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Retinal Vessel Functional Responses in South Africans Living With and Without HIV: The EndoAfrica-NWU Study

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Keywords: antiretroviral therapy | flicker light provocation | human immunodeficiency virus | retinal vessel analysis

ABSTRACT

Objectives: The effects of HIV and antiretroviral therapy (ART) on microvascular function are poorly explored. We compared retinal vessel functional responses to flicker light-induced provocation (FLIP) in people living with HIV (PLWH) and people living without HIV (PLWoutH).

Methods: We included 115 PLWH and 51 PLWoutH with a median age of 41 years. Treated PLWH received similar first-line fixed-dose combination ART. Clinical characteristics and retinal vessels functional responses to FLIP were compared in (a) PLWH and PLWoutH; and (b) PLWH groups stratified by the median of (i) CD4-count (511 cells/mm^3), (ii) viral load (50 copies/mL), and (iii) ART duration (57.6 months).

Results: PLWH were older, smoked more, and had a lower prevalence of hypertension than PLWoutH ($p < 0.05$). Almost 64% of PLWH were infected for more than 5 years. Retinal vessel responses to FLIP were similar between PLWH and PLWoutH after taking confounders into account. In addition, PLWH subgroups stratified according to immuno-virological status by CD4-count, viral load, and ART duration showed no differences in retinal vessel responses to FLIP.

Conclusion: Living with HIV and receiving ART were not associated with altered microvascular function as assessed with dynamic retinal vessel analysis in a South African case–control study.

Abbreviations: ANCOVA, analysis of covariance; ART, antiretroviral therapy; AUC_{FLIP}, area under the curve during flicker light-induced provocation; AUC_{Rest}, rest-after-flicker area under the reaction curve; CD4, cluster of differentiation; CRP, c-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FLIP, flicker light-induced provocation; HbA1c, glycated hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus; Il-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; MAP, mean arterial pressure; NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; NWU, North-West University; PLWH, people living with HIV; PLWoutH, people living without HIV; PP, pulse pressure; SBP, systolic blood pressure; SCOPE, study of the consequences of the protease inhibitor era.

1 | Introduction

A bi-directional relationship between viral infections and cardiovascular disease (CVD) is evident, where viral infection may increase CVD risk, and the presence of CVD may increase the susceptibility to viral infection [1]. Sub-Saharan Africa has a high prevalence of CVD, and it has the highest number of people living with the human immunodeficiency virus (PLWH) [2, 3]. CVD and HIV, the most critical noncommunicable and communicable diseases, respectively, in South Africa, significantly strain the country's public healthcare system and economy [4, 5]. A meta-analysis of CVD risk among PLWH found that the virus accounted for more than 15% of South Africa's CVD burden [6]. The virus and specific antiretroviral therapies (ART) particularly contribute to, among others, chronic inflammation, dyslipidemia, and hypertension which creates a milieu primed for vascular endothelial injury and dysfunction [2, 5, 7].

The interaction between HIV, ART, and vascular function is complex, evidenced by contradicting findings in the literature. Living with HIV and receiving ART have been related to arterial stiffness [8] and impaired macrovascular endothelial function [9] in developed countries. Long-term ART has been associated with markers of endothelial dysfunction in PLWH from Botswana [10], but endothelial activation appeared to be greater in ART naïve PLWH from a South African study [11]. However, recent studies reported no differences in macrovascular structure and function [12, 13] or markers of early vascular aging [14] between PLWH receiving treatment and HIV-free participants in South Africa.

Few studies investigating the vascular effects of HIV and ART have considered microvasculature effects. The optimal performance of the microcirculation ensures adequate tissue perfusion. By regulating vascular tone, inflammatory responses, solute exchange, and hemostasis, the microcirculation is essential in maintaining peripheral and cerebral tissue function [15]. The cerebral microvascular function relies on effective neurovascular coupling and optimal endothelial function, a process that couples changes in neural activity with increases in microvascular vasodilation (and later vasoconstriction) and local blood flow [16–18]. This process involves complex interactions between components of the neurovascular unit (neurons, glial cells, microvascular endothelium, vascular smooth muscle cells, and pericytes) [16, 18]. Dysfunction of the neurovascular unit has been associated with CVD incidence and development [19].

Data suggest that HIV-1 may impair microvascular function. Cell-based studies showed that the HIV proteins, Tat, gp120, and Nef induced microvascular endothelial cell apoptosis [20–23]. The immediate consequences may be reduced endothelium-dependent vasodilatory responses of the microvessels in response to increased metabolic demand [24]. In PLWH from the observational Study of the Consequences of the Protease Inhibitor Era (SCOPE), markers of T-cell dysfunction and inflammation were associated with peripheral microvascular dysfunction as assessed by reactive hyperemia [25]. Similarly, cerebral microvascular vasoreactivity, indicative of endothelial function, is reduced in virologically suppressed PLWH receiving ART [26]. While HIV infection and specific ART regimens that include efavirenz disrupt neurovascular coupling as related to increased blood–brain barrier permeability [7, 27, 28], the influence of HIV and ART on neurovascular coupling related to cerebral microvascular function is unknown. Additionally, previous studies investigated microvascular manifestations of HIV-1 subtype B, whereas the HIV-I subtype C dominates in South Africa [29], and PLWH follow a specific first-line ART regimen. Therefore, it is unclear whether the microvascular function of South African PLWH is similarly influenced.

