Arterial stiffness is associated with oxidative stress and endothelial activation among persons with treated HIV in Zambia

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Background: Cardiovascular disease (CVD) prevalence is rising among persons with HIV (PLWH) in sub-Saharan Africa. Oxidative stress and endothelial activation, resulting in reduced vascular compliance, are contributors to CVD risk. However, there is a paucity of vascular health data in this population.

Objectives: To assess the relationships of oxidative stress and endothelial activation with vascular stiffness among PLWH.

Method: Fifty-four PLWH on antiretroviral therapy > 5 years and 57 HIV-negative controls, all aged 18–45 years, were enrolled from the University Teaching Hospital, Lusaka, Zambia. Oxidative stress was measured by nitrotyrosine, a peroxynitrite biomarker, and endothelial activation by soluble intercellular adhesion molecule-1 (sICAM-1) plasma levels. Vascular compliance was measured using carotid-radial pulse wave velocity (crPWV) and arterial stiffness index (crASI).

Results: PLWH had higher sICAM-1 levels (median 345 ng/mL) compared to controls (275 ng/mL, p < 0.01), as well as higher nitrotyrosine levels (297 versus 182 nM; p = 0.02). Median crPWV was similar between the groups, but PLWH had higher crASI (2.4 versus 2.2 cm/ms; p < 0.05). After adjusting for age, fat mass, and blood pressure, the estimated effect of a one unit increase in nitrotyrosine on crPWV were twofold higher in the PLWH, but neither reached significance. In a model pooling all participants, there were significant differences in the relationship of nitrotyrosine with crPWV and crASI by HIV status.

Conclusion: PLWH in sub-Saharan Africa had significantly greater oxidative stress and endothelial activation compared to HIV-negative individuals. These factors may contribute to increased arterial stiffness and higher CVD prevalence in this population.

Keywords: Oxidative stress; endothelial activation, endothelial dysfunction; arterial stiffness, peroxynitrite.

Introduction

Globally, cardiovascular disease (CVD) is a leading cause of mortality and is estimated to comprise 54% of total deaths.1 The prevalence of CVD is on the rise in sub-Saharan Africa (SSA),2,3 and overlaps with a persisting epidemic of HIV infection.4 Evidence from high-income countries suggests that the incidence of CVD events in persons living with HIV (PLWH) is approximately 1.5–2.0-fold higher compared to age-matched HIV-negative individuals,5,6 and PLWH have a significantly higher risk of acute myocardial infarction (MI) compared with demographically and behaviourally similar HIV-negative individuals even after adjustment for Framingham risk factors, comorbidities and substance use.7,9,10 Notably, this risk differential persists despite suppression of plasma vireaemia by antiretroviral treatment (ART).4,7

At present, the vast majority of studies of CVD in PLWH are from cohorts in the United States of America (USA) and Europe,11 and there is a critical need to understand the factors contributing to CVD among PLWH in SSA, the region with the highest burden of HIV (comprising 61% of PLWH worldwide).12

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Persistent activation of the endothelium contributes to impaired vasodilatory properties, increased arterial stiffness and disrupted anticoagulatory pathways. These factors contribute to the development of CVD in the general population; they are heightened or increased in the setting of HIV infection and persist despite plasma viral suppression. Upon stimulation, the endothelium becomes activated, and there is upregulation of adhesion molecule expression, including soluble intercellular adhesion molecule 1 (sICAM-1). It has been observed that endothelial cell activation could also lead to endothelial dysfunction. Vascular tone is determined by many competing vasoconstrictor and vasodilator influences, as well as endothelial signalling factors such as nitric oxide. These are important regulators of arterial responsiveness. Among PLWH, there is increased expression of calcium-independent nitric oxide synthase, known as inducible nitric oxide synthase (iNOS); iNOS is induced in response to increased signalling molecules, for example pro-inflammatory cytokines.

In addition to higher levels of oxidative stress, PLWH have higher levels of free radicals, such as superoxide anion (O$_2^-$). There is considerable evidence that the vascular production of O$_2^-$ is increased in hypercholesterolaemia, diabetes mellitus, hypertension and cigarette use. The O$_2^-$ and nitric oxide react to produce the oxidant peroxynitrite. Peroxynitrite transits through cell membranes, in part through anion channels, and promotes DNA fragmentation, resulting in cellular dysfunction. Peroxynitrite affects both the structure and function of the endothelium and can trigger apoptosis and poly (adenosine diphosphate-ribose) polymerase (PARP)–dependent cell death. Peroxynitrite oxidises nitrates to form nitrotyrosine, which is the preferred biomarker for assessing plasma peroxynitrite levels, as observed by Maslov et al. These effects lead to a loss of endothelial response, which produces several observable changes in function, such as reduced vascular compliance and dilation resulting in increased arterial stiffness, which can be assessed in the clinical setting by measurement of the pulse wave velocity (PWV). Increased PWV has been reported among PLWH aged > 18 years compared to HIV-negative controls in a study in Cameroon, as well as Uganda. The prevalence of CVD is high among PLWH in Zambia, including lean individuals, unlike much of the evidence from the USA and Europe and among individuals with generally higher body mass indexes (BMIs). Given the rising rates of CVD among PLWH in SSA, it is important to understand endothelial dysfunction, a putative contributory mechanism. There are very few data on oxidative stress and vascular compliance or endothelial activation among PLWH in SSA. To address this research gap, we assessed the relationships of oxidative stress and endothelial activation with vascular function among persons living with and without HIV in Zambia.

**Methods**

We conducted a cross-sectional study among adults with and without HIV recruited from the University Teaching Hospital (UTH), an academic tertiary-care centre in Lusaka, Zambia, between September 2018 and June 2019. All participants with HIV were on an ART regimen of efavirenz, tenofovir and emtricitabine (i.e. the combination pill Atripla) for at least 5 years before enrolment. Plasma HIV-1 RNA testing was not available for routine care, but all participants met the clinical criteria for presumed viral suppression, including the absence of clinical disease progression, as well as reconstituted and stable CD4 cell counts. Participants without HIV were recruited from among the individuals who accompanied the PLWH to the ART clinic and from the UTH outpatient medicine department. They were confirmed negative for HIV using rapid tests (Uni-Gold HIV rapid test).

