dL or use of lipid-lowering medications with self-reported/clinical diagnosis of dyslipidemia; metabolic syndrome, as the presence of ≥3 of the following: waist circumference ≥102 cm, fasting triglycerides ≥150 mg/dL, fasting glucose ≥100 mg/dL, use of antidiabetic medications with a history of diabetes or self-report, low-density lipoprotein cholesterol <40 mg/dL, and systolic blood pressure (BP) ≥130 mm Hg, diastolic BP ≥85 mm Hg, or use of antihypertensive medications with a self-reported or clinical history of hypertension.

[b] Data represent No. (%) of subjects unless labeled as median (IQR).

[c] *P* values based on Kruskal-Wallis nonparametric rank sum for continuous variables,

[d] *P* values based on Fisher exact test for categorical variables.

Table 2. Univariate Comparison Biomarkers of Inflammation, T-Cell Activation and Senescence, and Hormonal Regulation by HIV Serostatus and Frailty Status[a]

Variable	HIV-Uninfected Men (n = 150)	HIV-Infected Men		*P* Value[b]	
		Nonfrail (n = 141)	Frail (n = 155)	Frail vs Nonfrail HIV-Infected Men	HIV-Infected Nonfrail vs HIV-Uninfected Men
Inflammation					
IL-6, pg/mL	1.2 (0.8–1.6)	1.4 (1.0–2.0)	2.3 (1.6–3.5)	<.001	.002
hsCRP, ng/L	0.8 (0.4–1.8)	1.4 (0.6–2.4)	2.2 (1.0–5.2)	<.001	.001
sTNFR1, pg/mL	1182 (1039–1300)	1203 (1061–1335)	1357 (1152–1618)	<.001	.21
sTNFR2, pg/mL	2505 (2040–2992)	3313 (2518–4247)	4203 (3028–6334)	<.001	<.001
T-cell activation ($CD38^+HLA\text{-}DR^+$ expression)					
$CD8^+$ cells, %	14.4 (10.1–20.7)	33.8 (20.1–49.3)	37.5 (23.1–53.7)	.26	<.001
$CD4^+$ cells, %	5.8 (3.9–8.35)	14.0 (7.8–21.4)	15.5 (8.4–29.1)	.08	<.001
T-cell senescence ($CD28^-$ expression)					
$CD8^+$ cells, %	40.3 (25.5–54.8)	57.7 (47.0–69.4)	55.2 (40.7–67.5)	.18	<.001
$CD4^+$ cells, %	11.5 (5.6–23.5)	19.9 (13.0–34.4)	17.4 (9.1–34.3)	.22	<.001
Hormonal regulation					
DHEA-S, ng/mL	1.2 (0.8–1.6)	1.2 (0.8–1.8)	0.8 (0.4–1.4)	<.001	.46
Free testosterone, pg/mL[c]	85 (71–106)	86 (70–106)	70 (48–99)	.045	.74
IGF-1, ug/L	118 (96–138)	116 (92–144)	107 (77–138)	<.001	.92
HOMA-IR	2.25 (1.64–3.01)	2.93 (2.17–4.89)	3.43 (2.24–6.8)	.06	<.001

Abbreviations: DHEA-S, dehydroepiandrosterone sulfate; HIV, human immunodeficiency virus; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; IGF-1, insulin-like growth factor 1; IL-6, interleukin 6; sTNFR1 and sTNFR2, soluble tumor necrosis factor α receptors 1 and 2.

[a] Data are presented as median (interquartile range) values, among subjects with available data.

[b] *P* values are based on the Kruskal-Wallis nonparametric rank sum test.

[c] Including men receiving testosterone replacement therapy

among the nonfrail HIV-infected than among the nonfrail HIV-uninfected men.

