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Research Paper

Monocyte bioenergetic function is associated with body composition in virologically suppressed HIV-infected women

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ABSTRACT

Women living with HIV may present with high levels of body fat that are associated with altered bioenergetic function. Excess body fat may therefore exacerbate the bioenergetic dysfunction observed with HIV infection. To determine if body fat is associated with bioenergetic function in HIV, we conducted a cross-sectional study of 42 women with HIV who were virologically suppressed on antiretroviral therapy. Body composition was determined via dual-energy x-ray absorptiometry. Oxygen consumption rate (OCR) of monocytes was sorted from peripheral blood mononuclear cells obtained from participants in the fasting state. Differences in bioenergetic function, as measured by OCR, was assessed using Kruskal-Wallis tests and Spearman correlations adjusted for age, race, and smoking status. Participants were 86% Black, 45.5 years old, 48% current smokers, and 57% were obese (body mass index $\geq$30). Nearly all women (93%) had > 30% total fat mass, while 12% had > 50% total fat mass. Elevated levels of total fat mass, trunk fat, and leg fat were inversely correlated with measures of bioenergetic health as evidenced by lower maximal and reserve capacity OCR, and Bioenergetic Health Index. Measures of extracellular acidification (ECAR) in the absence (basal) or maximal (with oligomycin) were positively correlated with measures of bioenergetics, except proton leak, and were negatively correlated with fat mass. Despite virological suppression, women with HIV present with extremely high levels of adiposity that correlate with impaired bioenergetic health. Without effective interventions, this syndemic of HIV infection and obesity will likely have devastating consequences for our patients, potentially mediated through altered mitochondrial and glycolytic function.

1. Introduction

Combination antiretroviral therapy (ART) has transformed HIV from a uniformly fatal disease to a manageable, chronic medical condition. Unfortunately, chronic HIV infection is associated with excess risk for cardiometabolic diseases including diabetes, hypertension, dyslipidemia, and atherosclerosis [1–5]. In previous work by our group and others, obesity contributes to the excess prevalence of cardiometabolic diseases, and obesity and excess adiposity more commonly affect women than men [6–8]. Black women in particular have a 2-fold increase in hypertension and diabetes compared to other groups, an association that was attenuated after adjusting for obesity [8]. Our data confirm previous reports that women living with HIV (WLH) are more likely to be obese than men and at particular risk for poor health outcomes related to obesity [7,9,10]. Given the significant gains in life expectancy and quality of life achieved with ART, understanding the negative health implications of excess adipose tissue deposition on health in WLH remains a critical area of focus.

Both HIV infection and obesity have negative consequences on bioenergetic health: each disease is characterized by persistent systemic inflammation and excess oxidative stress markers [11–15]. HIV infection and ART have detrimental effects on mitochondrial quality and function in adipose tissue [16–20]. In HIV-negative adults, lower mitochondrial DNA copy number is associated with higher mortality and chronic disease risk [21,22]. Additionally, HIV-negative obese individuals have significant derangements in mitochondrial function with resultant excess cardiometabolic disease incidence [23–25]. It has recently become possible to assess bioenergetics cellular function in circulating PBMC and platelets with high throughput [26,27]. Using this approach, it has been demonstrated that bioenergetic measures of

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circulating platelets, monocytes and lymphocytes can serve as biomarkers of metabolic dysfunction in patients following cardiac surgery, sickle cell disease, asthma and diabetes [28–31]. Importantly, in animal models and human subjects skeletal muscle mitochondrial function is strongly correlated with monocyte bioenergetic parameters [32,33]. The values obtained from these tests can be integrated to serve as a sensitive measure of bioenergetic health [26]. In the current study we tested the hypothesis that overlap of the HIV and obesity epidemics may have additive or synergistic toxicity mediated through altered bioenergetic function and oxidative stress.

Given our previous findings regarding obesity and cardiometabolic disease risk, we recruited HIV-infected women with suppressed HIV viremia on ART and performed measures of monocyte bioenergetic health and comprehensive body composition assessments to evaluate the relationship between HIV, obesity, and bioenergetic health. Our objective was to determine whether measures of bioenergetic function are associated with body composition in a cross-sectional study of WLH.

2. Methods

2.1. Study cohort and visit

All study protocols were reviewed and approved by the UAB Institutional Review Board. Consent was obtained from patients at the University of Alabama at Birmingham (UAB) 1917 HIV Clinic. Inclusion criteria for the study were (1) female; (2) ages 19–55; (3) white or African-American self-reported race/ethnicity; (4) body mass index (BMI) $\geq$18.5; (5) HIV viral load < 400 copies/mL; (6) negative pregnancy test prior to dual-energy x-ray absorptiometry (DXA); and (7) prescribed ART regimen for less than 10 years to avoid body composition changes associated with older generation regimens of ART. Data elements and blood samples were collected during a single study visit. Following informed consent, fasting blood samples were obtained, and a research technician verbally administered sociodemographic and lifestyle questionnaires. Race was self-reported by the women. Current smoking status was classified as (1) never, (2) former, or (3) current. Current age, CD4+ T-cell count, HIV viral load, and prescribed ART regimen at the time closest to the study visit were obtained through data query of the EMR. Each woman also completed DXA assessment.