We excluded persons with a prior diagnosis of hypertension, MI, heart failure, any CVD, elevated cholesterol, diabetes mellitus, respiratory diseases such as bronchitis and asthma, pregnant women, tobacco smokers and those with any self-reported acute infections. Information on non-communicable disease risk factors and general sociodemographic data were collected using the World Health Organization (WHO) STEPS survey instrument. Height and weight were measured using a Micro T3 PW-BMI digital physician scale. Blood pressure was measured in sets of three using an HEM-757 (Omron, Kyoto, Japan) blood pressure–measuring machine.

Enrolment was stratified by BMI to obtain a relatively uniform distribution of underweight (< 18.5 kg/m$^2$), normal weight (18.5 kg/m$^2$ – 24.9 kg/m$^2$) and overweight (≥ 25.0 kg/m$^2$) participants.

**Plasma biomarkers**

All participants fasted for at least 8 h. Blood was collected in ethylene-diamine-tetra-acetic acid (EDTA) tubes and spun at 2700 rpm for 15 min before storing plasma at –80 °C. The endothelial activation marker sICAM-1 was measured using an enzyme-linked immunosorbent assay (ELISA; RAB0219 human sICAM-1 ELISA kit, Sigma Aldrich, Burlington, MA, United States). To measure oxidative stress, nitrotyrosine, a surrogate measure of plasma peroxynitrite, was also measured by ELISA (ab210603; Abcam, Cambridge, Massachusetts, United States [US]). The absorbance of both ELISA kits was read at 450 nm wavelengths using a 96-well microplate reader (BioTek model EL800, BioTek Instruments, Winooski, VT, United States, US).

**Body composition**

Waist circumference and hip circumference were measured using flexible tape. The waist–hip ratio was calculated using the two values. Body fat mass was estimated using bioelectrical impedance analysis (Tanita body fat monitor, Tokyo, Japan). This method provides a detailed full-body and segmental-body composition analysis, including information on the weight, body fat percentage, body fat...
mass, BMI, fat free mass, estimated muscle mass, total body water and basal metabolic rate for the entire body, using bioelectrical impedance analysis or direct-segmental bioelectrical impedance analysis technology.

**Endothelial function measurement**

To assess arterial stiffness, we used the carotid–radial pulse wave velocity (crPWV), a measure of arterial stiffness for muscular arteries and an indicator of early alterations in peripheral vascular dynamics. The crPWV and carotid–radial arterial stiffness index (crASI) were obtained using the Compilior analyse unit (V1.9 beta version 2013; ALAM-Medical, Saint-Quentin-Fallavier, France) according to the manufacturer’s protocol. Participants rested for at least 5 min prior to measurement. The carotid probe was gently placed on the neck at the point where the carotid pulse was most palpable. The radial artery probe was applied to the radial artery and adjusted to obtain the best signal. Pressure signals from the sensors were displayed on the screen of the Compilior analysis unit to obtain the crPWV, heart rate and transit time. This was repeated two more times, and the mean of three readings was included in the analysis.

**Statistical analysis**

Demographic, clinical and oxidative stress variables, sICAM-1, crPWV and arterial stiffness index (ASI) were compared by HIV status using Wilcoxon rank-sum and chi-square tests (STATA V15.0). Continuous variables were expressed as medians (interquartile ranges). Multiple linear regression models were used to assess the relationships between the independent variable (nitrotyrosine) and the dependent variables (crPWV and crASI); these models were adjusted for BMI, blood pressure and age in both HIV+ and HIV– groups because of the sample size. The pooled data (HIV+ and HIV– groups) were adjusted for age, sex, body fat per cent and haemodynamic measures of pulse, heart rate and systolic blood pressure (SBP).

We included an interaction term between HIV status and nitrotyrosine level in the pooled models to assess whether the relationship of nitrotyrosine with the outcomes differed by HIV status. The covariates were selected *a priori* based on the published risk factors for arterial stiffness, including age, sex, body fat per cent and the haemodynamic measures of pulse, heart rate, SBP and diastolic blood pressure (DBP). The association models were constructed stepwise, adjusting for variables to note which model had the best associative relationship with the outcome variable. The models with the highest $R^2$ (the proportion of the variance for a dependent variable that is explained by an independent variable) and lowest Akaike information criterion selected for final interpretation. Partial effect plots were also constructed to assess the relationship of nitrotyrosine with arterial stiffness by sex. The normality of the outcome data was assessed using Q–Q plots and validated using the Shapiro–Wilk test. Neither required transformation.

**Ethical considerations**

All participants gave written informed consent. Ethical approval for the study was obtained from the University of Zambia Biomedical Research Ethics Committee (UNZABREC – IRB00001131 of IORG0000774) and Zambia’s National Health Research Authority (NHRA).

**Results**

We recruited 111 participants, 54 (36 female; 18 male) with HIV and 57 (37 female; 20 male) without HIV, between 18 and 45 years old (Table 1). The participants with HIV were older ($p < 0.001$) and had lower body weight ($p = 0.04$). The medians for most of the other clinical characteristics were comparable.

The two cohorts had comparable medians of crPWV, but a higher crASI of 2.4 cm/s (interquartile range [IQR] 2.1 cm/s – 2.7 cm/s) was observed among PLWH vs 2.2 cm/s (IQR 1.8 cm/s – 2.5 cm/s) ($p < 0.05$) compared to HIV-negative participants. PLWH had higher levels of nitrotyrosine (297 nM [IQR 171 nM – 558 nM] vs 182 nM [IQR 156 nM – 290 nM]; $p = 0.02$) as well as sICAM-1 (345 nM [IQR 252 nM – 412 nM] vs 275 nM [IQR 164 nM – 350 nM]; $p < 0.01$) compared to uninfected controls.