Adjusted differences between the groups in markers of inflammation, immune activation and senescence, and hormone biomarkers are shown in Figures 1, 2, and 3, respectively. Among HIV-infected men, frailty was associated with 52% higher IL-6 ($P < .001$) and 69% higher hsCRP concentrations ($P < .001$; Figure 1*A*). Only sTNFR2 levels were significantly higher among HIV-infected versus HIV-uninfected nonfrail men (22% higher; $P < .001$; Figure 1*B*). Cellular markers of immune activation or senescence did not differ significantly by frailty status among HIV-infected men (Figure 2*A*). In contrast, HIV-infected men had significantly higher percentages of $CD38^+HLA\text{-}DR^+$ and $CD28^-$ T cells than HIV-uninfected men (Figure 2*B*). Among HIV-infected men, the presence of frailty was significantly associated with lower free testosterone (17% lower; $P = .02$) and lower DHEA-S (18% lower; $P = .04$), with a trend toward worsened insulin resistance (20% higher HOMA-IR; $P = .051$; Figure 3*A*). In contrast, among nonfrail men, HIV infection was significantly associated with greater insulin resistance (26% higher HOMA-IR; $P = .003$) but not with DHEA-S, free testosterone, or IGF-1 levels (Figure 3*B*).

To further investigate the potential impact of detectable compared with undetectable HIV-1 RNA, a sensitivity analysis was performed, restricting the analysis to HIV-uninfected men, and HIV-infected frail (n = 76) and nonfrail (n = 86) men with an undetectable viral load (Supplemental Table 1). Univariate comparisons were similar to Table 2 and did not change significance level, except that the percentage of $CD4^+$ T cells with $CD28^-$ expression was significantly lower among frail versus nonfrail HIV-infected men. In the adjusted comparison (Supplemental Table 2), similar to the overall findings (Figures 1*A*–*C*), the HIV-infected frail men had higher hsCRP ($P = .005$), lower free testosterone ($P = .01$) and greater insulin resistance ($P = .01$) than HIV-infected nonfrail men. The difference in IL-6 was attenuated and no longer significant ($P = .15$), and frail HIV-infected men had a significantly lower percentage of $CD4^+CD28^-$ T cells ($P = .02$). Among HIV-infected vs HIV-uninfected men, the difference in sTNFR2 was attenuated but remained significant ($P = .049$); differences in markers of immune senescence persisted (all $P < .001$).

DISCUSSION

The degree to which multisystem regulation in older, HIV-infected men is altered by HIV infection versus the presence of frailty has not previously been described, to our knowledge. In the current study, by analyzing both HIV status and frailty status together, we have shown that IL-6 and hsCRP levels were associated with frailty among HIV-infected men. We have also shown for the first time that lower DHEA-S and testosterone levels were also associated with frailty in those men, consistent with the concept of multisystem dysregulation. In

