Smoking Cessation Is Associated With Short-Term Improvement of Vascular Health in a Cohort of People Living With HIV in Rio de Janeiro, Brazil

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Smoking is highly prevalent in people living with HIV/AIDS (PLHA), leading to detrimental effects in different tissues. We examined the effects of nicotine replacement therapy (NRT) on smoking cessation and vascular health. From December 2019 to October 2021, we prospectively enrolled PLHA who were actively smoking. The primary outcome was endothelial function measured by brachial artery flow-mediated dilatation (FMD). We evaluated the percent change in FMD compared to the baseline measure (Δ%FMD) to detect improvements among participants who quit smoking. To confirm the results, we used linear regression models to account for classical cardiovascular (CV) confounders. We included 117 participants with median age of 45.5 years (IQR= 36.4-54.8); 22 (20.4%) had hypertension, 9 (8.3%) had diabetes, almost half were smoking 20+ cigarettes/day (41.7%). After 12 weeks 30.76% participants quit smoking. Comparison of Δ%FMD change from baseline to week 12 showed that among participants adherent to therapy, there has been an increase in Δ%FMD when compared to those who relapsed (1.17% [0.29-2.98] vs -0.19% [-1.95-0.91], p<0.001). After adjustment for CV factors, multiple linear regression showed that Δ%FMD in participants who quit smoking presented a 2.54 mean increase in comparison to those who continued smoking (p=0.007). In conclusion, this study provides evidence that a strategy of NRT and counseling is modestly effective for smoking cessation in PLHA and improves vascular health in a short period of time. This reinforces the importance of the widespread anti-tobacco programs in HIV clinics and the expected impact lowering the incidence of future cardiovascular events. **© 2024 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/)** **(Am J Cardiol 2024;214:157−166)**

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Cigarette smoking is the leading cause of preventable diseases and deaths worldwide[1]; hence, advancing tobacco control is a paramount health priority. Although 1 of 6 noncommunicable disease−related deaths is attributable to smoking, ending the global tobacco epidemic is a critical step if reducing the global burden of morbidity and mortality is to be achieved.[2]

Recently, the World Health Organization showed that worldwide, smoking prevalence in people aged over 15 years has declined from 22.7% to 17.5% in 2021.[3] Driven mainly by governmental efforts, Latin America has experienced a substantial decrease in the absolute number of smokers over the past few decades.[4]

Unfortunately, those efforts seem not to be translated into benefits in people living with HIV (PLHIV) and the picture seems to have gotten even worse. Because smoking rates in PLHIV are 2 to 3 times higher than for the general population, it is unclear whether these treatment strategies are effective for this subpopulation regarding tobacco control.[5−7]

The relation between smoking and cardiovascular (CV) disease (CVD) is well established, even at very low doses.[8] As an example, smokers who consume, on average, 1 cigarette per day have a 50% excess risk for coronary artery disease.[8,9]

Smoking promotes atherogenesis through multiple mechanisms that include vasomotor, neurohormonal, and hematologic dysfunction; increased oxidative stress; and the development of dyslipidemia, which, ultimately, contributes to atherosclerosis.[9,10] Nicotine, a product of tobacco also leads to temporary endothelial dysfunction by reducing nitric oxide bioavailability, promptly measured by ultrasound imaging of the brachial artery after reactive hyperemia or endothelium-dependent flow-mediated dilatation (FMD), a widely accepted noninvasive technique and an early marker of atherosclerosis.[11] For instance, a cohort study

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See page 165 for Declaration of Competing Interest.

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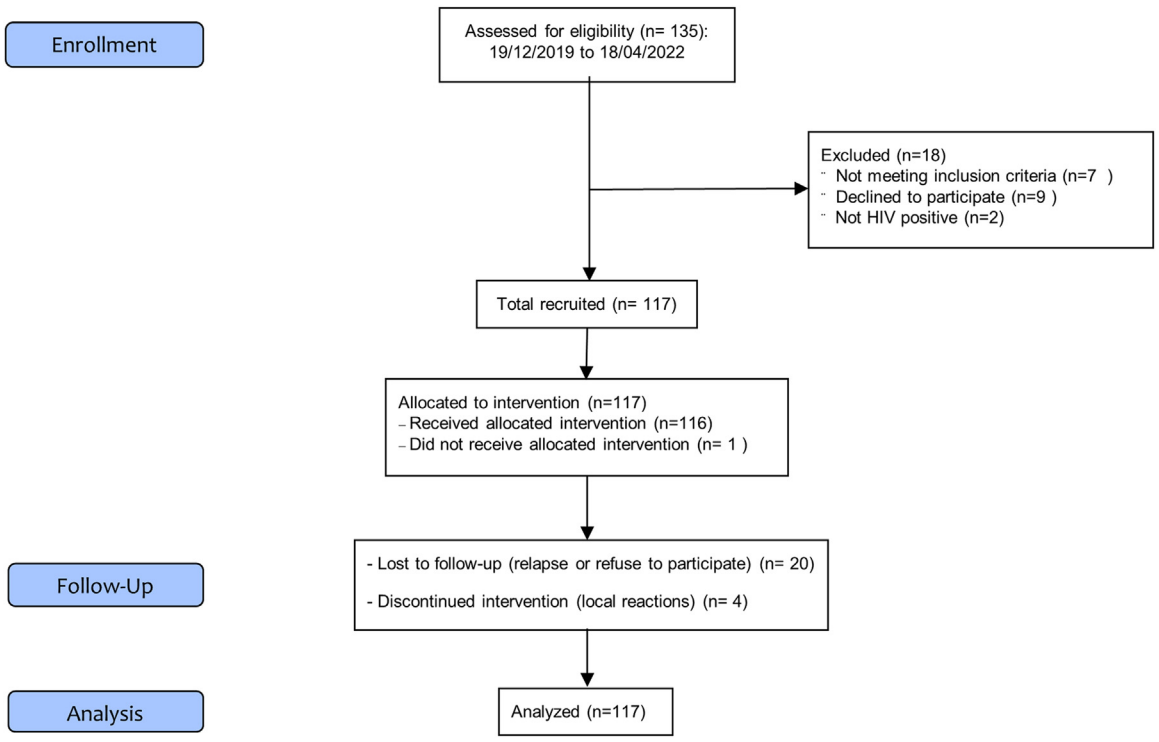