The retina allows for noninvasive assessment of microvascular structure and function [17, 18]. Structural retinal vessel parameters, such as narrower central retinal artery equivalent, wider central retinal vein equivalent, and reduced arterio-venous ratio, have been associated with CD4 count decline [30], higher viral loads, and highly active ART duration [31]. Research regarding the effect of HIV and ART on retinal microvascular *function* is, however, lacking. The exposure of retinal vessels to a metabolic challenge, namely flicker light-induced provocation (FLIP), evokes a vascular response characterized by vessel dilation and constriction followed by a return to baseline vessel diameter. This method is widely accepted for assessing microvascular endothelial function, and the ability of the retinal vasculature to respond to FLIP has been thought to be influenced by endothelial function, local metabolic demand, and neurovascular coupling [17, 32–34]. The value of dynamic retinal vessel analysis in the assessment of CVD risk is highlighted by studies that reported associations between attenuated retinal vessel dilation in response to FLIP and various CVD outcomes, such as coronary artery disease [35] and stroke [36]. To our knowledge, the EndoAfrica-NWU study is the first study to conduct dynamic retinal vessel assessments on PLWH and people living without HIV (PLWoutH) [5]. To elaborate on the microvascular function and CVD risk of South African PLWH, we aimed to compare retinal vessel baseline diameters and functional responses in PLWH and PLWoutH, and to consider the influence of HIV-related immune-virological markers (CD4 count, viral load, and ART duration) on microvascular function.

2 | Methodology

2.1 | Study Design and Participant Selection

The current study forms part of the EndoAfrica-NWU (vascular endothelial dysfunction: the putative interface of emerging cardiovascular risk factors affecting populations living with and without HIV in sub-Saharan Africa) case–control study, previously introduced by Fourie et al. [5]. Baseline data collection took place at the Hypertension Research and Training Clinic of the North-West University (NWU) between August 2017 and June 2018. The study adhered to the Declaration of Helsinki and was approved by the Health Research Ethics Committee of the NWU (NWU-00045-15-A1) and the North-West Department of Health.

Participants were recruited from clinics and HIV-support groups in and near the town of Potchefstroom (North West province of South Africa). Eligible PLWoutH were recruited from the Hypertension Research and Training Clinic database of individuals interested in future research projects. The study included 382 participants of African descent (aged 18–60 years)

with a confirmed HIV diagnosis ($n = 278$) and PLWoutH ($n = 104$). Most of the PLWH ($n = 247$) received the same first-line ART regimen, which consisted of a fixed-dose combination of two nucleoside reverse transcriptase inhibitors (NRTI) (emtricitabine and tenofovir) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) (efavirenz). Furthermore, of the 278 PLWH taking part in the study, $n = 9$ individuals were on ART for <4 weeks or were newly diagnosed with HIV infection, whereas $n = 22$ PLWH defaulted from their ART before data collection commenced by either interrupting or discontinuing ART. Individuals were excluded from the EndoAfrica-NWU study if they were on second-line ART, had co-infections such as tuberculosis or Pneumocystis pneumonia, were pregnant, or <3 months postpartum. In the EndoAfrica-NWU study, various measurements were performed as previously described [5]. The protocol was based on that of the parent EndoAfrica Study [37]. Functional retinal vessel measurements described in this manuscript were an additional measurement to the EndoAfrica-NWU study and not part of the protocol of the parent study. Because of time constraints in the study protocol and to accommodate study participants time spent in the clinic, on each given examination day, retinal vessel functional measurements were only performed when the time schedule allowed for it, resulting in measurements obtained from $n = 166$ participants (43% of the cohort). Figure 1 provides a summary of the participant selection.

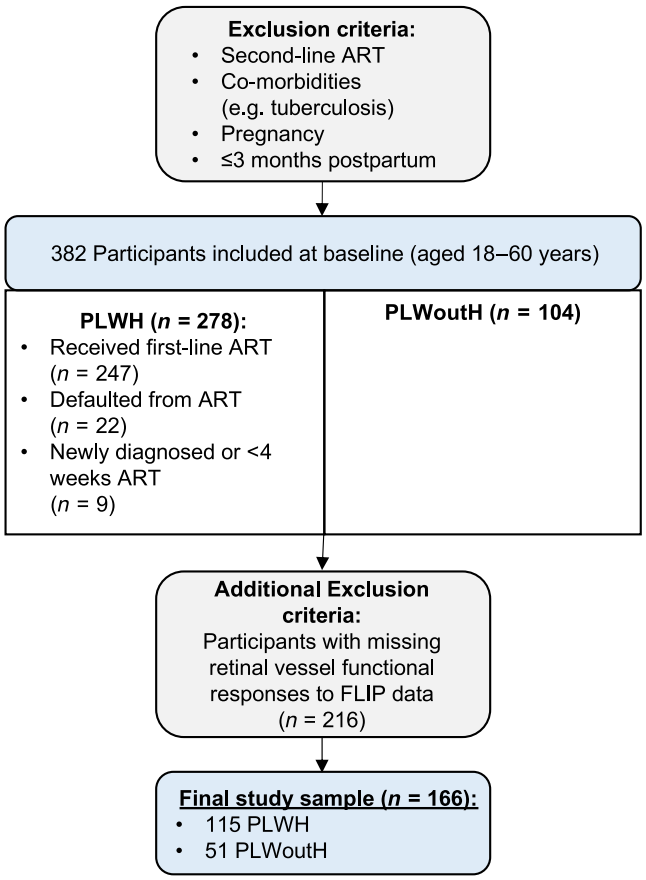

FIGURE 1 | Participant selection for the EndoAfrica-NWU case–control study. ART, anti-retroviral therapy; FLIP, flicker light-induced provocation; PLWH, people living with human immunodeficiency virus; PLWoutH, people living without with human immunodeficiency virus.