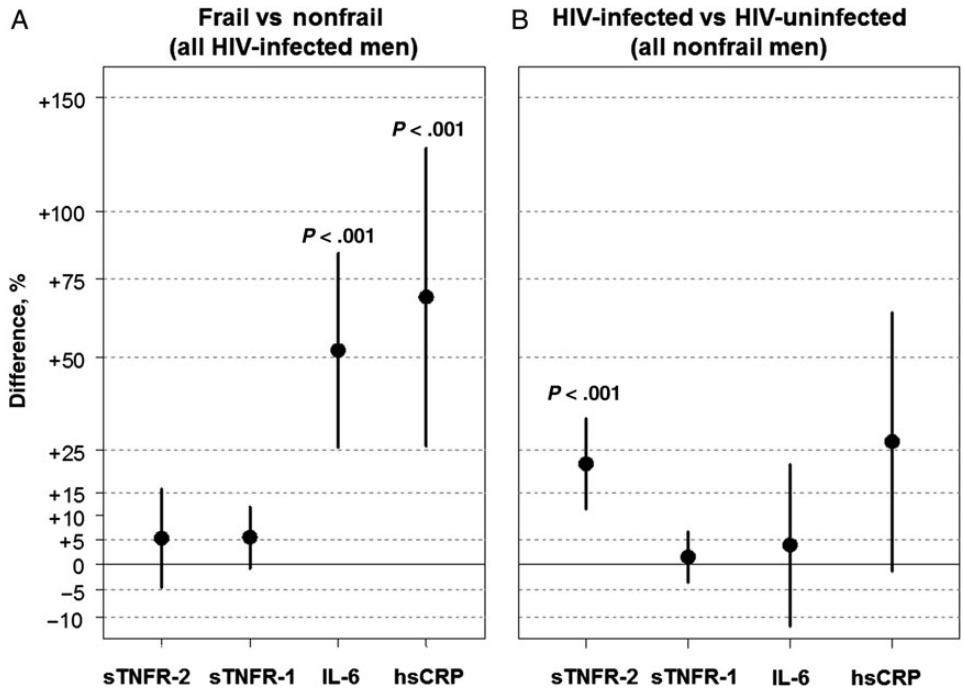

Figure 1. Adjusted differences in inflammatory marker levels by frailty status among human immunodeficiency virus (HIV)–infected men (*A*) and by HIV serostatus among nonfrail men (*B*), represented by point estimates (*dots*) and 95% confidence intervals (*vertical lines*). The study design included matching by age, calendar year of visit, and antiretroviral therapy (ART) use (among HIV-infected men); models were additionally adjusted for age, black race, body mass index in the log scale, obesity, smoking status (never, former, or current smoker), hepatitis C virus infection, diabetes, dyslipidemia, hypertension, and presence of depressive symptoms (Center for Epidemiologic Studies–Depression score >16). Among HIV-infected men (*A*), models were further adjusted for nadir $CD4^+$ T-cell count <350 cells/μL (yes or no), current $CD4^+$ T-cell count <350 cells/μL (yes or no), current detectable HIV-1 RNA (yes or no), and type of antiretroviral therapy (none, non–highly active ART, or highly active ART). Abbreviations: hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; sTNFR1 and sTNFR2, soluble tumor necrosis factor α receptors 1 and 2.

contrast, HIV serostatus but not frailty was associated with cellular immune activation and immunosenescence.

Even with effective ART, inflammatory markers remain elevated among HIV-infected compared with HIV-uninfected controls [21]. Although pronounced differences by HIV serostatus were expected, the adjusted differences were driven more by frailty for IL-6 and hsCRP. Overall, the association between inflammation and frailty is consistent with findings in several prior studies in HIV-infected populations, irrespective of the frailty definition, age range, or HIV risk factors of the population studied. Erlandson et al [22] found significantly higher levels of IL-6 but not CRP among frail versus nonfrail HIV-infected adults, perhaps owing to differences in liver function or other downstream inflammatory signaling between the populations. In a separate analysis of men in the MACS [23], HIV-infected men who were frail according to the criteria of Fried et al [2] had 50% higher CRP concentrations than nonfrail HIV-infected men. In the AIDS linked to the intraVenous experience cohort of HIV-infected and HIV-uninfected injection drug users, both IL-6 and sTNFR1 levels increased with increasing frailty, and this relationship was slightly stronger among HIV-infected participants [24]. In contrast, in the VACS, inflammation was associated with a higher score on the VACS Index, an index of HIV-related variables and other comorbid conditions, but not with a subjective frailty index [25]. In addition, in the AGEhiv Cohort, frailty was not associated with markers of inflammation or monocyte activation [26]. Differences in HIV disease severity, frailty definitions, comorbid conditions, coinfections, and other unrecognized confounders may account for discrepancies between studies, but as a whole these studies add to the growing literature on the relationship between frailty and the chronic inflammation of HIV infection.