Figure 1. Flow diagram of study.

demonstrated that immediately after smoking, FMD decreased to 1.6% and remained significantly depressed for 60 minutes.[12]

In People Living with HIV/AIDS (PLHA), there are a number of factors corroborating to CVD, such as viral load, cluster of differentiation (CD)4 cell count, and some antiretroviral therapy (ART) regimens, promoting persistent inflammation and endothelial damage, which results in an increased rate of CV complications.[13] Nevertheless, information about endothelial function through FMD after smoking cessation and the degree of nicotine dependence and determining factors for relapse are scarce in this population.

We aimed to evaluate the effectiveness of nicotine replacement therapy (NRT) with transdermal nicotine patches for smoking cessation, study factors associated with relapse, and describe and compare the changes of the vascular endothelium by measuring FMD in PLHA at baseline and after 12 weeks.

Methods

The study was carried out within a large, urban, prospective cohort of PLHIV at the Instituto Nacional de Infectologia Evandro Chagas (INI). Located in Rio de Janeiro, Brazil, INI is a national public referral center for PLWHA receiving primary care since 1998. From December 2019 to April 2022, 117 adults who had attitudes toward quitting smoking and had no contraindications to study pharmacotherapy were enrolled by active site searching (Figure 1). The included participants were deemed as regular smokers, defined as the consumption of 1 or more pack (20 cigarettes) per week in the past 6 months, and had expired carbon monoxide (CO) measurement >10 parts per million at the screening visit. The exclusion criteria were (1) previous use of transdermal nicotine patches or bupropion in the last 6 months, (2) contraindication to nicotine patches, (3) pregnancy, and (4) breastfeeding. The study was reviewed and approved by the ethics review board of the Evandro Chagas National Institute of Infectious Diseases at Oswaldo Cruz Foundation (INI/Fiocruz) in Rio de Janeiro. All participants signed a written informed consent form before study procedures. This study is registered in *Clinicaltrials.gov* under ID 3.171.692.

The study included a 1-week screening and inclusion period, with face-to-face individual smoking cessation counseling and a proactive brief telephone advice every 4 weeks, a 12-week open-label treatment with transdermal patch of NRT, and a 12-week on-site final follow-up visit. FMD measurements were performed at baseline and week 12. At baseline, participants took objective face-to-face interviews using questionaries to (1) determine the degree of depression symptoms (9-item depression module, patient health questionnaire-9 [PHQ-9]); (2) assessment of tobacco withdrawal regarding craving, negative affect, hunger, sleep, restlessness, and concentration (Wisconsin smoking withdrawal scale [WSWS]); and (3) nicotine dependence (Fagerstrom test). We collected data on sociodemographic characteristics, tobacco, and alcohol use and clinical evaluation including history of hypertension, dyslipidemia, diabetes mellitus, CVD, acute myocardial infarction, stroke, family CVD, and hepatitis coinfection. We retrieved medication history with direct questions regarding use of antihypertensive, antihyperglycemic, lipid-lowering medicine, and ART use. At the baseline visit, we also assessed anthropometric measurements and fasting blood samples were obtained.

Laboratory tests included glucose, glycated hemoglobin (HbA1c), lipid profile, high-sensitivity C reactive protein (hs-CRP), hepatogram, hemogram, and renal function.

Eligible participants were assigned to receive treatment with NRT by the application of transdermal patches, changed by the patient every 24 hours for a period of 12 weeks. Patients who smoked more than 20 cigarettes a day used the dosage in weeks 1 to 4 of 1 patch of 21 mg, in weeks 5 to 8 of 1 patch of 14 mg and in weeks 9 to 12 of 1 patch of 7 mg. For patients who smoke ≤20 cigarettes per day, the following scheme was used: in weeks 1 to 8, 1 patch of 14 mg every 24 hours and weeks 9 through week 12, 1 patch of 7 mg.

Smoking status was assessed by self-reported 7-day point-prevalence abstinence during telephone and site visits and was confirmed by an expired CO level at week 12 using the carbon monoxide monitor (Micro+TM Smokerlyzer, Bedfont (coVita, Cottage Grove Ave, Suite C, Santa Barbara, CA 93101- US)).