2.2. Bioenergetic assessment of monocytes

Whole blood was collected in 8.5 mL vacutainers (BD Biosciences) containing 1.5 mL ACD solution (trisodium citrate, 22.0 g/L; citric acid, 8.0 g/L; and dextrose 24.5 g/L) and processed within 2 h of collection for monocyte cell separation using MACS beads using established protocols [34]. Cells were counted and plated at 150,000 cells/well onto a 96 well Extracellular Flux analyzer (Seahorse Bioscience) to determine the cellular bioenergetic parameters in human monocytes as previously described [27]. After establishing the basal monocyte oxygen consumption rate (pmoles/min; OCR), sequential injections of 1 μg/mL oligomycin, 0.6 μM FCCP, and 10 μM antimycin A were used to measure ATP-linked respiration, maximal OCR, and non-mitochondrial respiration. Reserve capacity was calculated as the difference in basal and maximal OCR and proton leak was measured as the difference between the OCR following oligomycin and the non-mitochondrial respiration. The Bioenergetic Health Index (BHI) was computed as [BHI = (ATP-linked × reserve capacity)/(proton leak × non-mitochondrial)] as previously described [26,35].

During the same protocol, the extracellular acidification rate (ECAR) was measured. This parameter reflects proton production from a number of sources, including glycolysis and the TCA cycle [27,36]. For this study we have included in the analyses the basal ECAR measurement prior to oligomycin addition (Basal ECAR) and that immediately after (maximal ECAR). OCR and ECAR values are reported for the 150,000 cells per well.

2.3. Body composition

Weight was measured to the nearest 0.1 kg using an electronic scale (Seca 869, Seca, Columbia, MD). Height with shoes removed was measured to the nearest 0.1 cm using a portable stadiometer (Seca 217, Seca, Columbia, MD). Participants were classified as (1) normal weight (BMI 18.5–24.9 kg/m^2); (2) overweight (BMI 25.0–29.9 kg/m^2); or (3) obese (BMI $\geq$ 30 kg/m^2). Total body fat, soft lean tissue mass, trunk fat, leg fat, and visceral adipose tissue (VAT) were analyzed via a single DXA whole-body scan using a Lunar iDXA densitometer with CoreScan software (GE-Lunar Corporation, Madison, WI). The Relative Skeletal Muscle Index was measured by DXA as skeletal muscle mass (kg)/height (m^2) [37]. Percent body fat cutpoints were categorized as the frequency of women with total % fat mass of < 30, 30–39, 40–49, or $\geq$50. Excess visceral fat was classified as VAT $\geq$1359 cm^3, as defined in Framingham study women participants [38]. Lipoatrophy (loss of fat mass in the limbs) was assessed via DXA as a fat mass ratio (trunk fat/lower limb fat) > 1.329 [39].

2.4. Statistical analysis

Demographic and body composition characteristics were summarized as median and range or frequency (percent). Unadjusted differences in median monocyte bioenergetics values were presented by tertiles of total % fat mass, % leg fat, and % trunk fat, with tertile 3 indicating the highest fat levels, and analyzed using Kruskal-Wallis test with Dunn's multiple correction test. For total % fat mass category, the total numbers of women with less than 40% or greater than 50% fat mass were low; thus data for this variable was also analyzed by tertiles. The relationships between monocyte bioenergetics parameters and continuous body composition measures were assessed using the Spearman correlations coefficient. The first models for each body composition measure were unadjusted. The second models were adjusted for factors that may impact mitochondrial function: age, race, and smoking status. All data were analyzed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) with a significance level of $P < 0.05$.

3. Results

3.1. Participant characteristics and body composition

Forty-two women completed fasting blood draws and DXA analysis. Demographic characteristics are shown in Table 1. The median age was 45.5 (range 23–55) years, and 86% of women were Black. Almost half (48%) of women were current smokers, while 24% were former smokers. Median CD4+ T-cell count was 720.0 cells/μL (range 220–1468). The median BMI of participants was 32 kg/m^2 (Table 1), with 8 (19%) normal weight, 10 (24%) overweight, and 24 (57%) obese women.

Overall, participants had high levels of adiposity: women presented with a median 43.9% fat mass, 56.2% lean mass, and 48.4% trunk fat. Only 3 (7%) women had < 30% fat mass (all within a BMI range of 18.5–20 kg/m^2), while only 10 (25%) women had between 30–39% fat mass (BMI range 21–29 kg/m^2). Five (12%) women had $\geq$50% fat mass (BMI range 35–41 kg/m^2). Lipoatrophy was identified in 7 (16%) women – of these, all had > 40% fat mass, and 6 had excess visceral adiposity. In the total sample, 14 (34%) women had excess visceral adiposity (VAT $\geq$1359 cm^3), all of whom also presented with > 40% fat mass.