It is well recognized that markers of T-cell senescence and activation are higher among HIV-infected [27] than among HIV-uninfected persons, but the degree to which these elevations are attributable to frailty or to HIV is not clear. The lack of association of these cellular markers with frailty in our population differed from findings in a prior study [22], which found a strong association between frailty and immune activation (the percentage of $CD38^+HLA\text{-}DR^+$ $CD8^+$ T cells) [22]. In the prior study, the median percentage of $CD38^+HLA\text{-}DR^+$ $CD8^+$ T cells was 15%, all participants were receiving ART, and 96% were virologically suppressed [22]. In contrast, in the current study the $CD38^+HLA\text{-}DR^+$ $CD8^+$ T cells were much higher for HIV-infected men, even when restricted to those with virologic suppression (Supplemental Tables). The

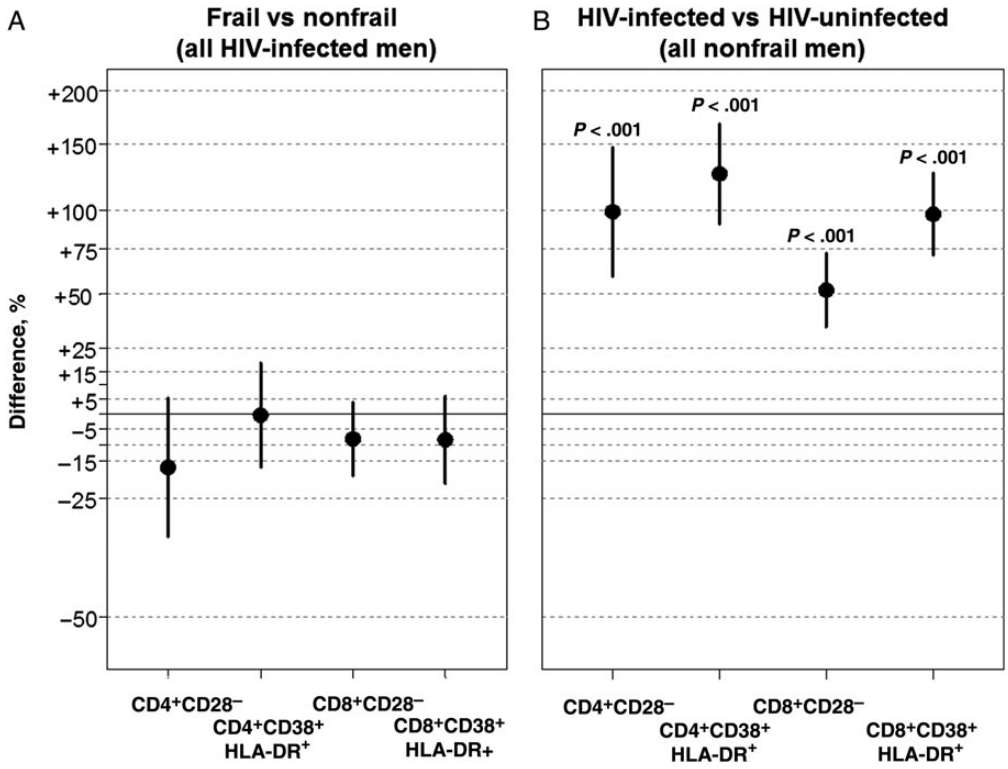

Figure 2. Adjusted differences in markers of immune activation and senescence (T-cell expression) by frailty status among human immunodeficiency virus (HIV)–infected men (*A*) and by HIV serostatus among nonfrail men (*B*), represented by point estimates (*dots*) and 95% confidence intervals (*vertical lines*; see Figure 1 legend for covariate adjustment).