Participants were asked to fast for at least 8 to 12 hours before being examined in a quiet, temperature-controlled room as per the latest guidelines.[14–16] In addition, participants were advised to avoid caffeine and foods high in fat for at least 12 hours before the examination.

The images were acquired using the Logiq S8 dimension device (GE Healthcare, Little Chalfont, United Kingdom), with a high-frequency vascular transducer (12 MHz). The device is equipped with 2 2-dimensional color and vascular Doppler imaging, with an internal electrocardiogram monitor. Gain and depth were adjusted so that the recorded images have resolution enough for subsequent analysis by the validated Brachial Analyzer software (Brachial Tools, Medical Imaging Applications (Medical Imaging Applications LLC832 Forest Hill Dr. Coralville, Iowa 52241, USA)).[17] The images were captured in Audio Video Interleave format from the ultrasound machine video output to a computer dedicated to this purpose, using a capture card and a vascular image software in timed mode at 30 frames/second.

On the day of the examination, the patient was placed supine in a temperature-controlled room (20°C), with the right or left arm immobilized on a table and the shoulder abducted to 70 to 90°, with the elbow fully extended in a comfortable position for viewing the brachial artery. A pressure cuff was placed around the forearm (E20 cuff inflator and AG101 air system (Hokanson, Inc. 12840 NE 21st Place Bellevue, WA 98005, USA)) and the transducer was fixed by an articulated arm with a stereotaxic clamp (Noga Engenharia Ltd.) fixed on a small platform with micrometric manual sliding (Velmex, New York) to maintain constant positioning and make fine adjustments to the artery image during the procedure.[18] The brachial artery was visualized 5 to 10 cm above the antecubital fossa in the longitudinal plane for at least 1 minute to acquire the baseline image for 30 seconds. A segment with clear anterior and posterior vessel interface was selected for Duplex mode (2-dimensional pulsed Doppler) imaging. A movie was recorded for the simultaneous measurement of the basal artery diameter and basal flow velocity measurements with a 1.5-mm window in the center of the brachial artery and an angle of 60° parallel to the arterial flow with the sample volume in the center of the artery.

The protocol started with 30-second baseline ultrasound recording, followed by 5 minutes of forearm ischemia (not recorded), induced by inflating the pressure cuff to 200 mm Hg. Hyperemic blood flow was induced by deflating the cuff after 5 minutes when flow velocity and artery diameters were recorded continuously from 15 seconds before cuff deflation up to 3 minutes.

For the follow-up measurement, the distance of the ultrasound transducer with respect to the medial epicondyle was measured to ensure that the same segment of the brachial artery of the initial examinaiton was studied.

Off-line analyzes of brachial artery diameter was assessed by a single trained operator using automated edge detection software. The Brachial Analyzer software (Medical Imaging Applications LLC832 Forest Hill Dr. Coralville, Iowa 52241, USA) quantified a brachial artery lumen diameter for each image of the scan before and after cuff occlusion. Once the vascular region of interest was defined, the application automatically identifies the diameters M-line to M-line in each frame continuously. The maximum blood velocity and blood velocity integral were calculated for each cardiac cycle. To detect R-wave gated frames, we used an additional R gated wave module that detect frames at the end of diastolic diameter. The following parameters were evaluated as a result: (1) average baseline diameter (Dbase) measured in millimeters and (2) the maximum postcuff deflation diameter ($D_{max.}$) used to calculate %FMD = 100 (Dmax_Dbase) / Dbase, (3) average vessel area in square centimeters as $\pi \left(\frac{Diameter}{2}\right)^2$, and (4) mean flow calculated as flow integral multiplied by vessel area.

The main end point was to describe and compare endothelial function between those who relapsed and those who continuously stopped smoking within 12 weeks of the smoking cessation date. Secondary outcomes were to investigate factors associated with relapse and predictors of endothelial disfunction.

Explanatory variables were divided into demographic, traditional CV risk factors, and variables related to HIV status. Self-reported race/skin color was categorized as "White," "Black," or "Mixed/Other." We dichotomized the self-reported number of years of formal education as ≤9 years of schooling or >9 years. Family history of premature CVD was considered as history of sudden death or any episode of acute myocardial infarction or stroke in fathers or brothers aged <55 years or mothers or sisters aged <65 years. The definitions of hypertension, diabetes, and dyslipidemia were based either on self-reporting or on current results/measurements. Hypertension was defined if the mean of the last 2 from 3 measurements showed systolic blood pressure of 140 mm Hg or higher or diastolic blood pressure of 90mm Hg or higher.[19] Diabetes was defined as plasma nonfasting glucose of 200 mg/100 ml or higher or HbA1c of 6.5% or higher.[20] Dyslipidemia was defined by use of lipid-lowering therapy or low-density lipoprotein cholesterol >130 mg.[21] For depression, we summed up the PHQ-9 scale to obtain a total score ranging from 0 to 27, with higher scores indicating greater severity of depression. Based on that, we evaluated 2 levels of severity ranging from 0 to 10 and 11 to 27 points indicating "minimal to mild" and "moderate to severe" depression. WSWS contains 7 factors: anger, anxiety, sadness, concentration,

craving, sleep, and hunger consisting of 28 items that are rated on a 5-point Likert scale (strongly disagree to strongly agree). Scale scores were ranked from 0 to 4 and calculated as the sum of respective items.