3.2. Bioenergetic health

Median monocyte basal OCR and ECAR, ATP-linked, proton leak, maximal OCR (after FCCP) and ECAR (after oligomycin), reserve capacity, and non-mitochondrial OCR along with BHI are presented in Table 1. Fig. 1 shows unadjusted monocyte OCR by percent total fat

Table 1
Demographics, body composition, and bioenergetics of women living with HIV.[a]

Characteristics	Total Sample (n = 42)	Range
Demographics:		
Age (years)	45.5	23.0–55.0
Black race	85.7	
Smoking history:		
Never	28.6	
Former	23.8	
Current	47.6	
CD4+ T cell count (cells/μL)	720.0	220–1468
Body Composition:		
Height (cm)	164.5	142.2–180.3
Weight (kg)	81.4	47.3–129.5
Body mass index (BMI)	32.0	18.0–42.0
% Obese (BMI ≥ 30)	57.1	
Total fat mass (kg)	34.8	13.0–61.0
Total lean mass (kg)	45.4	35.0–63.8
VAT mass (gm)	975.0	76.0–3955.0
VAT volume (cm^3)	43.9	81.0–4192.0
Percent fat mass	56.2	23.9–53.3
Percent lean mass	48.4	46.7–76.1
Percent trunk fat	40.5	20.6–60.3
Percent leg fat	40.1	23.8–53.4
Relative Skeletal Muscle Index	164.5	5.2–10.3
Percent fat mass category:		
< 30	7.2	
30–39	23.8	
40–49	57.1	
≥ 50	11.9	
VAT category (≥ 1359 cm^3)	34.0	
Lipoatrophy (yes)	16.7	
Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR)[b]:		
Monocyte OCR:		
Basal	46.7	16.9–70.1
Proton Leak	7.3	1.7–15.2
ATP-Linked	38.3	15.3–59.5
Maximal	101.0	30.5–178.2
Reserve Capacity	49.1	−11.2–117.3
Non-Mitochondrial	21.5	12.9–41.5
Bioenergetic Health Index	10.9	0.9–24.7
ECAR:		
Basal glycolysis	53.3	23.7–95.7
Maximal glycolysis	59.0	34.2–112.9

VAT: Visceral Adipose Tissue; Lipoatrophy: ratio trunk fat to lower limb fat ≥1.329.
[a] Values are median (range) or percent.
[b] Oxygen Consumption Rate (pmol/min/10,000 cells).

category tertile. Women in the highest tertile of fat (tertile 3) had lower ATP-linked (p = 0.04), maximal (p = 0.02), reserve capacity (p = 0.04), and BHI (p = 0.05) OCR compared to tertiles 1 and 2. Non-mitochondrial OCR was lower in tertile 3 compared to tertile 1 (p = 0.020. A similar pattern was seen when we evaluated % leg fat and % trunk fat. For % leg fat (Fig. 2), women in tertile 2 had a lower basal (p = 0.03), ATP-linked (p = 0.02), and non-mitochondrial (p = 0.02) OCR compared to those in tertile 1. Women in tertile 3 had a lower maximal (p = 0.04), reserve capacity (p = 0.02), and non-mitochondrial (p = 0.02) OCR and a lower BHI (p = 0.04) compared to those in tertile 1. When comparing % trunk fat (Fig. 3), women in tertiles 2 and 3 presented with lower basal (p = 0.07), ATP-linked (p = 0.05), maximal (p = 0.04), and non-mitochondrial (p = 0.02) OCR compared to tertile 1, while women in tertile 2 had a lower reserve capacity OCR (p = 0.05) and BHI (p = 0.04) compared to tertile 1.

The correlation coefficients of monocyte OCR with each body composition variable are presented in Table 2. In unadjusted models, total fat mass% was negatively correlated with maximal, reserve capacity, non-mitochondrial OCRs ($p < 0.05$ for all), and with BHI ($p < 0.01$). In models adjusted for age, race, and smoking status, total fat mass% remained negatively correlated with basal ($R = -0.36$; $p < 0.05$), ATP-linked ($R = -0.39$; $p < 0.05$), maximal ($R = -0.37$; $p < 0.05$), reserve capacity ($R = -0.35$; $p < 0.05$), non-mitochondrial ($R = -0.33$; $p < 0.05$) OCRs, and with BHI ($R = -0.37$; $p < 0.05$). Total lean mass% was positively correlated with the above variables as the inverse of fat mass%.