**Association between endothelial dysfunction and oxidative stress**

There was no significant association between nitrotyrosine level and crPWV or crASI in the HIV-positive or HIV-negative groups, as highlighted in Table 2. We found that BMI and SBP were associated with both arterial stiffness outcomes in the PLWH. Among the HIV-negative participants, only age was associated with arterial stiffness, as summarised in Table 2.

**Association between endothelial dysfunction and oxidative stress in pooled data of HIV groups**

The relationships between oxidative stress and outcomes of endothelial dysfunction as measured by PWV and the ASI differed between the HIV-negative participants and PLWH. Comparisons among the models constructed were evaluated using AIC and coefficient of determination ($R^2$) values to determine the best performing model. No significant associations were observed using the linear regression models (non-splined), with or without the interaction terms (Appendix 1: Tables 1-A1 and 2-A1). At a glance, the relationships in the model included some that were not straight enough for linear regression, as shown in the *dfbeta* analysis in the Appendix 1 data (Figure 1-A1). There were apparent bends, clumps and outliers in some plots. It appeared that examination of the multiple regression model using splines may give a better inference of the outcome. Models 4 and 8 (with splines) for crPWV and crASI, respectively, were therefore selected in this study, as they...
TABLE 1: Clinical characteristics comparing persons living with HIV and the control (HIV-negative persons).

| Variable                      | PLWH            | HIV negative       |
|-------------------------------|-----------------|--------------------|
|                               | n   | %   | Median | IQR | n   | %   | Median | IQR | P       |
| Population size               | 54  | 49  | -     | -   | 57  | 51  | -     | -   |         |
| (female)                      | 36  | 67  | -     | -   | 37  | 65  | -     | -   | 0.85    |
| Age (years)                   | -   | -   | 40    | 20–45 | -   | -   | 24    | 18–43 | < 0.001* |
| Weight (kg)                   | -   | -   | 54.8  | 49.1–62.2 | -   | -   | 60.5  | 53.2–77.9 | 0.04    |
| Length (cm)                   | -   | -   | 164   | 158–159 | -   | -   | 162   | 158–172 | 0.77    |
| BMI (kg/m²)                   | -   | -   | 20.4  | 17.8–26.8 | -   | -   | 22.8  | 18.4–28.3 | 0.11    |
| Trunk fat (%)                 | -   | -   | 20.1  | 11.8–28.2 | -   | -   | 21.2  | 12.1–32.2 | 0.68    |
| Body fat per cent (Tanita (%))| -   | -   | 23.3  | 15.0–31.7 | -   | -   | 23.3  | 14.1–34  | 0.75    |
| Total body fat mass (Tanita) (kg)| -   | -   | 12.3  | 7.1–23.1 | -   | -   | 14.4  | 7.0–25.7 | 0.50    |
| Total body fat mass (BodPod) (kg)| -   | -   | 12.0  | 9.2–25.5 | -   | -   | 14.0  | 6.6–29.2 | 0.75    |
| Waist–hip ratio               | -   | -   | 0.81  | 0.81–0.90 | -   | -   | 0.798 | 0.70–0.80 | 0.02*   |
| Central SBP (mmHg)            | -   | -   | 113   | 103–121 | -   | -   | 112   | 102–124 | 0.65    |
| SBP (mmHg)                    | -   | -   | 110   | 103–123 | -   | -   | 115   | 110–123 | 0.06    |
| DBP (mmHg)                    | -   | -   | 80.0  | 74–87  | -   | -   | 79.8  | 75–87   | 0.84    |
| Mean arterial pressure (mmHg) | -   | -   | 93    | 86–101 | -   | -   | 90    | 87–98   | 0.48    |
| Pulse (bpm)                   | -   | -   | 78    | 69–85  | -   | -   | 73    | 65–80   | 0.03*   |
| Alcohol consumption (yes)     | 30  | -   | -     | 24   | -   | -   | -     | -     | 0.16    |
| crPWV (m/s)                   | -   | -   | 10    | 9–11   | -   | -   | 10    | 8–11    | 0.19    |
| crASI (cm/ms)                 | -   | -   | 2.4   | 2.1–2.7 | -   | -   | 2.2   | 1.8–2.5 | 0.048*  |
| sICAM-1 (ng/mL)               | -   | -   | 345   | 252–412 | -   | -   | 275   | 164–350 | < 0.01* |
| Nitrotyrosine (nM)            | -   | -   | 297   | 171–558 | -   | -   | 182   | 156–290 | 0.02*   |

*, P-values < 0.05.
IQR, interquartile range; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PLWH, persons living with HIV; crPWV, carotid–radial pulse wave velocity; crASI, carotid–radial arterial stiffness index; sICAM-1, soluble intercellular adhesion molecule 1.

TABLE 2: Multiple linear regression analyses of arterial stiffness and oxidative stress.

| Outcome variable                          | Coef  | CI     | P      | Coef  | CI     | P      |
|------------------------------------------|-------|--------|--------|-------|--------|--------|
| crPWV as outcome variable                |       |        |        |       |        |        |
| Age                                       | 0.055 | -0.030 | 0.140 | 0.197 |        |        |
| Nitrotyrosine                             | -0.014| -0.036 | 0.009 | 0.225 |        |        |
| BMI                                       | -0.093| -0.185 | 0.002 | 0.046*|        |        |
| SBP                                       | 0.052 | 0.018  | 0.086 | 0.003*|        |        |
| crASI as outcome variable                 |       |        |        |       |        |        |
| Age                                       | 0.011 | -0.010 | 0.033 | 0.290 |        |        |
| Nitrotyrosine                             | -0.003| -0.009 | 0.002 | 0.270 |        |        |
| BMI                                       | -0.047| -0.070 | 0.023 | < 0.001*|        |        |
| SBP                                       | 0.013 | 0.005  | 0.022 | 0.003*|        |        |

*, P-values < 0.05.
Note: Model adjusted for age, SBP and BMI in both groups.
PLWH, persons living with HIV; CI, confidence interval; coef, β coefficient; crPWV, carotid–radial pulse wave velocity; crASI, carotid–radial arterial stiffness index; BMI, body mass index; SBP, systolic blood pressure.