Fagerstrom test consists of 6 questions. We categorized each question as follows: (1) how soon after waking up do you smoke your first cigarette? (within 5 minutes, within 6 to 30 minutes, within 31 to 60 minutes or after 60 minutes). (2) Do you find it difficult to refrain from smoking in places where it is forbidden? (yes/no). (3) Which cigarette of the day is the hardest to give up? (the first 1 in the morning or any other). (4) How many cigarettes per day do you smoke? (10 or less, 11 to 20, 21 to 30, or 31 or more). (v) Do you smoke more frequently during the first hours after waking up than during the rest of the day? (yes/no). (6) Do you smoke even when you are sick and need to stay in bed most of the day? (yes/no).

Lymphocyte CD4 count was performed by flow cytometry (Becton-Dickinson (BD Biosciences 2350 Qume Drive San Jose, CA 95131 US)) and HIV viral load quantification was measured by the NUCLISENS method, with a minimum detection limit of 40 copies/ml. Substance abuse was identified as use of cocaine, cannabis, or downers (defined as a depressive or sedative drug) in the last 12 months. Cumulative specific antiretroviral drugs and classes were calculated. Nucleoside reverse transcriptase inhibitor, nonnucleoside reverse transcriptase inhibitor, protease inhibitor, and integrase inhibitor classes were included. No minimum exposure was required. The cumulative exposure is reported as the summation of a participant's time on a given agent or drug class up to the end of their study follow-up. The time since HIV diagnosis was calculated as the difference between self-reported laboratory documented date of HIV diagnosis and the study start date. Nadir $CD4^+$ T lymphocyte count was defined as the lowest recorded measurement obtained at least 7 days before inclusion.

Relapse refers to a self-report return to regular smoking >5 cigars per day for more than 3 days after some period of abstinence and confirmed by an expired CO level of >10 parts per million at week 12.[22] To evaluate the main outcome of the study, change in %FMD, we calculated the Δ%FMD% as %FMD at week 12 − %FMD at week1.

General descriptive characteristics of participants, shown as counts, percentages, medians, and interquartile ranges (IQRs), were compared according to smoking relapse (yes/no). We applied nonparametric Mann−Whitney tests for continuous variables and chi-square tests for categorical variables and the results are shown with the corresponding p values. To test the hypothesis that endothelial function would improve at week 12 in those participants who quit smoking, we performed multivariable linear regression models with the Δ%FMD as the main outcome and clinical and laboratory covariables as predictors, with the estimated 95% confidence intervals (CIs). In the secondary analysis, to investigate factors associated with relapse, we calculated odds ratios (ORs) with their respective 95% CIs using logistic regression models. To explore the predictors of endothelial disfunction, we have fit a linear regression model with the respective betas and 95% CIs. The final selection of covariates in each model was based on the best fit measured by the Akaike information criterion, using an automated model selection based on a genetic algorithm.[23] The candidate set for each model included traditional CV risk factors, such as age, gender, race, history of CVD, family history of premature CVD, education, monthly per-person income, hypertension, diabetes, dyslipidemia, body mass index (BMI), and hs-CRP. A 2-tailed level of significance was set at 5%. Analyses were performed using the R environment version 4.2.2 (Free Software Foundation 51 Franklin Street, Fifth FloorBoston, MA 02110, USA).

Results

Of the 135 screened participants for eligibility from December 2019 to April 2022, a total of 117 (86.6%) were included and allocated to intervention. At the end of 12 weeks, 20 (17.09%) were lost to follow-up because of refusal continue participating; however, they were included in the final analysis as relapse participants. Figure 1 depicts the flow diagram of study. The baseline characteristics of the study population according to relapse are listed in Table 1. Overall, they presented a median age of 45.4 years (IQR 36.54 to 54.8), predominantly men (72.17%) and smoking <20 packs of cigarettes a day (58.12%). Mostly had high grade of schooling (71.79%), in contrast with low monthly income (36.21%). Mental health assessed by PHQ-9 depression scale showed that almost half presented with moderate to severe depression (41.38%). Dyslipidemia was present at least in 1/3 of participants and most of them reported regular alcohol use (62.93%). Regarding nicotine dependence, most participants smoke the first cigarette within 6 minutes after waking, smoke in places where it is forbidden, or smoke even when they are sick in bed (Table 2). Regarding laboratory and HIV-related characteristics (Table 3), subjects had a 10.87-year median time of HIV infection (IQR 5.64 to 17.29) and 7.38 years of cumulative use of ART (IQR 3.28 to 13.06), exhibiting increased levels of hs-CRP (0.34, IQR 0.18 to 0.85). In the crude analysis, participants with relapse were younger (43.53 vs 54.2, p = 0.01) and had less co-morbidities such as hypertension (16% vs 36.11%, p = 0.01) and dyslipidemia (22.2 vs 44.4, p = 0.1). However, they had better management of diabetes measured by HbA1c (5.3 vs 5.7, p = 0.02) than those who had no relapse. Furthermore, at baseline, assessment of symptoms of tobacco withdrawal by WSWS in PLHA and the median points in domains of craving (12.5 [IQR 10 to 15] vs 11.5 [IQR 9 to 14]), p = 0.04) and hunger (12 [IQR 9 to 16] vs 9.5 [IQR 6.75 to 12.25], p <0.01) were higher in participants experiencing relapse than those who did not (Table 4).