In adjusted models, trunk fat% was negatively correlated with basal ($R = -0.40$; $p < 0.01$), ATP-linked ($R = -0.43$; $p < 0.01$), maximal ($R = -0.34$; $p < 0.05$), and non-mitochondrial ($R = -0.32$; $p = 0.05$) OCR. However, no correlation was observed between VAT and monocyte bioenergetics. Leg fat% was negatively correlated with reserve capacity ($R = -0.35$; $p < 0.05$) and the BHI ($R = -0.45$; $p < 0.01$).

3.3. Extracellular acidification

Median basal and maximal ECAR are presented in Table 1. Changes in ECAR, measured in parallel to the mitochondrial stress test, are presented in Fig. 4 and Table 2. Basal ECAR was not significantly different between tertiles of total fat mass% (Fig. 4a), while women in the lowest tertile of total fat (tertile 1) had significantly higher maximal ECAR (Fig. 4b) than women in tertiles 2 and 3. A positive association was observed of basal ECAR with basal OCR ($r = 0.38$, $p = 0.01$; Fig. 4c), and of maximal ECAR with maximal OCR ($r = 0.62$, $p < 0.001$; Fig. 4d). When correlations between ECAR and body compositions were assessed (Table 2), basal ECAR was correlated with total fat mass% in unadjusted ($R = -0.31$; $p < 0.05$) and adjusted ($R = -0.32$; $p < 0.05$) models. Maximal ECAR was also correlated with total fat mass% in unadjusted ($R = -0.33$; $p < 0.05$) and adjusted ($R = -0.33$; $p < 0.05$) models. Total lean mass% was positively correlated with both basal and maximal ECAR as the inverse of fat mass%. Maximal ECAR was negatively correlated with unadjusted leg fat% ($R = -0.31$; $p < 0.05$); however, this correlation was not significant after adjusting for covariates.

4. Discussion

Here, we demonstrate that WLH with excess adiposity have altered bioenergetic function despite successful HIV suppression. While this was a convenience sample from a single clinic, the striking excess adiposity, even in women with normal BMI, raises serious concerns about long term health consequences. Furthermore, given the previously described relationship between this altered bioenergetic phenotype and cardiometabolic disease risk and aging-related conditions such as frailty, these findings raise critical concerns about long term health for women living with HIV [17–21].

The high prevalence of obesity (57%) and excess fat mass (93%) is striking, particularly given the similarity to previously reported data from the 1917 Clinic Cohort (49% obesity among black HIV-infected women) [8]. The very high percentage of total body fat is concerning with nearly 70% had > 40% total body fat mass. In this cohort, 63% of normal weight and 100% of overweight women could be classified with "normal weight obesity" (NWO), a condition in which someone with a BMI between 18.5 and 24.9 kg/m^2 has elevated percent total body fat. Among HIV-uninfected women and men, NWO was identified in 30% of normal weight and 80% of overweight participants, and NWO is associated with systemic inflammation and increased cardiovascular disease risk in women [40–42]. Of particular concern are the 14% of women with lipoatrophy, NWO, and excess visceral fat. The Fat Redistribution and Metabolic Change in HIV Infection (FRAM) study identified increased lipoatrophy in WLH compared to HIV-negative controls, and found that men and women with lipoatrophy did not experience improvements in subcutaneous adipose tissue levels within 5 years of follow-up [43,44]. Taken together, these findings highlight a crucial need to explore the impact of body composition beyond BMI among today's population of individuals living with HIV, who are experiencing an epidemic of obesity and possible excess adiposity that can significantly contribute to metabolic derangements.

Even among this small sample of women with predominately high body fat levels, excess adiposity was negatively correlated with several