Performed better at showing the relationship between the predictive variables and the outcome variables. All interpretations were based on these models. After adjusting for age, sex, waist circumference, hip circumference, per cent body fat, SBP, DBP and HIV status, the associations of nitrotyrosine levels with crPWV and crASI as the outcomes were as shown in Table 3.

Nitrotyrosine levels were significantly associated with crPWV (β = 0.12; p = 0.03). Participants’ HIV status had a significant influence on this relationship, as observed by the significant interaction terms between nitrotyrosine level and HIV status (Table 3). The crASI model showed that HIV status also affected the relationship between nitrotyrosine level and crASI, as illustrated by the significant interaction term between nitrotyrosine level and HIV status in the model (Table 3).

In the partial effect plots, crPWV and crASI values rose with increases in nitrotyrosine levels in all participants (Figure 1). Higher nitrotyrosine levels have been associated with decreased nitric oxide and thus with reduced ability for vasodilation. This could represent a mechanism for the arterial stiffness, as noted.

In PLWH with nitrotyrosine levels below 167 nM, nitrotyrosine levels were positively associated with crPWV and crASI, as shown in Figure 1. The crPWV value reached a climax of 10.30 m/s and 9.70 m/s in male and female participants, respectively. The crASI value reached a climax of 2.40 cm/ms and 2.43 cm/ms in male and female participants, respectively. Above 167 nM, an inverse association was observed between nitrotyrosine level and crPWV as well as crASI, until 250 nM. Beyond this, an increase in nitrotyrosine level was positively associated with crPWV and crASI.
Among the HIV-negative participants, nitrotyrosine levels were positively associated with crPWV and crASI below 260 nM, as shown in Figure 1. After this point, there was an inverse association between nitrotyrosine and arterial stiffness. Similar observations were noted for the male and female participants. The arterial stiffness values at any level of plasma nitrotyrosine were higher in male participants than those in the female participants.

**Discussion**

Our cohort of PLWH showed higher oxidative stress compared to the HIV-negative controls, which was associated with increased arterial stiffness. Specifically, we observed that a unit increase in plasma nitrotyrosine was associated with a significant increase in crPWV of 0.12 m/s. This suggests that endothelial dysfunction in PLWH may be associated with oxidative stress-related factors. Nitrotyrosine is a biomarker of peroxynitrite, which causes DNA fragmentation, resulting in cellular dysfunction and affecting both the structure and function of the endothelium. This reduces vascular compliance, as conceptualised in Figure 2. Other studies have fortified the idea of peroxynitrite having detrimental effects on the endothelium, which may lead to a disturbance in vascular function. Such observations could result from the activity of HIV. They also observed that ART for 1 month was associated with a significant increase in PWV and ASI in arteries. Furthermore, increased pulse pressure and augmentation index have been observed among ART-experienced patients compared to untreated PLWH. Because our study cohort comprised PLWH on stable ART who were assumed to be virally suppressed, endothelial cell damage and the lack of ability to execute its physiological functions could, in part, explain the observations in the PLWH. Our results comparing plasma nitrotyrosine levels in healthy Zambian (black African) populations and PLWH are, as far as we know, the first to be documented. Further, we observed contributory effects of oxidative stress to endothelial dysfunction among PLWH.

Endothelial dysfunction can be measured by the rate of transmission of the arterial pulse pressure wave from the carotid artery, an upstream pressure point, to a defined downstream pressure point (e.g. the radial artery – carotid–radial). The crPWV provides information about the peripheral muscular arteries.

The PLWH in our study had no significant difference in crPWV compared to the HIV-negative participants, implying that virally suppressed PLWH have crPWV values similar to HIV-negative persons. These findings were similar to those of Fourie et al., who did not find significant differences in mean crPWV by HIV status. However, Pretorius observed that crPWV values were significantly higher among PLWH compared to HIV-negative persons (11.26 m/s vs 10.68 m/s; p = 0.03) at baseline. And in a follow-up study, after 3 years of ART, no significant differences were found in crPWV values between the two populations. This supports the idea that virally suppressed PLWH have similar crPWV values to HIV-negative persons after at least 5 years of viral suppression. It may also mean that crPWV is not a good marker for endothelial dysfunction in PLWH.

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**TABLE 3: Multiple linear regression analyses of endothelial function and oxidative stress.**

| Outcome variable | crPWV | crASI |
|------------------|-------|-------|
| Sex (male)       | 0.444 | –0.347 | 0.236 |
| Age              | 0.063 | 0.008 | 0.013 |
| Waist circumference | –0.023 | –0.064 | 0.018 |
| Hip circumference | 0.022 | –0.020 | 0.016 |
| Body fat         | –0.033 | –0.080 | 0.180 |
| SBP              | –0.028 | –0.064 | 0.118 |
| DBP              | 0.121 | 0.062 | 0.018 |
| rcs_Nitrotyrosine_1 | 0.122 | 0.101 | 0.032 |
| rcs_Nitrotyrosine_2 | –0.213 | –0.463 | 0.067 |
| rcs_Nitrotyrosine_3 | 0.472 | –0.308 | 0.236 |
| HIV status (positive) | 3.866 | 0.403 | 0.029 |
| HIV interaction rcs_Nitrotyrosine_1 | –0.196 | –0.354 | 0.014 |
| HIV interaction rcs_Nitrotyrosine_2 | 0.393 | 0.055 | 0.023 |
| HIV interaction rcs_Nitrotyrosine_3 | –1.072 | –2.065 | 0.035 |
| Constant         | –0.393 | –3.879 | 0.825 |

| R²               | 0.356 | – | 0.428 |
| F-test           | 3.627 | – | 4.872 |
| AIC              | 437.620 | – | 124.220 |
| Number of observations | 107 | – | 106 |
| Prob > F         | 0.000 | – | 0.000 |
| BIC              | 477.720 | – | 164.180 |