At the end of treatment period, 20 participants (17.09%) were lost to follow-up and were analyzed as relapse. A total of 36 (30.76%) abstained from smoking completely and 81 (69.24%) returned to smoking habits; most of those who returned to smoking did at the beginning of treatment in week 4 (62.9%). However, in those who relapsed, there was a reduction from week 1 to week 12 on the average reported number of smoked cigarettes (20 [IQR 16.5 to 30] vs 11.5 [IQR 7 to 20], p <0.001), although no difference in the exhaled CO concentration was observed (19 [IQR 15 to 24.25] vs 18 [14 to 24.25]). Furthermore, at the follow-up

Table 1
Baseline general clinical and sociodemographic characteristics of people living with HIV according to relapse

	Relapse				
	-	+	Total	Missing	p value
Total	36	81	117		
Age in years median (IQR)	54.02 (39.61-63.48)	43.53 (36.14-51.99)	45.47 (36.54-54.8)	0	0.0106
Sex				0	0.648
Male	27 (75%)	56 (70.89%)	83 (72.17%)		
Years of education				0	0.3377
≤9	8 (22.22%)	25 (30.86%)	33 (28.21%)		
>9	28 (77.78%)	56 (69.14%)	84 (71.79%)		
Monthly Income*				0	0.9885
< US$ 367,50	23 (63.89%)	51 (63.75%)	74 (63.79%)		
US$ 367,50+	13 (36.11%)	29 (36.25%)	42 (36.21%)		
Self-declared race/color				0	0.7083
Black	8 (22.22%)	23 (28.75%)	31 (26.72%)		
Mixed/other	14 (38.89%)	31 (38.75%)	45 (38.79%)		
White	14 (38.89%)	26 (32.5%)	40 (34.48%)		
Weight median (IQR)	73 (61.01-81.93)	74.35 (61.33-86)	74 (61.1-84.45)	0.85%	0.7114
High median (IQR)	169 (165.75-173.25)	171 (165.5-176.5)	170 (165.5-175.5)	1.70%	0.1594
Waist − median (IQR)	93 (85.75-99.5)	89.5 (81-100)	91 (81-100)	0.85%	0.4243
BMI median (IQR)	25.93 (22.43-28.47)	25.4 (22.29-27.87)	25.53 (22.29-28.39)	1.70%	0.7129
Heart rate median (IQR)	71 (63-91.25)	74.5 (65.75-82)	73.5 (64-85)	0.85%	0.779
SBP median (IQR)	121 (114-136.25)	120.5 (112-130)	121 (112-131)	0.85%	0.268
DBP − median (IQR)	75 (66.75-80)	76 (68-81.25)	76 (68-81)	0.85%	0.6499
Baseline CO median (IQR)	16.5 (11.75-27.25)	19 (15.5-24.5)	19 (14-25)	0.85%	0.1358
Packs/day				0.85%	0.6619
<20	22 (61.11%)	46 (56.79%)	68 (58.12%)		
20+	14 (38.89%)	35 (43.21%)	49 (41.88%)		
C hepatitis	1 (2.78%)	5 (6.17%)	6 (5.13%)	0	0.6649
B Hepatitis	3 (8.33%)	6 (7.41%)	9 (7.69%)	0	0.9
Depression (PHQ9) scale				0.85%	0.653
Minimum-mild	20 (55.56%)	48 (60%)	68 (58.62%)		
Moderate-severe	16 (44.44%)	32 (40%)	48 (41.38%)		
Family History of premature CVD	16 (44.44%)	29 (35.8%)	45 (38.46%)	0	0.3752
History of CVD	3 (8.33%)	7 (8.64%)	10 (8.55%)	0	
Hypertension	13 (36.11%)	13 (16.05%)	26 (22.22%)	0	0.016
Diabetes mellitus	5 (13.89%)	7 (8.64%)	12 (10.26%)	0	0.5097
Dyslipidemia	16 (44.44%)	18 (22.22%)	34 (29.06%)	0	0.0145
Alcohol use	23 (63.89%)	50 (62.5%)	73 (62.93%)	0	0.8861
Medication for Hypertension	16 (45.71%)	14 (17.5)	30 (26.09%)	1.70%	0.0015
Hypoglycemic drugs	4 (11.43%)	7 (8.75)	11 (9.57%)	1.70%	0.7334
Aspirin	5 (14.29%)	5 (6.33%)	10 (8.77%)	2.56%	0.2793
Calcium channel blockers	4 (11.43%)	4 (5.06%)	8 (7.02%)	2.56%	0.2471
Benzodiazepines	9 (25.71%)	16 (20.25%)	25 (21.93%)	2.56%	0.5157
Beta blockers	4 (11.43%)	8 (10.13%)	12 (10.53%)	2.56%	1
ARBs	4 (11.43%)	6 (7.59%)	10 (8.77%)	2.56%	0.4932
Diuretic	6 (17.14%)	4 (5.06%)	10 (8.77%)	2.56%	0.066
ACE inhibitors	7 (20%)	3 (3.8%)	10 (8.77%)	2.56%	0.009
Statins	7 (20%)	11 (14.86%)	18 (16.51%)	2.56%	0.5002

ACE = angiotensin-converting enzyme; ARBs = angiotensin-2 receptor blockers; BMI = body mass index; CVD = cardiovascular disease; SBP = systolic blood pressure; DBP = diastolic blood pressure; PHQ9 = patient health questionnaire-9.