2.2 | The General Procedure of Investigation

Data collection took place between approximately 08:00 and 12:00 each workday. Six to eight participants, who were interested in the study and met the inclusion criteria, were scheduled per day for data collection. They were requested to refrain from any food or fluid intake from 22:00 the previous evening. On the day of data collection, the participants were transported to the Hypertension Research and Training Clinic and arrived at approximately 08:00. After obtaining informed consent, the participants provided morning urine spot samples and a nurse obtained blood samples. Anthropometric and cardiovascular measurements (clinic blood pressure and retinal vascular imaging) followed, and participants also completed a questionnaire about their lifestyle (e.g., smoking habits and alcohol consumption), and medication use.

A professional HIV counselor interacted with the participants in a private room. If they were unsure about their HIV status, an HIV test was performed after the participant gave permission. Immediate feedback on available results was provided, and participants were referred to a medical professional or local clinic if necessary.

2.3 | Biochemical Analyses

Blood samples were taken to the onsite laboratory immediately after collection. The blood samples were prepared using standardized procedures and stored at −80°C until analyses were performed [5]. The National Health Laboratory Services determined CD4 count (Beckman Coulter FC500 MPL/CellMek, Miami, FL, USA) and viral load (Cobas AmpliPrep/COBAS TaqMan HIV-1 test, version 2.0) from EDTA blood samples. The remaining blood samples were analyzed at the onsite laboratory.

Serum levels of total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured with the Cobas Integra 400 plus (Roche, Basel, Switzerland) using homogeneous enzymatic colorimetric assays. The total cholesterol to HDL-C ratio (total cholesterol: HDL-C) was also calculated. The Cobas Integra 400plus (Roche, Basel, Switzerland) was used to measure plasma glucose, glycated hemoglobin (HbA1c), and high-sensitivity C-reactive protein (CRP). The enzymatic reference method with hexokinase and a turbidimetric inhibition immunoassay were used to analyze plasma glucose and HbA1c, respectively. CRP was analyzed with the high-sensitive particle enhanced turbidimetric assay. The Cobas e411 analyzer (Roche, Basel Switzerland) was used to measure Interleukin-6 (IL-6) with the electrochemiluminescence immunoassay. Serum creatinine was quantified with the kinetic colorimetric assay (Cobas Integra 400plus) and used in The Chronic Kidney Disease Epidemiology formula to calculate the estimated glomerular filtration rate (eGFR).

2.4 | Anthropometric Measurements

Standardized procedures were followed for anthropometric measurements [38]. These measurements included waist circumference (Lufkin steel anthropometric tape, W606PM;

Lufkin, Apex, USA), body weight (SECA 813 Electronic scale, SECA, Hamburg, Germany), and body height (SECA 213 Stadiometer, SECA, Hamburg, Germany). The participants' body mass indexes were calculated by dividing their body weight (in kilogram) by the square of their height (in meter).

2.5 | Clinic Blood Pressure

Brachial systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements were obtained in duplicate with the OMRON M6 automatic digital blood pressure monitor (Omron Healthcare, Kyoto, Japan) with the participants in a seated position. The final blood pressure measurements were used in this study. When a participant had a mean office SBP of ≥140 mmHg and/or a DBP ≥90 mmHg and/or was using antihypertensive medication, they were classified as being hypertensive according to the 2021 European guidelines for managing arterial hypertension [39].

2.6 | Retinal Imaging

The intraocular pressure of the participants was measured with a Tono-Pen Avia applanation tonometer (Reichert 7–0908, ISO 9001, New York, USA) after local anesthetic eye drops (Novasine Wander 0.4% Novartis) were administrated to both eyes. Participants with a history of epilepsy or high intraocular pressure were excluded from this procedure. An eye drop containing 1% tropicamide and bensalconiumchloride 0.01% (m/v) (MYDRIACYL 1% ophthalmic solution; Alcon Laboratories, South Africa) was administered to the right eye approximately 30 min prior to retinal imaging to induce mydriasis. The IMEDOS Dynamic Retinal Vessel analyzer (DVA) fitted with a Zeiss Fundus Camera FF-450 Plus (Imedos Systems GmbH, Jena, Germany) was used for dynamic retinal vessel analysis according to a standardized FLIP protocol [40], using RVA 4.61 software (Imedos Systems GmbH, Jena, Germany). The assessment consisted of three flicker light provocation cycles, and the measurement lasted 350 s. Each cycle had three phases, namely a 50-s baseline phase, followed by 20 s of FLIP, and an 80-s recovery/baseline phase. The last 30 s of the previous cycle was used as the baseline phase of the subsequent cycle. For the measurement, arteriolar and venular segments between 0.5 and 2.0 optic disc diameters from the optic nerve head border in the upper (mostly) or lower temporal quadrant of the fundus image were used. Measurements were performed with the camera set at a 30° angle, and the participant focused on the tip of a fixation rod. After the measurement, the raw numerical data that the DVA generated were exported to a Microsoft Excel template with macros. Retinal arteriolar and venular parameters in response to flicker light provocation were determined as previously described [41] and are summarized in Table 1. Variables listed include those describing retinal vessel dilation, constriction (arteriolar) or minimum response (venular), and rest-after-flicker phases. Of note, changes in vessel diameters during each cycle were calculated as a percentage of the corresponding baseline diameter. Absolute vessel diameters (measured in measuring units [MU]) of the vessel segment selected in the measurement were calculated individually as the median value over the last 30 s of the first baseline phase prior to flicker stimulation.

TABLE 1 | Parameters derived from the smoothed averaged flicker response curve.