* P-values < 0.05.

crPWV, carotid–radial pulse wave velocity; crASI, carotid–radial arterial stiffness index; DBP, diastolic blood pressure; SBP, systolic blood pressure; rcs, restricted cubic spline; var, variable; HIV interaction rcs_Nitrotyrosine, the interaction between HIV status and nitrotyrosine levels with restricted cubic spline with 3 knots; sex interaction rcs_Nitrotyrosine, the interaction between sex and nitrotyrosine levels with restricted cubic spline with 3 knots; coef, beta coefficient; CI, confidence interval; AIC, Akaike information criterion; Prob, probability; BIC, Bayesian information criterion.
The ASI is another measure of arterial stiffness quantifying the contour of the oscillometric envelope of peripheral pulse waveform. With increasing arterial stiffness, the arteries become harder to collapse by the application of external pressure. Therefore, with increased arterial stiffness, the oscillometric envelope becomes much flatter. In this study, the crASI, unlike crPWV factors in the participant’s height, was significantly higher among the PLWH (+0.2 cm/ms), implying that there may be an increase in arterial stiffness in this group. Persons living with HIV may have a significantly higher stiffness of the medium, muscular-type arteries.

In the SSA population, crASI seems to be more sensitive in detecting arterial stiffness of the peripheral arteries compared to crPWV. Stiffening of these arteries predominantly results in functional variation, such as increased diastolic blood pressure because of increased muscle tone. Stiffness in the peripheral muscular arteries is of potential clinical importance. Peripheral arterial stiffness may be specifically associated with peripheral vascular disease, which is linked to hypertension and diabetes, both of which are risk factors for CVD. We further observed that oxidative stress as measured by plasma nitrotyrosine level was associated with increased arterial stiffness after adjusting for blood pressure, age and body fat mass, in line with the conceptual model in Figure 2. Our models showed that increased oxidative stress is associated with higher arterial stiffness as measured by crPWV. Further, the significant interaction term between HIV and both crPWV and crASI, specifically nitrotyrosine’s significantly different association with crASI and crPWV between PLWH and HIV-negative persons, suggests that the association between oxidative stress and arterial stiffness was influenced by HIV status. Gurovich et al. also reported an association between plasma nitrotyrosine and PWV in PLWH. We also observed that the male participants had higher arterial stiffness compared to the female participants, possibly suggesting the need for a more robust study to ascertain the predictability of arterial stiffness by nitrotyrosine level stratified by sex.

The endothelial activation marker, sICAM-1, was higher among PLWH. Stimulation of the endothelium results in
Conclusion

Persons living with HIV in Zambia exhibit increased endothelial dysfunction, as shown by their significantly higher crASI values compared to HIV-negative persons. The PLWH in our study also showed significantly higher endothelial activation markers. The nitrotyrosine levels were elevated among PLWH, signifying an increase in oxidative stress in this group of individuals. Our models revealed that a high nitrotyrosine level was associated with arterial stiffness (crPWV) in a non-linear relationship. Further studies evaluating crPWV, crASI and nitrotyrosine levels from the time of HIV diagnosis will help elucidate how the observed results may vary with time and potentially reveal causal relationships and mechanisms. This will facilitate the individualisation of the treatment of PLWH, considering oxidative stress levels (nitrotyrosine levels) and endothelial activation (sICAM-1) to lower arterial stiffness. There is also a need to determine if these findings are representative of PLWH in Zambia or are more broadly generalisable.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors’ contributions

T.C., F.G., D.C.H. and J.R.K. conceived and planned the research, analysis and manuscript. T.C., F.G., D.C.H. and J.R.K. contributed to the sample preparation. T.C. and L.K. carried out the experiments. T.C., F.G., D.C.H. and J.R.K. contributed to the interpretation of the results. T.C. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Data availability

The data that support the findings of this study are available from the corresponding author, T.C., upon request.
Paisible AL, Chang CC, So-Armah KA, et al. HIV infection, cardiovascular disease and HIV infection, cardiovascular disease and risk factors in Africa: A systematic review and meta-analysis. J Intern Med. 2018;284(3):249–262.

Triant VA, Lee H, Hadigan C, et al. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus infection. J Am Coll Cardiol. 2017;24(10):1043–1050. https://doi.org/10.1016/j.jacc.2017.07.029

Martin-Igualacel R, Llibre JM, Fris-Moller N. Risk of cardiovascular disease in an HIV-infected population: Where are we now? Curr HIV/AIDS Rep. 2015;12(4):375–387. https://doi.org/10.1007/s11904-015-0295-9

Freiberg MS, Chang CC, Kuhler LH, et al. HIV infection and the risk of acute myocardial infarction. JAMA Intern Med. 2013;173(8):614–622. https://doi.org/10.1001/jamainternmed.2013.3728

Triant VA, Lee H, Hadigan C, et al. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus infection. J Clin Endocrinol Metab. 2007;92(7):2505–2512. https://doi.org/10.1210/jc.2006-1590

Paasivirta AI, Chang CC, So-Armah KA, et al. Risk of acute myocardial infarction. J Am Coll Cardiol. 2017;24(16):1746–1758. https://doi.org/10.1016/j.jacc.2017.07.029

So-Armah K, Benjamin LA, Bloomfield G, et al. HIV and cardiovascular disease. Lancet. HIV 2020;7(4):e279–e293. https://doi.org/10.1016/S2352-3018(20)30036-9

UNAIDS. UNAIDS data 2019 [homepage on the Internet]. UNAIDS; 2019 [cited 2019 July 25]. Available from: https://www.unaids.org/sites/default/files/media_asset/2019-UNAIDS-data_en.pdf

Houe P. Impact of HIV infection on diabetic function and left ventricular mass. Circ Heart Fail. 2010;3(1):132–139. https://doi.org/10.1161/CIRCHEARTFAILURE.109.859493

Drexler H, Hornig B. Endothelial dysfunction in human disease. J Mol Cell Cardiol. 1999;31(1):53–60. https://doi.org/10.1016/S0022-2828(98)00084-8

Mazzcuotto P, Caruso A, Caccur F. HIV-1 infection, microenvironment and endothelial cell dysfunction. New Microbiol. 2016;39(3):163–173.