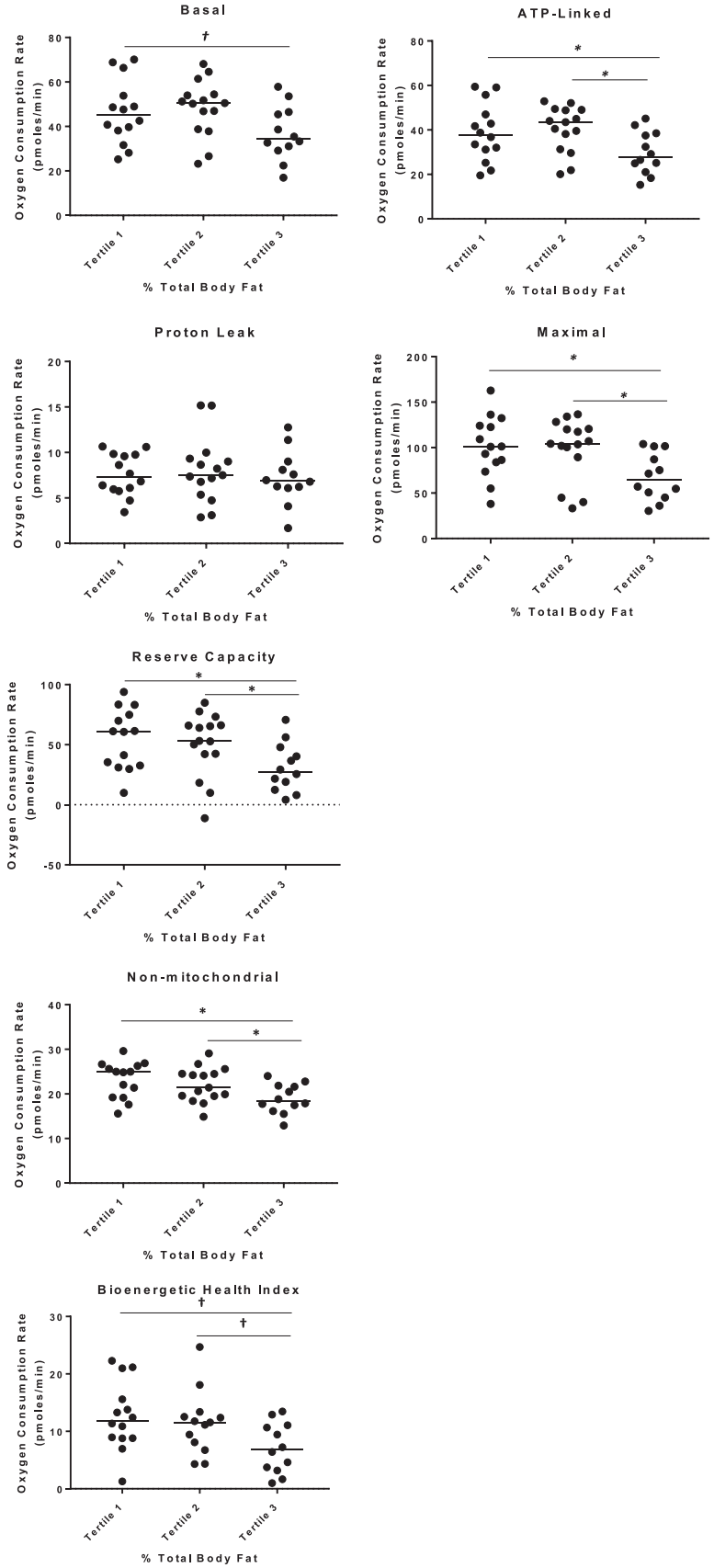

Fig. 1. Distribution of monocyte oxygen consumption rate by percent total body fat tertiles in women living with HIV. Medians are indicated by the solid line. $^{\dagger} = P < 0.07$ Kruskal-Wallis result with Dunn test. $^{*} = P < 0.05$ Kruskal-Wallis result with Dunn test.