* Monthly income of 2 minimum salary R$1,996 equals to US$367.50.

Data are median (interquartile range [IQR]), n (%), unless otherwise specified.

examinations, the overall weight did not change significantly (74 [IQR 61.1 to 84.45]) vs 73 [61 to 82.25], p = 0.66).

After adjustment with multivariable logistic regression models (Table 5), increased age (OR 0.90, 95% CI 0.82 to 0.97) and presence of hypertension (OR 0.9, 95% CI 0.82 to 0.97) were associated with reduced chance of relapse and, although not significant, participants with more years of smoking exhibited an increased chance of relapse (OR 1.07, 95% CI 1.00 to 1.17).

Overall, %FMD showed improvement in the follow-up examinations from 6.31 (IQR 4.54 to 9.52) at week 1 to 6.75 (IQR 4.74 to 8.74) at week 12, resulting in a Δ%FMD of 0.29 (IQR −1.09 to 1.81). Participants who successfully quit smoking exhibited some improvement in the median average baseline vessel diameter from week 1 (3.78 [IQR 3.29 to 4.31]) to week 12 (3.87 [IQR 3.22 to 4.3]) compared with those presenting relapse (3.52 [IQR 3.18 to 4.09] to 3.45 [IQR 3.07 to 3.95]), In addition, participants who quit

Table 2
Fagerstrom test for nicotine dependence

	Relapse				
	-	+	Total	Missing	p value
Total	36	81	117		
First cigarette				0.85%	0.1634
>60 min	3 (8.33%)	4 (5%)	7 (6.03%)		
31-60 min	9 (25%)	9 (11.25%)	18 (15.52%)		
6-30 min	13 (36.11%)	30 (37.5%)	43 (37.07%)		
< 6 min	11 (30.56%)	37 (46.25%)	48 (41.38%)		
Smoking in places where it is forbidden	15 (41.67%)	34 (42.5%)	49 (42.24%)	0.85%	0.933
The First cigarette in the Morning	26 (72.22%)	63 (78.75%)	89 (76.72%)	0.85%	0.4415
Smoke more frequently after waking	14 (38.89%)	34 (42.5%)	48 (41.38%)	0.85%	0.7149
Smoke when ill	24 (66.67%)	46 (57.5%)	70 (60.34%)	0.85%	0.3505
Cigars/day				0.85%	0.6833
< 10	6 (16.67%)	8 (9.88%)	14 (11.97%)		
11 a 20	16 (44.44%)	38 (46.91%)	54 (46.15%)		
21 a 30	7 (19.44%)	21 (25.93%)	28 (23.93%)		
30+	7 (19.44%)	14 (17.28%)	21 (17.95%)		

smoking had an increase in median %FMD from 5.08 (IQR 3.53 to 7.69) to 6.72 (IQR 4.88 to 8.46). However, in those showing relapse, %FMD ranged from 6.96 (IQR 5.03 to 9.74) at week 1 to 6.79 (IQR 4.69 to 9.06) at week 12. Consequently, at the final follow-up evaluation, Δ%FMD was significantly higher in those who quit smoking (1.17 [IQR 0.3 to 2.98] vs −0.19 [−1.95 to 0.91], p <0.01) (Table 6). After adjustment using multiple linear regression, accounting for traditional CV factors such as age, gender, gender, history of CVD, and BMI, participants who quit smoking

Table 3
Laboratory and HIV related characteristics of study population according to relapse