No.	Parameter	Unit	Description/Explanation
1	Percentage maximal dilation in response to FLIP. Termed maximal dilation	%	The absolute maximum of the reaction curve taken between the start of the flicker stimulation and 30 s after flicker initiation. Expressed as a percentage of baseline vessel diameter
2	Time to maximal vessel dilation	s	Taken with time of flicker as 0 s
3	Area under the curve during flicker light-induced provocation (AUC_{Flip})	%.s	Describes the curve form during flicker stimulation (0–20 s). For values under the 100% line, the area was negative
4	Percentage absolute maximal constriction after flicker stimulation, termed maximal constriction; in venules, it is referred to as the minimum reaction	%	The minimum value occurring after maximum flicker induced dilation. Expressed as a percentage of baseline vessel diameter
5	Time to maximal vessel constriction. In venules, it will be referred to as the time to minimum reaction	s	Taken with time of flicker as 0 s
6	The area under the reaction curve during vessel constriction. In veins, it is referred to as the area under	%.s	Calculated between 15 and 45 s after the end of the flicker stimulation. Describes the arterial constriction response or venous behavior after cessation of flicker. For values under the 100% line, the area was negative
7	Rest-after-flicker area under the reaction curve (AUC_{Rest})	%.s	Provided information on the vessel behavior long term after the flicker calculated between 50 and 80 s after the completion of flicker stimulation. For values under the 100% line, the area was negative

Abbreviations: AUC_{FLIP}, area under the curve during flicker light-induced provocation; AUC_{Rest}, rest-after-flicker area under the reaction curve; FLIP, flicker light-induced provocation.

2.7 | Statistical Analyses

All statistical analyses were performed with IBM SPSS version 26.0 (IBM Corporation, Armonk, NY, USA) software. Continuous data's normality was visually inspected using Q-Q plots and numerically assessed according to the skewness and kurtosis coefficients, as well as the results of Kolmogorov–Smirnov tests. Non-normally distributed data were $\log_{10}$ transformed. Basic characteristics and retinal vessel functional responses to FLIP were compared between PLWH and PLWoutH with independent *t*-tests (results reported as arithmetic means and standard deviations) or Mann–Whitney *U* tests (results reported as medians with 25th and 75th percentiles) for normally distributed and skewed continuous data, respectively. Pearson's chi-square test was used to compare categorical data, and results were reported as proportions. Similarly, basic characteristics were compared between groups that were stratified based on the median values of (i) CD4 count (511 cells/mm^3), (ii) viral load (50 copies/mL), and (iii) ART duration (57.6 months). Retinal vessel functional responses were compared between PLWH and PLWoutH, and between the respective median split groups with analysis of covariance (ANCOVA), while adjusting for confounders. We initially adjusted for age and smoking status when comparing PLWH with PLWoutH, as these confounders have been associated with attenuated microvascular dilatory responses [42] and a disruption in neurovascular coupling [43]. In subsequent analysis when comparing PLWH and PLWoutH and when comparing the median split groups, we adjusted for basic characteristics that differed significantly between the respective groups. Lastly, we determined relationships between ART duration, CD4 count, and viral load with Spearman rank correlation analyses.

3 | Results

3.1 | Comparisons of Participant Characteristics for Those With Retinal Data Compared With Those Without

We excluded 216 participants with missing retinal vessel functional data from the main EndoAfrica-NWU study. Table S1 shows a comparison of basic characteristics between those with versus those without data within the PLWoutH and PLWH groups. For PLWoutH, those without retinal vessel functional data were older with higher HDL-C levels. In PLWH, those with missing retinal vessel functional data had statistically higher prevalence of hypertension, higher total cholesterol to HDL-C ratio, lower HDL-C and higher HbA1c levels. Despite these differences, the mean values for these parameters (total cholesterol, HDL-C, total cholesterol to HDL-C and HbA1c) in the PLWH all fell within the same clinical category. Nevertheless, comparisons between PLWH and PLWoutH reported in the present study were mostly comparable to the group differences reported in the overall EndoAfrica-NWU study population [5]. The exception was that hypertension status and eGFR, only tended to be higher in PLWoutH compared with PLWH ($p=0.070$ and 0.055, respectively) in the parent study, but reached statistical significance in the present study.

3.2 | Basic Characteristics and Retinal Vessel Functional Responses in PLWH Compared With PLWoutH

Table 2 shows the basic characteristics of the study population. PLWH were older ($p<0.001$), smoked more ($p=0.006$), and had a lower hypertension prevalence ($p=0.038$) and body mass index ($p=0.012$) than the PLWoutH. Sex distribution between the two groups was similar. Total cholesterol ($p=0.004$) and HDL-C levels ($p<0.001$) were higher in PLWH, but their total cholesterol: HDL-C ($p=0.027$), HbA1c levels ($p=0.015$), and eGFR ($p=0.014$) were lower than that of PLWoutH. Almost 64% of the PLWH were infected for more than 5 years. The median duration of ART was 57.6 (26.6; 98.9) months, but 13.9% of PLWH defaulted from ART by either interrupting or discontinuing their treatment.

An unadjusted comparison between the raw retinal vessel diameter responses to FLIP is presented in Figure 2. Retinal vessel functional responses to FLIP were comparable between groups prior to any adjustment, and following adjustment for age and smoking status (data not shown). We subsequently adjusted for all basic characteristics that differed significantly between the respective groups (identified in Table 2) with the findings presented in Table S2 and Figure 3. Arteriolar ($p=0.338$) and venular baseline diameters ($p=0.300$) were similar between PLWH and PLWoutH (Figure 3a). Maximal dilation of the retinal arteriole ($p=0.847$) and venule ($p=0.225$), as well as maximal arteriolar constriction ($p=0.345$) and venular minimum reaction ($p=0.874$) were also comparable between the two groups (Figure 3b). No differences were evident between the groups for arteriolar and venular AUC parameters (AUC_{FLIP}, AUC during constriction, rest-after-flicker value), or for the time to maximal dilation and constriction/minimum reaction (Figure 3c,d) (all *p*-values > 0.05). We repeated the analysis between the two groups in a sensitivity analysis, additionally adjusting for defaulting from ART, but the results remained unchanged (data not shown). Retinal vessel functional responses to FLIP did not differ between hypertensive PLWH and PLWoutH, or between normotensive PLWH and PLWoutH (Table S3).