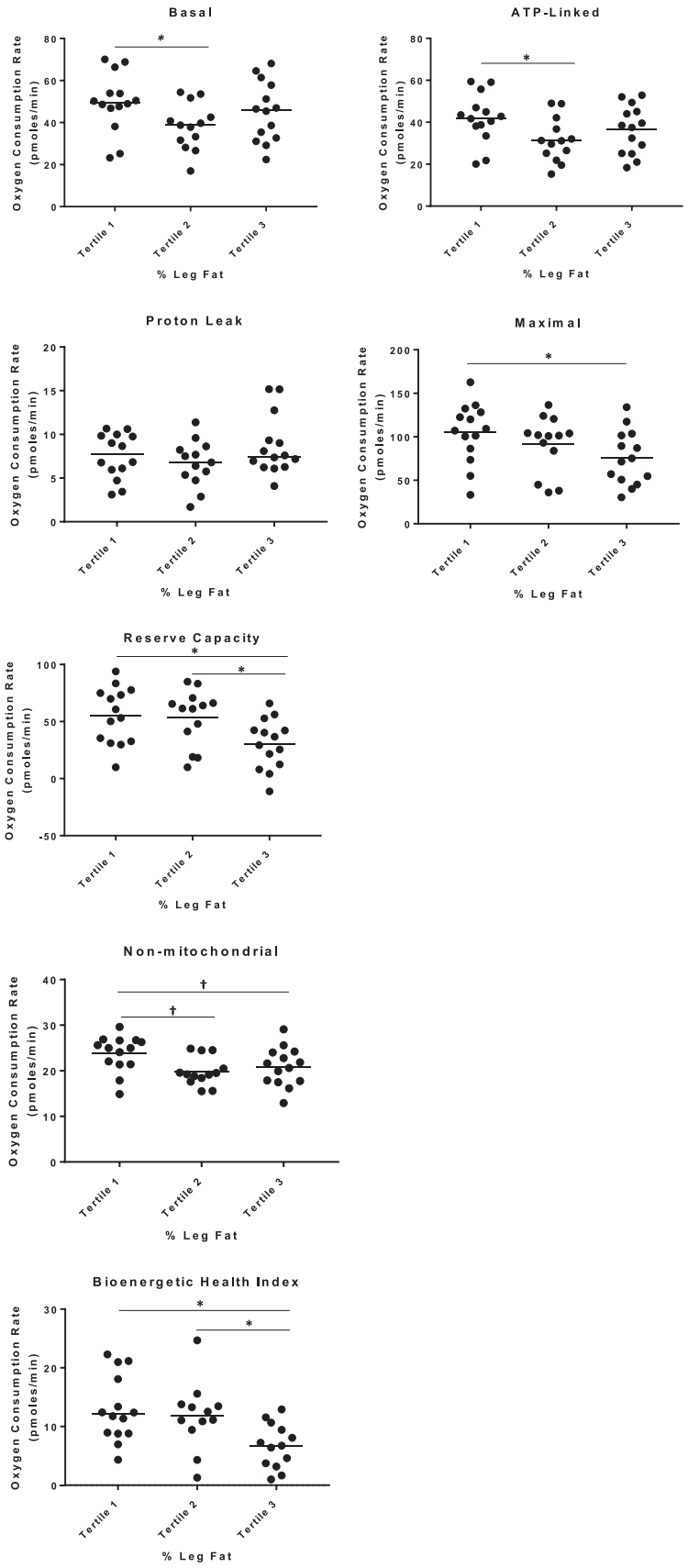

Fig. 2. Distribution of monocyte oxygen consumption rate by percent leg fat tertiles in women living with HIV. Medians are indicated by the solid line. † = P < 0.07 Kruskal-Wallis result with Dunn test. * = P < 0.05 Kruskal-Wallis result with Dunn test.