Iborrián FJ, Komarow L, Parker RA, et al. Endothelial function in human immunodeficiency virus-infected patients with and without antiretroviral therapy. J Am Coll Cardiol. 2002;40(3):521–528. https://doi.org/10.1016/S0735-1097(02)01991-5

Ye C, Pan Y, Xu X, et al. Pulse wave velocity in elastic and muscular arteries: Tracking stability and association with anthropometric and hemodynamic measurements. Hypertens Res. 2016;39(11):786–791. https://doi.org/10.1038/hr.2016.67

Solages A, Vita JA, Thornton DJ, et al. Endothelial function in HIV-infected persons. Clin Infect Dis. 2006;43(1):1325–1332. https://doi.org/10.1086/503261

Torres-Narvaez JC, Perez-Torres I, Castrenjon-Tellez V, et al. The role of the activation of the TRPV1 receptor and of nitric oxide changes in endothelial and cardiac function and biomarker levels in hypertensive rats. Int J Environ Res Public Health. 2019;16(19):3576.

Phiri S, Goma F, MS SM. Effects of ART on endothelial dysfunction: Role of superoxide anion. J Hypertens. 2001;19(5):891–897. https://doi.org/10.1097/00042140-200109000-00008

Ivanov AV, Valuev-Elliston VT, Ivanova ON, et al. Oxidative stress during HIV infection: Possible mechanisms and consequences. Oxid Med Cell Longev. 2016;2016:8910396. https://doi.org/10.1155/2016/8910396

Oghara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide production. J Clin Invest. 1993;91(6):2546–2551. https://doi.org/10.1172/JCI11464

Guzik TJ, Mussa S, Gastaldl D, et al. Mechanisms of increased vascular superoxide production in human diabetes mellitus: Role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation. 2002;105(14):1656–1662. https://doi.org/10.1161/01.CIR.000002748.58444.08

Kumar KV, Das UN. Are free radicals involved in the pathobiology of human essential hypertension? Free Radic Res Commun. 1993;19(1):59–66.
51. Gurovich A, Avery J, Holtgrieve N, et al. Flow-mediated dilation is associated with endothelial oxidative stress in human venous endothelial cells. Vasc Med. 2014;19(4):251–256. https://doi.org/10.1177/1358863X14537546

52. Ren Z, Yao Q, Chen C. HIV-1 envelope glycoprotein 120 increases intercellular adhesion molecule-1 expression by human endothelial cells. Lab Invest. 2002;82:245–255. https://doi.org/10.1038/labinvest.3780418

53. Abdi Al MM, Kiragu E, Bull PC. Measuring soluble ICAM-1 in African populations. PLoS One. 2014;9(10):e108956. https://doi.org/10.1371/journal.pone.0108956

54. James HM, Julian HE, Silvia B, et al. Viral suppression after 12 months of antiretroviral therapy in low- and middle-income countries: A systematic review. WHO. 2013;91:313–388. https://doi.org/10.2471/BLT.12.112946

55. Van Vonderen MG, Hassink EA, Van Agtmael MA, et al. Increase in carotid artery intima-media thickness and arterial stiffness but improvement in several markers of endothelial function after initiation of antiretroviral therapy. J Infect Dis. 2009;199(8):1186–1194. https://doi.org/10.1086/597475

Appendix starts on the next page→
Appendix 1
Supplementary material

The following models were used to observe the effect of nitrotyrosine level on crPWV and crASI. All the models include adjustments for age, sex, waist circumference, hip circumference, per cent body fat, SBP, DBP and HIV status. Among the models are one with splines included to note the more fluid (more robust) relationship between the changes in nitrotyrosine levels and those in crPWV or crASI values, as well as one model with the inclusion of an interaction term of nitrotyrosine and HIV status. The final models used to represent these relationships were those showing a higher $R^2$ value and lower Akaike information criterion (AIC) estimator.

![Scatter plot matrix of data showing all possible plots for specified variables with dfbeta for linear regression.](http://www.sajhivmed.org.za)