	Relapse				
	-	+	Total	Missing	p value
Time of HIV median (IQR)	13.05 (5.58-21.02)	10.47 (5.71-16.31)	10.87 (5.64-17.29)	0.85%	0.4667
Abacavir use	1 (2.86)	2 (2.5)	3 (2.61)	1.70%	0.9
Time on NNRTI (years) median (IQR)	0.65 (0-4.92)	2.27 (0-6.64)	1.68 (0-5.89)	7.69%	0.2297
Time on NRTI (years) median (IQR)	5.33 (1.72-13.54)	5.52 (2.4-9.97)	5.42 (2.04-11.25)	7.69%	0.6817
Time on PI in years (years) median (IQR)	1.38 (0-6.15)	0 (0-4.29)	0.08 (0-5.34)	7.69%	0.3633
Cumulative ART median (IQR)	7.48 (2.4-14.6)	7.28 (3.36-12.54)	7.38 (3.28-13.06)	7.69%	0.7287
TGO (mg/dl) median (IQR)	23 (19.5-27.5)	22 (18-27)	22 (18-27)	1.70%	0.3537
TGP (mg/dl) median (IQR)	30.5 (24.75-43.25)	31 (24-43)	31 (24-43)	1.70%	0.8872
Alkaline Phosphatase (mg/dl) median (IQR)	87 (63.5-114)	94 (74.5-113)	92 (72-114)	1.70%	0.2789
GGT median (IQR)	57 (35-78)	51 (35-76)	53 (35-77)	1.70%	0.5605
Total bilirubin (mg/dl) median (IQR)	0.46 (0.34-0.78)	0.37 (0.25-0.55)	0.4 (0.26-0.62)	1.70%	0.1086
Direct bilirubin (mg/dl) median (IQR)	0.13 (0.08-0.18)	0.1 (0.08-0.14)	0.11 (0.08-0.15)	1.70%	0.2711
Indirect bilirubin (mg/dl) median (IQR)	0.32 (0.2-0.68)	0.28 (0.17-0.41)	0.29 (0.17-0.43)	1.70%	0.1625
Glucose (mg/dl) median (IQR)	94 (86.75-105)	89 (85-98)	91 (85-101)	3.41%	0.051
HbA1c median (IQR)	5.7 (5.4-5.88)	5.3 (5-5.55)	5.4 (5.1-5.7)	3.41%	0.0254
Creatinin (mg/dl) median (IQR)	27.5 (24,37.75)	25.5 (21,29)	26 (21,31.75)	2.56%	0.4139
hs-CRP (mg/l) median (IQR)	0.26 (0.18-0.67)	0.39 (0.18-0.86)	0.34 (0.18-0.85)	12.82%	0.4383
Total cholesterol median (IQR)	176 (143.5-199.25)	166 (138-190)	172 (139-193)	3.41%	0.3732
LDL (mg/dl) median (IQR))	102 (68.5-117.25)	95 (74-117)	97 (72-117)	3.41%	0.622
HDL (mg/dl) median (IQR)	39 (33.75-55.5)	40.5 (34-49)	40 (34-50.5)	3.41%	0.8286
Triglycerides (mg/dl) median (IQR)	130.5 (78.5-192)	130 (92-174)	130 (85-176)	3.41%	0.8946
Viral load (IQR)	0 (0-0)	0 (0-0)	0 (0-0)	5.12%	0.512
Current CD4 counts (cells/mL) median (IQR)	852 (605-1020)	818 (603.5-1008)	828 (604.5-1014)	5.12%	0.8714
Nadir CD4 median (IQR)	334 (172,516)	298 (126,466)	307 (153,490.5)	5.12%	0.6692
RBC ($x10^6$/uL) median (IQR)	4.53(3.88-4.98)	4.58 (4.22-4.94)	4.57(4.13-4.97)	3.41%	0.49
Hemoglobin (g/dL) median (IQR)	14.8 (13.23-15.93)	14.6 (13.7-15.6)	14.6 (13.6-15.7)	3.41%	0.8775
Hematocrit (%) median (IQR)	42.95 (38.8-46.05)	42.3 (40.4-45.4)	42.4 (40.1-45.5)	3.41%	0.7815
WBC ($x10^3$/uL) median (IQR)	6.88 (4.94-7.74)	7.12 (5.49-9.04)	6.97 (5.39-8.74)	3.41%	0.1327
Platelets $x10^3$ median (IQR)	225 (179-254)	243(206-295)	236 (203-293)	3.41%	0.0817

ART = antiretroviral therapy. GGT- gamma-glutamyl transferase; HbA1c- glycated hemoglobin; HDL: high-density lipoprotein; hs-CRP: high-sensitivity C reactive protein; LDL- low-density lipoprotein; IQR: interquartile range; NNRTI- Non-nucleoside reverse transcriptase inhibitors; NRTI- nucleoside reverse transcriptase inhibitors; PI-protease inhibitors; TGO - alanine aminotransferase; TGP - aspartate aminotransferase.

Table 4
Baseline Wisconsin Smoking Withdrawal Scale (WSWS) among people living with HIV

	Relapse				
	-	+	Total	Missing	p value
Anger median (IQR)	6 (3-8)	7(3-9)	6 (3-8.25)	0.85%	0.5746
Anxiety median (IQR)	12 (8.75-13.25)	12 (9.75-14)	12 (9-14)	0.85%	0.7642
Sadness median (IQR)	7 (5-8.5)	7 (4-10.25)	7 (4-10)	0.85%	0.7464
Concentration median (IQR)	6 (4-7)	6 (4-7)	6 (4-7)	0.85%	0.7203
Craving median (IQR)	11.5 (9-14)	12.5 (10-15)	12 (10-15)	0.85%	0.0417
Sleep median (IQR)	13 (7.75-15)	12 (7-16)	12 (7-15.25)	0.85%	0.8834
Hunger median (IQR)	9.5 (6.75-12.25)	12 (9-16)	11 (8-15)	0.85%	0.0071
Total median (IQR)	62 (51.75-73)	68 (55-80.25)	66 (54-78)	0.85%	0.1077

Table 5
Final multiple logistic regression model with predictors of relapse among people living with HIV