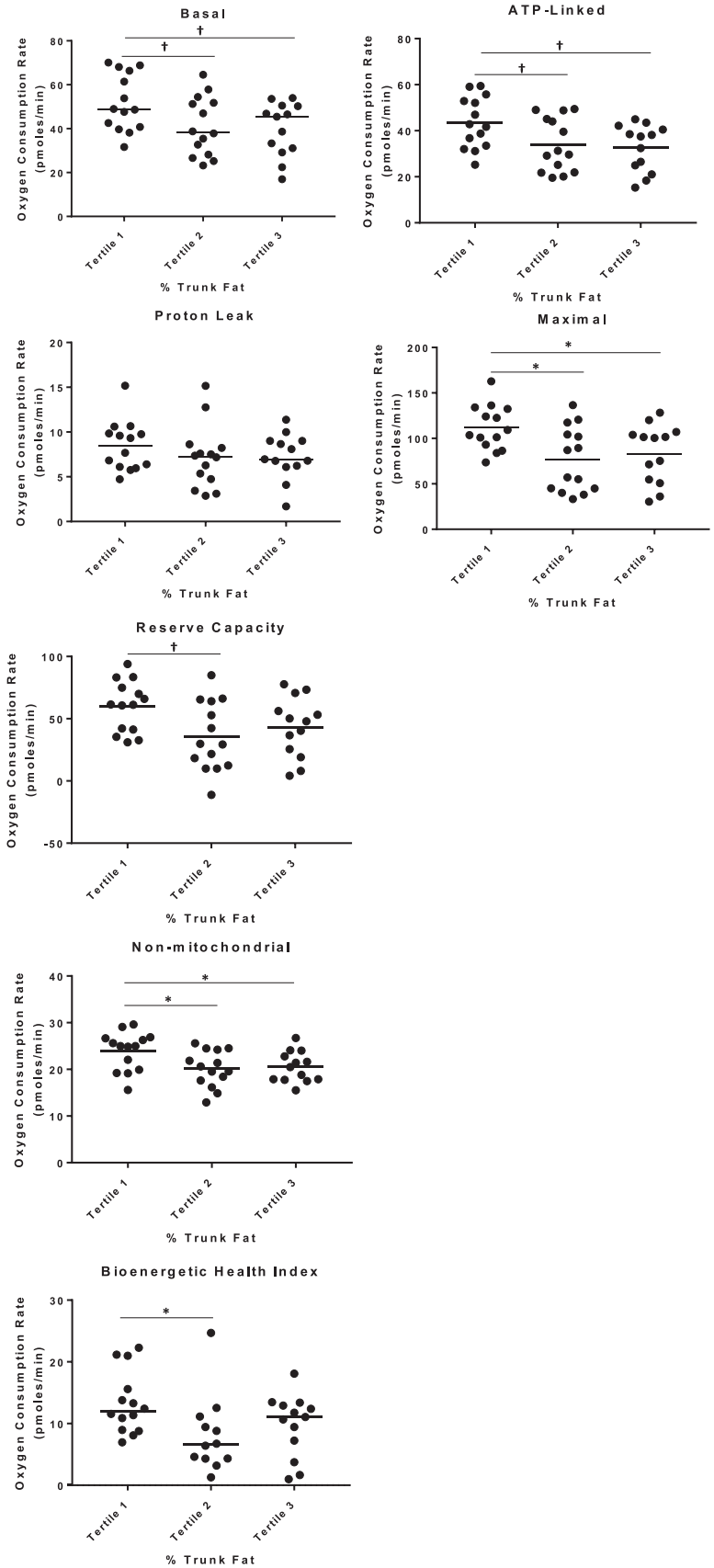

Fig. 3. Distribution of monocyte oxygen consumption rate by percent trunk fat tertiles in women living with HIV. Medians are indicated by the solid line. †=P < 0.07 Kruskal-Wallis result with Dunn test. *=P < 0.05 Kruskal-Wallis result with Dunn test.

Table 2
Correlation coefficients of monocyte oxygen consumption rate (pmol/min/10,000) cells with body composition in women living with HIV.

	Basal		Proton Leak		ATP-Linked		Maximal	
	Spearman	Partial[a]	Spearman	Partial[a]	Spearman	Partial[a]	Spearman	Partial[a]
BMI	−0.18	−0.23	−0.18	−0.05	−0.20	−0.25	−0.20	−0.24
Total fat mass (kg)[b]	−0.25	−0.02	−0.11	0.16	−0.27	0.01	−0.18	−0.06
Total lean mass (kg)[b]	−0.11	0.01	−0.07	−0.01	−0.11	0.01	−0.06	0.20
Visceral fat (gm)[b]	−0.20	0.06	−0.17	−0.15	−0.20	−0.09	−0.12	−0.07
Fat mass%	−0.23	**−0.36****	−0.08	−0.06	−0.25	**−0.39****	**−0.30***	**−0.37****
Lean mass%	0.23	**0.36****	0.08	0.06	0.25	**0.39****	**0.30***	**0.37****
Trunk fat%	−0.26	**−0.40****	−0.14	−0.14	−0.27	**−0.4*****	−0.22	**−0.34****
Leg fat%	−0.16	−0.19	0.07	0.17	−0.18	−0.22	**−0.35****	−0.29
RSMI[c]	−0.26	**−0.37****	−0.19	−0.14	−0.27	**−0.39****	−0.08	−0.17

	Reserve Capacity		Non-Mitochondrial		Bioenergetic Health Index		Basal ECAR		Maximal ECAR	
	Spearman	Partial[a]	Spearman	Partial[a]	Spearman	Partial[a]	Spearman	Partial[a]	Spearman	Partial[a]
BMI	−0.18	−0.28	**−0.29****	−0.21	−0.15	−0.30	−0.24	−0.27	−0.26	−0.30
Total fat mass (kg)[b]	−0.16	−0.10	−0.19	−0.01	−0.24	−0.13	−0.15	−0.15	−0.14	−0.14
Total lean mass (kg)[b]	0.08	0.21	0.10	0.21	0.03	0.24	0.14	0.14	0.18	0.19
Visceral fat (gm)[b]	−0.07	−0.04	−0.16	0.01	−0.09	0.18	−0.19	−0.18	−0.09	−0.09
Fat mass%	**−0.30***	**−0.35****	**−0.32****	**−0.33***	**−0.34****	**−0.3****	**−0.31****	**−0.32***	**−0.33****	**−0.33****
Lean mass%	**0.30***	**0.35****	**0.32****	**0.33***	**0.34****	**0.37****	**0.31****	**0.32***	**0.33****	**0.33****
Trunk fat%	−0.18	−0.30	−0.27	**−0.32***	−0.24	−0.27	−0.25	−0.29	−0.27	−0.29
Leg fat%	**−0.40*****	**−0.35****	**−0.32****	−0.27	**−0.44*****	**−0.45*****	−0.27	−0.23	**−0.31****	-0.27
RSMI[c]	−0.01	−0.11	−0.18	−0.22	−0.12	−0.13	−0.09	−0.11	−0.12	−0.13

* P = 0.05.
** P < 0.05.
*** P < 0.01.
[a] adjusted for age + race + smoking status.
[b] partial models also adjusted for BMI.
[c] Relative Skeletal Muscle Index.