3.3 | Retinal Vessel Functional Responses Between CD4-Count, Viral Load, and ART Duration Groups

Figures S1–S3 and Table S5 demonstrate adjusted comparisons where we explored whether certain HIV-related characteristics, including CD4 count, viral load and ART duration, may influence retinal microvascular function in response to FLIP. In these analyses, we adjusted only for baseline characteristics that differed significantly between the median split groups, as reported in Table S4. We found no differences in the retinal vessel parameters between the respective groups. Furthermore, we did not find any relationship between duration of ART in PLWH and their CD4 counts ($r_s=0.11$, $p=0.267$) or viral loads ($r_s=0.09$; $p=0.361$) (*results not shown*).

4 | Discussion

This was the first study in a South African population to describe microvascular function in PLHW by comparing retinal vessel functional responses between PLWH and PLWoutH, and to

TABLE 2 | Comparison of characteristics between people living with HIV and those living without HIV.

	PLWoutH ($n=51$)	PLWH ($n=115$)	p
Demographic characteristics			
Age (years)	35.6 ± 9.8	41.8 ± 8.6	**<0.001**
Male, *n* (%)	14/51 (27.5)	29/115 (25.2)	0.762
HIV-related characteristics			
CD4 count (cells/mm^3)	—	511 (337; 741)	—
Viral load (copies/mL)	—	50 (10; 197)	—
HIV duration >5 years, *n* (%)	—	73/115 (63.5)	—
ART duration (months)	—	57.6 (26.6; 98.9)	—
Defaulted from ART or no ART, *n* (%)	—	16/115 (13.9)	—
Anthropometric measurements			
Waist circumference (cm)	87.3 ± 16.1	83.1 ± 12.4	0.103
Body mass index (kg/m^2)	29.0 ± 8.1	25.7 ± 6.7	**0.012**
Lifestyle characteristics			
Smoked, *n* (%)	19/51 (37.3)	68/114 (60.5)	**0.006**
Consumed alcohol, *n* (%)	34/51 (66.7)	82/114 (71.9)	0.494
Physically active, *n* (%)	41/51 (80.4)	102/114 (89.5)	0.113
Cardiovascular profile			
SBP (mmHg)	120 ± 15	119 ± 21	0.643
DBP (mmHg)	87 ± 15	83 ± 13	0.061
MAP (mmHg)	98 ± 14	95 ± 15	0.245
PP (mmHg)	32 (28; 39)	33 (28; 41)	0.459
Hypertensive, *n* (%)	25/51 (49.0)	37 (32.2)	**0.038**
Antihypertensive medication use, *n* (%)	9/51 (17.6)	15/115 (13.0)	0.437
Biochemical markers			
Total cholesterol (mmol/L)	2.70 ± 0.67	3.1 ± 0.74	**0.004**
HDL-C (mmol/L)	0.85 ± 0.23	1.17 ± 0.44	**<0.001**
Total cholesterol:HDL-C	3.34 ± 1.20	2.90 ± 1.17	**0.027**
LDL-C (mmol/L)	1.51 (1.22; 2.04)	1.67 (1.38; 2.16)	0.271
Triglycerides (mmol/L)	0.60 (0.46; 0.85)	0.69 (0.51; 1.02)	0.134
HbA1c (%)	5.44 (5.20; 5.68)	5.31 (5.12; 5.50)	**0.015**
Glucose (mmol/L)	3.30 (3.00; 3.97)	3.59 (3.16; 3.87)	0.289
CRP (mg/L)	1.72 (0.52; 4.87)	2.38 (1.09; 5.50)	0.257
IL-6 (pg/mL)	2.33 (0.75; 4.38)	2.77 (1.82; 4.38)	0.267
eGFR (mL/min/1.73m^2)	124.30 ± 13.51	117.80 ± 16.38	**0.014**

Note: Parameter values are displayed as arithmetic mean ± standard deviation for normally distributed data or median (25th; 75th percentiles) for variables that were not normally distributed. The chi-square (χ^2) tests were used to compare proportions and prevalence and are indicated as frequencies (%).
Abbreviations: ART, anti-retroviral therapy; CRP, C-reactive protein; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; PLWH, people living with HIV; PLWoutH, people living without HIV; SBP, systolic blood pressure; total cholesterol:HDL-C, total cholesterol to high-density lipoprotein cholesterol ratio. Bold *p*-values highlight comparisons that were statistically significantly different at $p < 0.05$.

consider the influence of HIV-related immune-virological markers on microvascular function. We observed no significant differences in retinal vessel functional responses to FLIP in PLWH and PLWoutH. In addition, retinal vessel functional responses to FLIP within the PLWH group were not influenced by CD4 count, viral load, or ART duration.