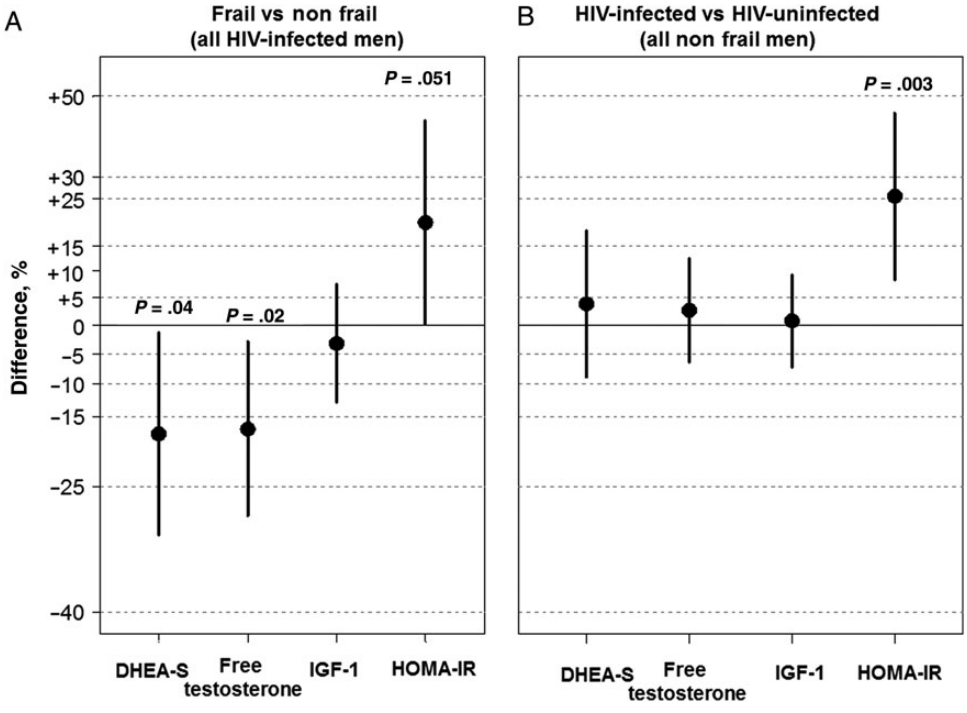

Figure 3. Adjusted differences in markers of hormonal regulation by frailty status among human immunodeficiency virus (HIV)–infected men (*A*) and by HIV serostatus among nonfrail men (*B*), represented by point estimates (*dots*) and 95% confidence intervals (*vertical lines*; see Figure 1 legend for covariate adjustment). Abbreviations: DHEA-S, dehydroepiandrosterone sulfate; HOMA-IR, homeostatic model assessment of insulin resistance; IGF-1, insulin-like growth factor 1.

reasons for these differences are unclear, but they could be partially explained by different characterizations of frailty.

Insulin resistance or diabetes and low DHEA-S and testosterone levels have been associated with frailty and components of frailty, including fatigue, weakness, and low energy, in multiple studies of HIV-uninfected cohorts [10, 28–33]. In HIV-infected populations, frailty (by varying definitions) has been associated with diabetes [34, 35], low testosterone levels [36], and low IGF-1 levels [37], each in separate cohorts. Although low levels of DHEA-S were seen in asymptomatic HIV disease before HAART [38] and have been associated with progression to AIDS [39], no prior studies in HIV-infected populations had shown an association with frailty. Whether treatments to replace or normalize these hormones might result in improvement in HIV-infected adults remains to be clearly established in either HIV-infected or HIV-uninfected populations. Data on the effects of DHEA supplementation on physical function in the general elderly population are inconclusive [40], with some studies [41], but not all [42], demonstrating improved strength and function. Furthermore, the benefit of testosterone replacement therapy on components of frailty remains controversial [43].

Several limitations of our study should be mentioned. First, we used the frailty-related phenotype to establish a classification of frailty. This phenotype has been used in several prior studies within the MACS Cohort, because grip strength and gait speed were not added to the MACS until 2007. The phenotype is associated with increased mortality rates and poor outcomes [8], but it relies on subjective report and therefore may reflect a greater predominance of depressive symptoms, subjective fatigue, and self-image than the frailty phenotype described by Fried et al [8]. Second, the median year of assessment was 2006, so some of our data preceded the HAART era and were subject to a lack of viral suppression. Furthermore, relatively few observations were from the modern ART era of the potent integrase-strand transfer inhibitors. Matching by calendar year and ART use, with further adjustment for virologic suppression, minimized the ART differences while maximizing the available data across diverse HIV treatment periods. The MACS includes only men who have sex with men, and the present study included predominantly middle-aged white men; thus, generalizability to women, much older populations, or populations with greater racial/ethnic diversity or differing HIV risk factors may be limited. Furthermore, no HIV-uninfected frail control group was included. Differences in body fat and muscle mass beyond BMI may have influenced markers of inflammation, activation, and hormone dysfunction, but image-based body composition measures were not available for most participants. Similarly, additional measures of immune senescence, such as CD57 or proliferation assays, may have provided a better assessment of the true senescence status. Finally, the cross-sectional nature of the data does not allow inferences to be made regarding causality.