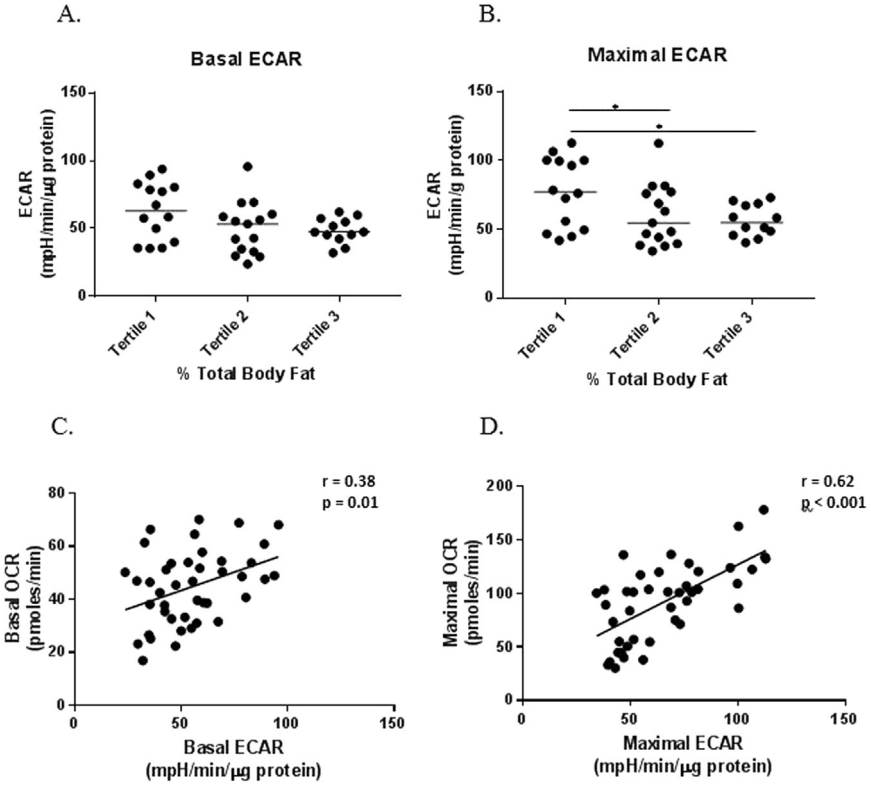

Fig. 4. Monocyte extracellular acidification rate (ECAR) of monocytes in women living with HIV. (A and B) Distribution of basal and maximal ECAR by percent trunk fat. Medians are indicated by the solid line. † = P < 0.06 Kruskal-Wallis result with Dunn test. * = P < 0.05 Kruskal-Wallis result with Dunn test. (C) Correlation of basal oxygen consumption rate (OCR) with basal ECAR. (D) Correlation of maximal OCR with maximal ECAR.

parameters of bioenergetic function in monocytes, including the BHI. This is consistent with recent finding that increased adiposity is associated with decreased mitochondrial function from lower body skeletal muscle biopsies [23].

Of particular interest is the decrease in the bioenergetic reserve capacity associated with increased fat mass and the positive association with lean mass. Reserve capacity is important because it will likely decrease the threshold at which the effects of oxidative stress result in bioenergetic dysfunction and the onset of fatigue syndromes [33,35]. In addition, we observed a negative correlation with ATP linked respiration with fat mass which suggests that mitochondrial energetic capacity is decreased with obesity. Using the same protocol for measurement of OCR the ECAR measurements were also obtained under basal conditions and after the addition of oligomycin, and our results may demonstrate a reliance on excess adiposity as a fuel substrate in this study population. Non-mitochondrial respiration is the term we ascribe to the oxygen consumption which remains after inhibition of mitochondrial function in the monocytes with antimycin, an inhibitor of mitochondrial electron transport. It is associated with greater obesity and could reflect inflammatory enzymes which consume oxygen such as cyclooxygenase [45]. However, it is important to note that we do not know the precise source of this oxygen consumption rate and indeed it could be a mitochondrial protein not inhibited by antimycin. The robust correlations between total body fat and mitochondrial dysfunction suggest that addressing excess adiposity may be as important as maintaining HIV viral suppression for the long term health of persons living with HIV infection.

Monitoring bioenergetic function in chronic diseases like HIV infection may facilitate early detection of disease risk in this population highly susceptible to complications from chronic inflammation [11–13]. However, certain limitations prohibit the generalization of these findings to all people living with HIV. Study participants were exclusively HIV+ women living in the Southeast and additional work comparing this population to an HIV− control group matched for BMI is a crucial next step; however, as this is an understudied population in HIV research our findings are critical to the care of a group experiencing increased HIV incidence. The cross-sectional nature of this study does not allow us to establish causality, and no corrections for multiple comparisons were done due to the small sample size. Additionally, as our cohort was aged < 55 years, we are unable to investigate the impact of expanded life span on bioenergetics health in WLH.

In summary, we found that WLH present with high levels of adiposity, and that greater total body fat, trunk fat, and leg fat are associated with an altered bioenergetic profile even among women with well-controlled HIV infection. Future studies that measure change in bioenergetic function over time and measure markers of inflammation will provide crucial information regarding the independent and interactive effects of body composition and HIV infection on bioenergetic health. The impact of lifestyle and pharmaceutical therapies designed to improve bioenergetic health among people living with HIV, thereby decreasing chronic disease risk, merits intense investigation.

Conflicts of interest

There are no conflicts of interest.

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