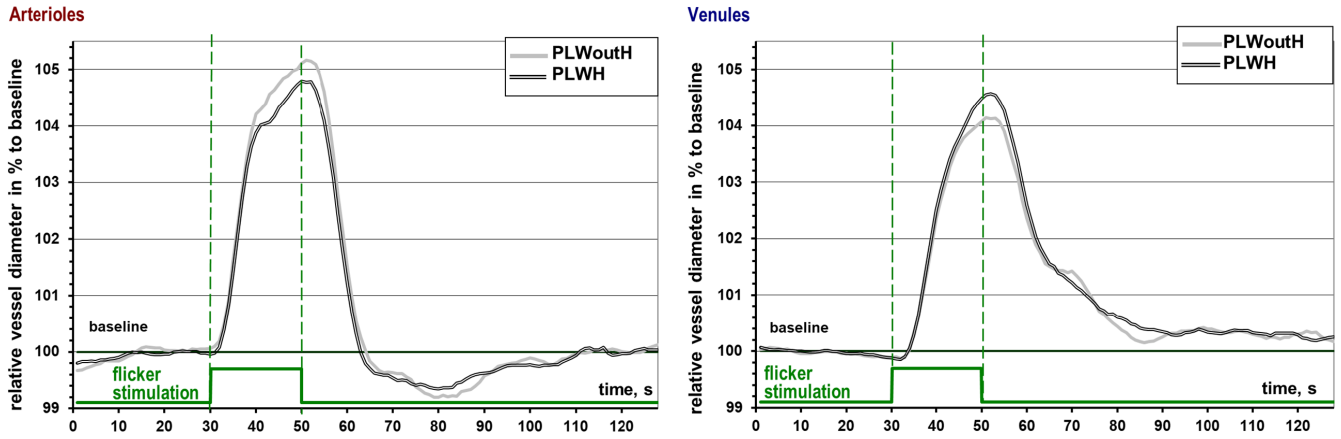

FIGURE 2 | Average reactions of retinal vessels to light flicker provocation in the people living without HIV (PLWoutH) ($n = 51$) and people living with HIV (PLWH) on ART ($n = 115$ for venules and arterioles). For each time point a median of vessel diameter values of all individuals in the cohort was computed to build the average curve.

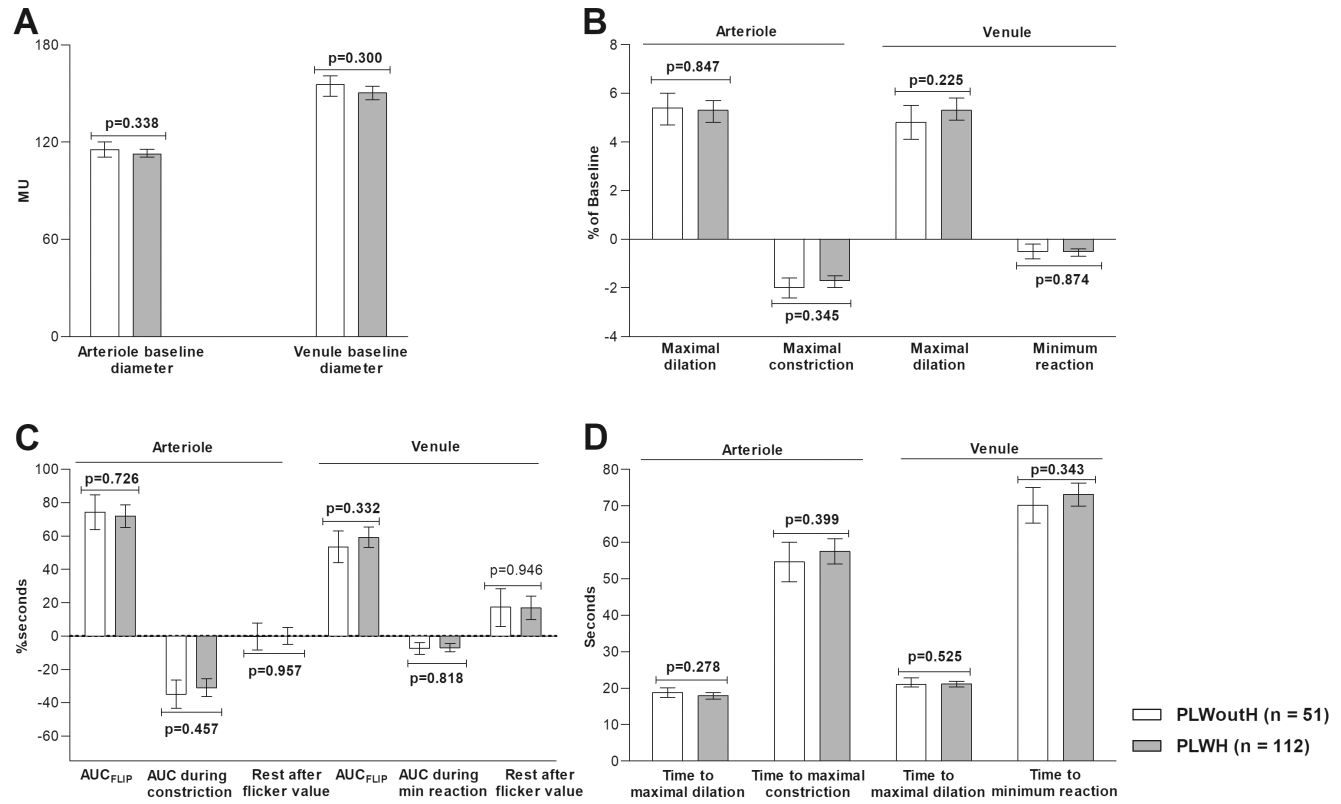

FIGURE 3 | Adjusted comparisons of retinal vessel functional responses to flicker light-induced provocation between people living with HIV and people living without HIV. Data are expressed as arithmetic or geometric mean (95% confidence intervals). Variables in (a) to (d) were compared with analyses of covariance, independent of age, body mass index, hypertension status, HbA1c, total cholesterol to high-density lipoprotein cholesterol ratio, estimated glomerular filtration rate and smoking status. AUC_{FLIP}, area under the curve during flicker light-induced provocation; AUC_{Rest}, Rest-after-flicker area under the reaction curve; HIV, human immunodeficiency virus; MU, measuring units; PLWH, people living with HIV; PLWoutH, people living without HIV.