Figure 1-A1 continues on the next page →
FIGURE 1-A1 (Continues...): Scatter plot matrix of data showing all possible plots for specified variables with df beta for linear regression.
| Variable                  | Model 1†                                  | Model 2‡                                  | Model 3§                                  | Model 4¶                                  | Model 5¶                                  |
|--------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|
|                         | Coef | CI          | F statistic | Coef | CI          | F statistic | Coef | CI          | p  | F statistic | Coef | CI          | F statistic | Coef | CI          | p  | F statistic |
| Sex (male)               | 0.491 | -0.479 – 1.461 | -          | 0.508 | -0.47 – 1.487 | -          | 0.477 | -0.511 – 1.465 | - | -          | 0.444 | -0.347 – 1.23 | - | -          |
| Age                      | 0.066* | 0.008 – 0.124 | -          | 0.065* | 0.007 – 0.124 | -          | 0.066* | 0.008 – 0.125 | - | -          | 0.063* | 0.008 – 0.133 | - | -          |
| Waist circumference      | -0.027 | -0.075 – 0.021 | -          | -0.028 | -0.078 – 0.021 | -          | -0.027 | -0.077 – 0.022 | - | -0.023 | -0.064 – 0.018 | - | -          |
| Hip circumference        | 0.020 | -0.018 – 0.058 | -          | 0.020 | -0.018 – 0.058 | -          | 0.021 | -0.018 – 0.059 | - | -0.022 | -0.020 – 0.064 | - | -          |
| Body fat                 | -0.024 | -0.082 – 0.034 | -          | -0.022 | -0.081 – 0.036 | -          | -0.026 | -0.085 – 0.033 | - | -0.033 | -0.080 – 0.015 | - | -          |
| SBP                      | -0.019 | -0.059 – 0.021 | -          | -0.019 | -0.059 – 0.022 | -          | -0.017 | -0.058 – 0.025 | - | -0.028 | -0.064 – 0.007 | - | -          |
| DBP                      | 0.102** | 0.037 – 0.168 | -          | 0.102** | 0.036 – 0.168 | -          | 0.101** | 0.033 – 0.169 | - | 0.121** | 0.062 – 0.180 | - | -          |
| HIV status               | -0.229 | -1.174 – 0.167 | 0.057 | -1.775 – 1.889 | 0.175 | -1.716 – 2.067 | 3.866* | 0.403 – 7.328 | - | -          | -          | -          |
| Nitrotyrosine            | -0.010 | -0.026 – 0.007 | -0.006 | -0.033 – 0.021 | -          | -0.009 | -0.085 – 0.067 | 0.122* | 0.101 – 0.234 | - | -          | -          | -          |
| rcs_Nitrotyrosine_1      | -    | -          | -          | -0.006 | -0.041 – 0.028 | -          | -0.007 | -0.029 – 0.043 | - | -0.196* | -0.354 – 0.039 | - | -          |
| rcs_Nitrotyrosine_2      | -    | -          | -          | -0.033 | -0.151 – 0.218 | -          | -0.213 | -0.463 – 0.038 | - | -          | -          | -          |
| rcs_Nitrotyrosine_3      | -    | -          | -          | 0.200 | -0.811 – 0.412 | -          | 0.472 | -0.308 – 1.252 | - | -          | -          | -          |
| HIV interaction nitrotyrosine | -   | -          | -          | -0.006 | -0.041 – 0.028 | -          | -0.007 | -0.029 – 0.043 | - | -0.196* | -0.354 – 0.039 | - | -          |
| HIV interaction rcs_Nitrotyrosine_1 | - | - | - | -0.033 | -0.151 – 0.218 | - | -0.213 | -0.463 – 0.038 | - | - | - | - |
| HIV interaction rcs_Nitrotyrosine_2 | - | - | - | -0.200 | -0.811 – 0.412 | - | 0.472 | -0.308 – 1.252 | - | - | - | - |
| HIV interaction rcs_Nitrotyrosine_3 | - | - | - | -0.006 | -0.041 – 0.028 | - | -0.007 | -0.029 – 0.043 | - | -0.196* | -0.354 – 0.039 | - | - |
| Constant                 | 3.083 | -1.052 – 7.219 | 2.989 | -1.198 – 7.176 | 2.527 | -2.116 – 7.170 | 3.866* | 0.403 – 7.328 | - | -0.393 | -3.879 – 3.092 | - | - |
| Regression details       | -    | -          | F(9, 97) = 4.61 | -0.001 | F(12, 94) = 3.56 | < 0.001 | F(14,92) = 3.63 | - | -0.001 | F(14,92) = 3.63 | - | - |
| R²                       | 0.300 | -          | 0.300 | -          | 0.313 | -          | 0.356 | -          | -0.001 | F(14,92) = 3.63 | - | - |
| F-test                   | 4.601 | -          | 4.123 | -          | 4.356 | -          | 4.356 | -          | -          | -          | -0.001 | F(14,92) = 3.63 | - | - |
| AIC                      | 436.56 | -          | 438.42 | -          | 440.53 | -          | 437.62 | -          | -0.001 | F(14,92) = 3.63 | - | - |
| No. of observations      | 107 | -          | 107 | -          | 107 | -          | 107 | -          | -          | -          | -0.001 | F(14,92) = 3.63 | - | - |

DBP, diastolic blood pressure; SBP, systolic blood pressure; rcs, restricted cubic spline; var, variable; HIV interaction rcs_Nitrotyrosine, the interaction between HIV status and nitrotyrosine levels with restricted cubic spline with 3 knots; sex interaction rcs_Nitrotyrosine, the interaction between sex and nitrotyrosine levels with restricted cubic spline with 3 knots; var, variable; HIV interaction rcs_Nitrotyrosine, the interaction between HIV status and nitrotyrosine levels with restricted cubic spline with 3 knots; sex interaction rcs_Nitrotyrosine, the interaction between sex and nitrotyrosine levels with restricted cubic spline with 3 knots; coef, beta coefficient; CI, confidence interval; AIC, Akaike information criterion; Prob, probability; BIC, Bayesian information criterion.

*, p < 0.5; **, p < 0.01.