Prior studies suggest a causal role of inflammation in muscle mass decline, muscle turnover, and weight loss, contributing to components of frailty [44–47]. In the present study, associations persisted in multivariate models adjusted for comorbid conditions, suggesting that the frailty-associated inflammation and hormonal dysregulation were not merely a result of greater comorbid disease burden among frail participants. Thus, our findings emphasize the potential importance of inflammation (IL-6 and hsCRP) and hormonal dysregulation in frailty among HIV-infected adults. However, considering the cross-sectional design and the small but significant difference, our findings cannot provide a basis for recommending testosterone or DHEA-S replacement or anti-inflammatory therapy as a treatment for frailty. Indeed, few interventions outside of exercise have been shown to effectively reverse the trajectory of frailty [48]. Results from ongoing studies on the anti-inflammatory roles of statins, angiotensin-converting enzyme inhibitors/angiotensin-receptor blockade, metformin, or probiotics may support a role for these therapies in the future [49]. For now, interventions should focus on limiting ongoing exposures to inflammation through lifestyle factors, such as maintenance of a healthy weight and diet, physical activity, and adequate sleep; optimizing management of comorbid conditions; and preservation of gonadal function (ie, early initiation of ART and avoidance of alcohol, marijuana, and opiates). Ultimately, early and long-lasting interventions that affect multiple pathways will probably prove most effective in slowing or preventing frailty, particularly in older HIV-infected adults.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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References