The analysis of possible functional responses of the retinal microvasculature to light flicker provocation reflects the state of neurovascular coupling [16–18]. In addition, retinal vessel functional responses to FLIP are considered an indicator of systemic microvascular endothelial function [17]. During flicker light exposure, stimulated neurons and astrocytes release vasoactive substances that induce relaxation of arteriolar and venular smooth muscle cells and pericytes surrounding the capillaries [18]. The resulting increase in blood flow also increases shear stress, which further facilitates endothelium-dependent flow-mediated dilation of the microvessels [18]. After maximal vasodilatory responses, the microvessels undergo reactive constriction before returning to baseline diameters [18]. Our results indicate for the first time that individuals living with HIV and receiving ART, in the EndoAfrica-NWU study, did not appear to show compromised microvascular function. This supports results from the brachial artery where flow-mediated dilation, a marker of

macrovascular endothelial function, did not differ between PLWH and PLWoutH from the same cohort [13].

Contrary to our findings, Yung et al. [44] suggested that autoregulation capabilities of the retinal microcirculation may be impaired in PLWH as they presented with a reduced perifoveal capillary blood flow velocity compared with age-matched controls. Furthermore, studies from developed countries, where the HIV-1 subtype B dominates, have found that the virus can adversely impact microvascular function by inducing microvascular endothelial cell apoptosis [20–23] and contributing to endothelial dysfunction [7]. Associations were also found between peripheral microvascular dysfunction, T-cell dysfunction, and inflammation in PLWH from the SCOPE study [25]. Moreover, in another SCOPE sub-study, cerebral vasoreactivity in response to inhaled carbon dioxide was reduced in virally suppressed PLWH who were receiving ART [26]. Callen et al. also found a reduction in cerebral vasoreactivity in response to intravenous administration of acetazolamide in a small virologically suppressed HIV-positive female cohort. The authors suggested that reduced cerebrovascular reactivity may be related to HIV-induced dysfunction of the neurovascular unit [45], but the precise mechanism is unclear. It has been shown that HIV can disrupt neurovascular coupling related to blood–brain barrier function by infecting and activating various components of the blood–brain barrier neurovascular unit, including pericytes and astrocytes [27].

Based on geographic region-specific information [29], PLWH in this South African cohort were most likely infected with the HIV-1 subtype C, and not subtype B, which has been the focus of most other vascular studies. Reported characteristic differences between the subtypes may explain why the virus might not directly contribute to altered microvascular function in PLWH from our study. Subtype C was found to have an overall slower rate of viral replication [46] and CD4 cell count decline compared with subtype B [47]. We suspect that attenuated viral replication may have protected our participants from microvascular dysfunction as it limits the number of virions that may contribute to microvascular endothelial cell damage. Our study's relatively low median viral load (50 copies/mL) and high CD4 count (511 cells/mm^3) in PLWH may reflect the subtype-specific characteristics. Alternatively, it could be argued that effective ART rather than the subtype itself may contribute to the aforementioned features. However, ART duration did not correlate with either viral load or CD4 count in our study. Persistent viral replication and CD4 cell decline generally induce chronic inflammation [48]. However, our study's systemic inflammatory markers were similar between PLWH and their controls, further reinforcing the possibility of low-level viral replication.

Interestingly, in PLWH from the same parent study that our cohort was drawn from low-level viremia (defined as a viral load of 50–999 copies/mL), which can be caused by ongoing viral replication, did not associate with overall cardiovascular risk [49]. In the current study, viremia status did not influence retinal vessel functional responses to FLIP, as we observed no differences in these responses between people with virological suppression (<50 copies/mL) [49] and those with low to high-level viremia (≥50 copies/mL). Similarly, Swart et al. [13] did not observe macrovascular differences between South African PLWH from the EndoAfrica study who were stratified similarly. However, it is possible that the period of HIV exposure might have been too short to induce notable effects on microvascular function. Prolonged viral exposure has been associated with greater vascular injury [2] and almost 64% of the PLWH from our study have been exposed for more than 5 years. However, in support of this, we did not see blood pressure differences between PLWH and PLWoutH over 10 years in a different cohort [50]. Nevertheless, additional longitudinal studies are required to determine if persistent low to high-level viremia may contribute to microvascular dysfunction and/or endothelial function changes.

Endothelial dysfunction of the macro- and microvessels was found to be more pronounced in participants with immunosuppressive CD4 counts (<200 cells/mm^3) [51]. Our study's 25th percentile value for CD4 count in PLWH was higher than 200 cells/mm^3, which may explain why microvascular function did not differ between median CD4 count groups. In another South African study, flow-mediated dilation did not differ between PLWH groups stratified by the median split of CD4 count (525 cells/mm^3) [13]. Moreover, Dysangco et al. [52] found no differences in microvascular endothelial function among HIV-free participants and PLWH who had CD4 counts >200 cells/mm^3. The high CD4 count of PLWH in their study also showed no correlation with markers of endothelial function [52]. We suggest that altered microvascular function may only occur at immunosuppressive CD4 counts. A slower rate of CD4 cell decline may delay the onset of microvascular dysfunction in the EndoAfrica-NWU cohort, but further investigation is warranted.

Except for the direct influence of the virus, we expected poorer microvascular function in PLWH as they were older and smoked more than their controls. Aging attenuates microvascular vasodilatory responses by reducing nitric oxide bioavailability, increasing oxidative stress, contributing to arteriolosclerosis, and enhancing endothelial cell apoptosis [42]. Similarly, smoking has also been associated with endothelial dysfunction of resistance arteries [53] and disruption of neurovascular coupling [43]. Despite the adverse impact of aging and smoking on microvascular function, retinal vessel functional responses remained similar between PLWH and PLWoutH, even after adjusting for these confounders. Whether this is due to a protective effect of ART (discussed briefly below) is unknown.