†, Model 1 was adjusted for age, sex, waist circumference, hip circumference, percentage body fat, SBP, DBP and HIV status; ‡, Model 2 was adjusted for age, sex, waist circumference, hip circumference, percentage body fat, SBP, DBP and HIV status with an interaction term between HIV status and nitrotyrosine; §, Model 3 was adjusted for age, sex, waist circumference, hip circumference, percentage body fat, SBP, DBP and HIV status with an interaction term between HIV status and nitrotyrosine (with splines on nitrotyrosine); ¶, Model 4 was adjusted for age, sex, waist circumference, hip circumference, percentage body fat, SBP, DBP and HIV status with an interaction term between HIV status and nitrotyrosine (with splines on both nitrotyrosine and interaction term).
| Variable                  | Model 5 |               | F statistic | Model 6 |               | F statistic | Model 7 |               | F statistic | Model 8 |               | F statistic |
|--------------------------|---------|---------------|-------------|---------|---------------|-------------|---------|---------------|-------------|---------|---------------|-------------|
| **Coef**                 | Cl      |               |             | **Coef** | Cl             | F statistic | **Coef** | Cl             | F statistic | **Coef** | Cl             | F statistic |
| Sex (male)               | 0.068   | -0.155 – 0.292| -           | 0.068   | -0.157 – 0.294| -           | 0.063   | -0.165 – 0.292| -           | 0.053   | -0.138 – 0.245| -           |
| Age                      | 0.019** | 0.006 – 0.032 | -           | 0.019** | 0.005 – 0.032 | -           | 0.019** | 0.005 – 0.033 | -           | 0.018** | 0.004 – 0.031 | -           |
| Waist circumference      | -0.012* | -0.023 – --0.001| -           | -0.012* | -0.023 – --0.001| -           | -0.012* | -0.023 – --0.001| -           | -0.011  | -0.022 – 0    | -           |
| Hip circumference        | 0.003   | -0.006 – 0.011 | -           | 0.003   | -0.006 – 0.011 | -           | 0.003   | -0.006 – 0.012 | -           | 0.002   | -0.008 – 0.013 | -           |
| Bodyfat                  | -0.004  | -0.017 – 0.01  | -           | -0.004  | -0.017 – 0.01  | -           | -0.004  | -0.018 – 0.01  | -           | -0.005  | -0.018 – 0.008 | -           |
| SBP                      | -0.005  | -0.015 – 0.004 | -           | -0.005  | -0.015 – 0.004 | -           | -0.005  | -0.015 – 0.004 | -           | -0.008  | -0.016 – 0.001 | -           |
| DBP                      | 0.028** | 0.012 – 0.043  | -           | 0.028   | 0.012 – 0.043  | -           | 0.027** | 0.012 – 0.043  | -           | 0.032** | 0.017 – 0.046  | -           |
| HIV status               | -0.055  | -0.274 – 0.164 | -           | -0.057  | -0.496 – 0.381 | -           | -0.034  | -0.499 – 0.431 | -           | 0.885   | 0.115 – 1.656  | -           |
| Nitrotyrosine            | -0.003  | -0.007 – 0.001 | -           | -0.003  | -0.001 – 0.003 | -           | -0.003  | -0.021 – 0.015 | -           | 0.029   | -0.001 – 0.058 | -           |
| rcs_Nitrotyrosine_1      | -       | -              | -           | -       | -              | -           | 0.005   | -0.039 – 0.049  | -           | -0.053  | -0.123 – 0.017 | -           |
| rcs_Nitrotyrosine_2      | -       | -              | -           | -       | -              | -           | -0.030  | -0.174 – 0.114 | -           | 0.120   | -0.102 – 0.343 | -           |
| rcs_Nitrotyrosine_3      | -       | -              | -           | -       | -              | -           | -0.000  | 0.999 – 0.009   | -           | 0.000   | -0.009 – 0.009 | -           |
| HIV interaction Nitrotyrosine | -       | -              | -           | 0.000   | 0.999 – 0.008  | -           | -0.044  | -0.082 – --0.006| -           | -0.083  | -0.02 – 0.187  | -           |
| HIV interaction rcs_Nitrotyrosine_1 | -       | -              | -           | 0.000   | -0.000 – 0.000 | -           | -0.036  | -0.174 – 0.114 | -           | 0.120   | -0.102 – 0.343 | -           |
| HIV interaction rcs_Nitrotyrosine_2 | -       | -              | -           | -0.044  | -0.082 – --0.006| -           | -0.036  | -0.174 – 0.114 | -           | 0.120   | -0.102 – 0.343 | -           |
| HIV interaction rcs_Nitrotyrosine_3 | -       | -              | -           | -0.036  | -0.174 – 0.114 | -           | -0.036  | -0.174 – 0.114 | -           | 0.120   | -0.102 – 0.343 | -           |
| Constant                 | 1.109   | 0.157 – 2.06   | -           | 1.109   | 0.143 – 2.075  | -           | 1.036   | 0.057 – 2.129  | -           | 0.239   | -1.233 – 1.818 | -           |
| Regression details       | F(9, 96) = 6.836 | -           | -           | F(10, 95) = 6.089 | -           | -       | F(12, 93) = 5.066 | -           | 0.428   | -                | -           |
| R²                       | 0.391   | -              | -           | 0.391   | -              | -           | 0.395   | -              | -           | 0.428   | -                | -           |
| F-test                   | 6.386   | -              | -           | 6.089   | -              | -           | 5.066   | -              | -           | 4.872   | -                | -           |
| AIC                      | 121.020 | -              | -           | 123.020 | -              | -           | 127.200 | -              | -           | 124.220 | -                | -           |
| No. of observations      | 106     | -              | -           | 106     | -              | -           | 106     | -              | -           | 106     | -                | -           |
| Prob > F                 | 0.000   | -              | -           | 0.000   | -              | -           | 0.000   | -              | -           | 0.000   | -                | -           |
| BIC                      | 137.660 | -              | -           | 152.320 | -              | -           | 160.830 | -              | -           | 164.180 | -                | -           |

DBP, diastolic blood pressure; SBP, systolic blood pressure; rcs, restricted cubic spline; var, variable; HIV interaction rcs_Nitrotyrosine, the interaction between HIV status and nitrotyrosine levels with restricted cubic spline with 3 knots; sex interaction rcs_Nitrotyrosine, the interaction between sex and nitrotyrosine levels with restricted cubic spline with 3 knots; sex interaction rcs_Nitrotyrosine, the interaction between sex and nitrotyrosine levels with restricted cubic spline with 3 knots; Coef, Beta coefficient; CI, confidence interval; AIC, Akaike information criterion; Prob, probability; BIC, Bayesian information criterion.

* p < 0.5; ** p < 0.01.

† Model 5 was adjusted for age, sex, waist circumference, hip circumference, percentage body fat, SBP, DBP and HIV status; ¶ Model 6 was adjusted for age, sex, waist circumference, hip circumference, percentage body fat, SBP, DBP and HIV status with an interaction term between HIV status and nitrotyrosine; § Model 7 was adjusted for age, sex, waist circumference, hip circumference, percentage body fat, SBP, DBP and HIV status with an interaction term between HIV status and nitrotyrosine (with splines on nitrotyrosine); ¶ Model 8 was adjusted for age, sex, waist circumference, hip circumference, percentage body fat, SBP, DBP and HIV status with an interaction term between HIV status and nitrotyrosine (with splines on both nitrotyrosine and interaction term).