1. Fried LP. Conference on the physiologic basis of frailty. April 28, 1992, Baltimore, Maryland, U.S.A. Introduction. Aging (Milano) **1992**; 4:251–2.
2. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci **2001**; 56:M146–56.
3. Rockwood K, Andrew M, Mitnitski A. A comparison of two approaches to measuring frailty in elderly people. J Gerontol A Biol Sci Med Sci **2007**; 62:738–43.
4. Robinson TN, Wu DS, Stiegmann GV, Moss M. Frailty predicts increased hospital and six-month healthcare cost following colorectal surgery in older adults. Am J Surg **2011**; 202:511–4.
5. Margolick JB, Chopra RK. Relationship between the immune system and frailty: pathogenesis of immune deficiency in HIV infection and aging. Aging (Milano) **1992**; 4:255–7.
6. Desquilbet L, Jacobson LP, Fried LP, et al. HIV-1 infection is associated with an earlier occurrence of a phenotype related to frailty. J Gerontol A Biol Sci Med Sci **2007**; 62:1279–86.
7. Desquilbet L, Margolick JB, Fried LP, et al. Relationship between a frailty-related phenotype and progressive deterioration of the immune system in HIV-infected men. J Acquir Immune Defic Syndr **2009**; 50:299–306.
8. Desquilbet L, Jacobson LP, Fried LP, et al. A frailty-related phenotype before HAART initiation as an independent risk factor for AIDS or death after HAART among HIV-infected men. J Gerontol A Biol Sci Med Sci **2011**; 66:1030–8.
9. Akgun KM, Tate JP, Crothers K, et al. An adapted frailty-related phenotype and the VACS index as predictors of hospitalization and mortality in HIV-infected and uninfected individuals. J Acquir Immune Defic Syndr **2014**; 67:397–404.
10. Fried LP, Xue QL, Cappola AR, et al. Nonlinear multisystem physiological dysregulation associated with frailty in older women: implications for etiology and treatment. J Gerontol A Biol Sci Med Sci **2009**; 64:1049–57.
11. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med **2008**; 5:e203.
12. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR Recomm Rep **1992**; 41:1–19.
13. DHHS/Henry J. Kaiser Family Foundation Panel on Clinical Practices for the Treatment of HIV infection. Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents. January 2016 revision. https://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-treatment-guidelines/0/. Accessed 5 August 2016.
14. Radloff LS. The CES-D scale: a self-report depression scale for research in the general population. Appl Psychol Measur **1977**; 1:385–401.
15. Ng DK, Jacobson LP, Brown TT, et al. HIV therapy, metabolic and cardiovascular health are associated with glomerular hyperfiltration among men with and without HIV infection. AIDS **2014**; 28:377–86.
16. Schenker EL, Hultin LE, Bauer KD, Ferbas J, Margolick JB, Giorgi JV. Evaluation of a dual-color flow cytometry immunophenotyping panel in a multicenter quality assurance program. Cytometry **1993**; 14:307–17.
17. Hultin LE, Menendez FA, Hultin PM, et al. Assessing immunophenotyping performance: proficiency-validation for adopting improved flow cytometry methods. Cytometry B Clin Cytom **2007**; 72:249–55.
18. Crawford KW, Li X, Xu X, et al. Lipodystrophy and inflammation predict later grip strength in HIV-infected men: the MACS Body Composition substudy. AIDS Res Hum Retroviruses **2013**; 29:1138–45.
19. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab **1999**; 84:3666–72.
20. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia **1985**; 28:412–9.
21. Wada NI, Jacobson LP, Margolick JB, et al. The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. AIDS **2015**; 29:463–71.
22. Erlandson KM, Allshouse AA, Jankowski CM, et al. Association of functional impairment with inflammation and immune activation in HIV type 1-infected adults receiving effective antiretroviral therapy. J Infect Dis **2013**; 208:249–59.
23. Margolick JB, Martinez-Maza O, Jacobson L, et al. Frailty and circulating markers of inflammation in HIV+ and HIV- men in the Multicenter AIDS Cohort Study. Presented at: Conference on Retroviruses and Opportunistic Infections; 3–6 March 2013: Atlanta, GA. Abstract 800.
24. Piggott DA, Varadhan R, Mehta SH, et al. Frailty, inflammation, and mortality among persons aging with HIV infection and injection drug use. J Gerontol A Biol Sci Med Sci **2015**; 70:1542–7.
25. Escota GV, Patel P, Brooks JT, et al. Short communication: the Veterans Aging Cohort Study index is an effective tool to assess baseline frailty status in a contemporary cohort of HIV-infected persons. AIDS Res Hum Retroviruses **2015**; 31:313–7.
26. Kooij KW, Wit FW, Schouten J, et al. HIV infection is independently associated with frailty in middle-aged HIV type 1-infected individuals compared with similar but uninfected controls. AIDS **2016**; 30:241–50.
27. Serrano-Villar S, Sainz T, Lee SA, et al. HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8+ T cell activation, and increased risk of non-AIDS morbidity and mortality. PLoS Pathog **2014**; 10:e1004078.
28. Baylis D, Bartlett DB, Syddall HE, et al. Immune-endocrine biomarkers as predictors of frailty and mortality: a 10-year longitudinal study in community-dwelling older people. Age **2013**; 35:963–71.
29. Afilalo J. Androgen deficiency as a biological determinant of frailty: hope or hype? J Am Geriatr Soc **2014**; 62:1174–8.
30. Voznesensky M, Walsh S, Dauser D, Brindisi J, Kenny AM. The association between dehydroepiandosterone and frailty in older men and women. Age Ageing **2009**; 38:401–6.
31. Cappola AR, Xue QL, Fried LP. Multiple hormonal deficiencies in anabolic hormones are found in frail older women: the Women's Health and Aging studies. J Gerontol A Biol Sci Med Sci **2009**; 64:243–8.
32. Sanders JL, Ding V, Arnold AM, et al. Do changes in circulating biomarkers track with each other and with functional changes in older adults? J Gerontol A Biol Sci Med Sci **2014**; 69:174–81.
33. Tajar A, O'Connell MD, Mitnitski AB, et al. Frailty in relation to variations in hormone levels of the hypothalamic-pituitary-testicular axis in older men: results from the European male aging study. J Am Geriatr Soc **2011**; 59:814–21.
34. Althoff KN, Jacobson LP, Cranston RD, et al. Age, comorbidities, and AIDS predict a frailty phenotype in men who have sex with men. J Gerontol A Biol Sci Med Sci **2014**; 69:189–98.
35. Erlandson KM, Allshouse AA, Jankowski CM, et al. Comparison of functional status instruments in HIV-infected adults on effective antiretroviral therapy. HIV Clin Trials **2012**; 13:324–34.
36. Rochira V, Diazzi C, Santi D, et al. Low testosterone is associated with poor health status in men with human immunodeficiency virus infection: a retrospective study. Andrology **2015**; 3:298–308.
37. Erlandson KM, Allshouse AA, Jankowski CM, MaWhinney S, Kohrt WM, Campbell TB. Functional impairment is associated with low bone and muscle mass among persons aging with HIV infection. J Acquir Immune Defic Syndr **2013**; 63:209–15.
38. Villette JM, Bourin P, Doinel C, et al. Circadian variations in plasma levels of hypophyseal, adrenocortical and testicular hormones in men infected with human immunodeficiency virus. J Clin Endocrinol Metab **1990**; 70:572–7.
39. Jacobson MA, Fusaro RE, Galmarini M, Lang W. Decreased serum dehydroepiandrosterone is associated with an increased progression of human immunodeficiency virus infection in men with CD4 cell counts of 200–499. J Infect Dis **1991**; 164:864–8.
40. Baker WL, Karan S, Kenny AM. Effect of dehydroepiandrosterone on muscle strength and physical function in older adults: a systematic review. J Am Geriatr Soc **2011**; 59:997–1002.
41. Kenny AM, Boxer RS, Kleppinger A, Brindisi J, Feinn R, Burleson JA. Dehydroepiandrosterone combined with exercise improves muscle strength and physical function in frail older women. J Am Geriatr Soc **2010**; 58:1707–14.