Other cardiovascular characteristics known to create a milieu for vascular injury and disruption of neurovascular coupling are systemic inflammation [54], dyslipidemia [55, 56], and hypertension [17]. These characteristics usually accompany HIV infection and specific ART use, such as protease inhibitors [2, 3, 5, 57]. However, PLWH from our study had similar levels of systemic inflammatory markers (CRP and IL-6), a more favorable lipid profile (higher HDL-C and lower total cholesterol:HDL-C), and lower BMI, Hba1c, hypertension prevalence compared with the PLWoutH. In a 10-year South African follow-up study, increases in HDL cholesterol and a lower hypertension prevalence at follow-up were also evident in PLWH compared with controls [50]. HDL-C has known anti-inflammatory, anti-thrombotic, anti-apoptotic, and antioxidant functions and may protect against microvascular endothelial dysfunction [58]. Interestingly, hypertension

did not appear to play a role in our participants' retinal vessel functional responses, as the additional adjustment for hypertension status upon the initial adjustment for age and self-reported smoking left the results unchanged (data not shown). This observation may be due to a proportion of the hypertensive participants (58%, data not shown) having controlled hypertension, since the group mean BP values were in the normal range. It could be argued that in the PLWH from our study, some of the beneficial determinants on vascular function noted outweigh or compensate for the detrimental determinants on vascular function noted. We accounted for this by adjusting the retinal vessel comparisons for all the basic characteristics that differed between our groups in Table 2.

The beneficial cardiovascular characteristics of PLWH may also be explained by the combined first-line ART that they received. This notion is supported by Nkeh-Chungag et al. [59] who found higher HDL-C levels in participants that followed the same ART regimen as our cohort, compared with ART-naive PLWH. Another study has also associated a longer duration of NNRTI use with a lower prevalence of hypertension [60]. Moreover, tenofovir disoproxil fumarate contributes to decreases in monocyte activation and systemic inflammation biomarkers after 48 weeks of treatment [61]. It may explain why chronic inflammation was not evident in PLWH from our study.

In contrast with these positive effects, efavirenz-based treatments can directly impair endothelial function [62] and increase cerebral microvascular endothelial cell permeability [7, 28]. As our cohort also received efavirenz as part of their ART regimen, the risk exists that long-duration ART may contribute to microvascular dysfunction. However, in the current study, a longer ART duration did not contribute to altered microvascular function compared with PLWH that followed ART for a shorter period.

The major strength of our study includes the method used for the functional assessment of the microvasculature. This study is the first to have conducted dynamic retinal vessel assessments on PLWH and PLWoutH. This methodology allowed the investigation of retinal microvascular endothelial function and neurovascular coupling of PLWH [17]. However, we also acknowledge that there were certain limitations in our study. This study did not include any biomarkers of neurovascular coupling, which prevented us from elaborating on this process. As most of the PLWH in our study received ART, the number of ART-naive participants was too small to compare microvascular function between treated and untreated PLWH. Furthermore, our study only included participants from one province in South Africa, and our findings may thus not be translatable to all South African PLWH. This study's cross-sectional design also prevented us from proving or elaborating on causality. Lastly, it could be argued that our study population was too small to observe significant findings between the groups. The percentage maximum retinal arteriolar dilation in response to flicker light is probably considered the most explored marker of this measurement. In our study, the unadjusted and adjusted mean difference between PLWoutH and PLWH was only 0.4% and 0.1%, respectively. Our study was not powered to detect a difference this small; however, we do not consider such a difference meaningful. Upon review of a few studies reporting maximal arteriolar dilation in response to FLIP between a "control" and "unhealthy" group, the average difference in this parameter between these groups was calculated as 1.33% [63–67]. Given this mean difference, for an unadjusted comparison between PLWH and PLWoutH, 48 participants per group would be required to achieve 80% power. It should also be mentioned that our study had many more PLWH than PLWoutH. In addition, the percentage maximal artery dilation in response to flicker light observed in our study was much higher than the 50th percentile (just under 3.5%) of the normalized data from a European population recently reported [68], suggesting that our group values may be well within normal limits. Nevertheless, since our study is to our knowledge the first to assess retinal vessel functional measurements in response to FLIP, additional studies are encouraged to test whether our findings are robust, but also to extend these findings to other microvascular beds.

5 | Conclusion

Compared with PLWoutH, living with HIV and receiving ART did not contribute to altered microvascular function as assessed with dynamic retinal vessel analysis in a South African case–control study embedded in the EndoAfrica-NWU cohort.

6 | Perspectives

Contradictory findings exist regarding the impact of HIV and ART treatment on vascular function, with even less information available on the microcirculation. This is an important gap in the field as microvascular parameters seem to be early markers of vascular risk. When assessed cross-sectionally, adults living with HIV (63.5% had HIV for a duration of 5 years), mostly on fixed-dose combination first-line ART, demonstrated similar retinal microvascular functional responses to FLIP when compared to people living without HIV. In the context of our study, retinal microvascular function appears to remain intact after a relatively short duration of HIV infection and ART treatment.

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Ethics Statement

The study adhered to the Declaration of Helsinki and was approved by the Health Research Ethics Committee of the NWU (NWU-00045-15-A1) and the North-West Department of Health.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The dataset is the property of the North-West University under supervision of Wayne Smith. In this regard, W. Smith should be contacted if, for any reason, the data included in this paper need to be shared.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.