42. Nair KS, Rizza RA, O'Brien P, et al. DHEA in elderly women and DHEA or testosterone in elderly men. N Engl J Med **2006**; 355:1647–59.
43. Seftel AD, Kathrins M, Niederberger C. Critical update of the 2010 Endocrine Society clinical practice guidelines for male hypogonadism: a systematic analysis. Mayo Clin Proc **2015**; 90:1104–15.
44. Janssen SP, Gayan-Ramirez G, Van den Bergh A, et al. Interleukin-6 causes myocardial failure and skeletal muscle atrophy in rats. Circulation **2005**; 111:996–1005.
45. Baltgalvis KA, Berger FG, Pena MM, Davis JM, Muga SJ, Carson JA. Interleukin-6 and cachexia in ApcMin/+ mice. Am J Physiol Regul Integr Comp Physiol **2008**; 294:R393–401.
46. White JP, Puppa MJ, Sato S, et al. IL-6 regulation on skeletal muscle mitochondrial remodeling during cancer cachexia in the ApcMin/+ mouse. Skeletal Muscle **2012**; 2:14.
47. van Hall G, Steensberg A, Fischer C, et al. Interleukin-6 markedly decreases skeletal muscle protein turnover and increases nonmuscle amino acid utilization in healthy individuals. J Clin Endocrinol Metab **2008**; 93:2851–8.
48. Ng TP, Feng L, Nyunt MS, et al. Nutritional, physical, cognitive, and combination interventions and frailty reversal among older adults: a randomized controlled trial. Am J Med **2015**; 128:1225–36 e1.
49. Erlandson KM, Campbell TB. Inflammation in chronic HIV infection: what can we do? J Infect Dis **2015**; 212:339–42.