	Univariable- OR (95%CI)	Multivariable- OR (95%CI)
Age in years	16.49 [3.32 - 96.93, p=0.01]	0.90 [0.82 - 0.97, p=0.01]
Sex: Female	1.23 [0.51 -3.14, p=0.65]	1.03 [0.38 -2.85, p=0.99]
Hypertension	0.34 [0.13 - 0.83, p<0.01]	0.31 [0.10 -0.94, p=0.04]
Years of smoking	0.97 [0.94 - 1.01, p=0.11]	1.07 [1.00 - 1.17, p=0.07]
BMI	0.99 [0.91 - 1.08, p=0.89]	1.05 [0.96- 1.11, p=0.30]

exhibited a mean 2.54-point increase in Δ%FMD (p <0.01) compared with those who continued smoking (Table 7). The information about Δ%FMD and percentage change %FMD according to relapse is depicted graphically. Supplementary Figure 1 express a boxplot displaying the difference in % flow dilatation of the brachial artery according to relapse status. The *x* axis has the relapse status category and the *y* axis has the % flow dilatation values. Supplementary Figure 1 depicts a scatterplot of %FMD values in week 1 and week 12 according to relapse. The plot contains dots representing individual participants, with different colors representing participants who relapsed versus those who did not. Exploring the predictors of endothelial dysfunction at initial examination, we found that when adjusted for the average baseline diameter, older age (−2.40 [−3.86 to −0.94, <0.01]), lower BMI (0.86 [0.18 to 1.54, 0.01]), and duration of ART (0.86 [0.06 to 1.67, 0.04]) were associated with %FMD (Supplementary Table 1)

Discussion

In the present cohort study, we found that in PLHIV who are motivated to quit smoking, brief counseling combined with NRT with dermal patches are moderately effective for smoking cessation. At the end of 3 months, 30.76% of the participants remained continuously abstinent from smoking, leading to an improvement of vascular health measured

Table 6
Vascular parameters of brachial artery among PLHA according to relapse

	Relapse				
	-	+	Total	Missing	p value
		Baseline measurement at Week 1			
Dbase (mm) median (IQR)	3.78 (3.29-4.31)	3.52 (3.18-4.09)	3.61 (3.18-4.12)	0.85%	0.2854
Area (cm^2) median (IQR)	0.11 (0.08-0.15)	0.1 (0.08-0.13)	0.1 (0.08-0.13)	0.85%	0.2397
Flow (ml/min) median (IQR)	59 (43.75-95.75)	52.5 (35.75-110.25)	54 (37-104.75)	0.85%	0.3306
		Post-ischemic measurement at Week 1			
Dmax median (IQR)	4.02 (3.47-4.45)	3.77 (3.42-4.35)	3.82 (3.45-4.36)	0.85%	0.4153
%FMD median (IQR)	5.08 (3.53-7.69)	6.96 (5.03-9.74)	6.31 (4.54-9.52)	0.85%	0.0112
		Baseline measurement at Week 12			
Dbase (mm) median (IQR)	3.87 (3.22-4.3)	3.45 (3.07-3.95)	3.56 (3.1-4.12)	17.09%	0.0806
Area (cm^2) median (IQR)	0.11 (0.09-0.14)	0.09 (0.07-0.12)	0.10 (0.08-0.13)	17.09%	0.0445
Flow (ml/min) median (IQR)	58 (33.75-119)	58 (33-97)	58 (33-102)	17.09%	0.7823
		Post-ischemic measurement at Week 12			
Dmax median (IQR)	4.14 (3.45,4.5)	3.64 (3.3,4.23)	3.81 (3.39,4.38)	17.09%	0.0554
%FMD median (IQR)	6.72 (4.88-8.46)	6.79 (4.69-9.06)	6.75 (4.74-8.74)	17.09%	0.8054
Δ%FMD median (IQR)	1.17 (0.3-2.98)	-0.19 (-1.95-0.91)	0.29 (-1.09-1.81)	17.09%	< 0.001

Dbase = maseline diameter; Dmax = maximum diameter; FMD = flow-mediated dilatation.

Table 7
Final linear regression model with associations of Δ%flow-mediated dilatation in people living with HIV smokers

	Univariable- estimate (95%CI, p-value)	Multivariable- estimate (95%CI, p-value)
Relapse: No	2.55 (1.30 - 3.80, <0.01)	2.54 (1.20 - 3.87, <0.01)
Age in years	0.05 (-0.01- 0.10, 0.06)	0.46 (-0.33 - 1.25, 0.25)
Sex: Female	-0.19 (-1.64 - 1.26, 0.80)	-0.36 (-1.81 - 1.08, 0.62)
History of CVD: Yes	1.97 (0.67 - 3.28, <0.01)	2.18 (0.82 - 3.54, <0.01)
BMI	-0.06 (-0.21 - 0.08, 0.39)	-0.35 (-0.98 - 0.28, 0.27)
Alcohol: Yes	-1.11 (-2.45 - 0.22, 0.10)	-1.53 (-2.87 - -0.19, 0.03)
Race: Mixed	-0.12 (-1.76 - 1.51, 0.88)	-0.89 (-2.49 - 0.71, 0.27)
Race: White	-0.45 (-2.16 -1.26, 0.60)	-1.38 (-3.10 - 0.34, 0.11)
Nadir CD4 $\times$ 10^2	0.03 (-0.19 -0.26, 0.77)	0.10 (-0.12 - 0.31, 0.38)
ART duration in years	-0.02 (-0.12 -0.07, 0.61)	-0.53 (-1.32 - 0.236, 0.19)

ART = antiretroviral therapy; BMI = body mass index; CVD = cardiovascular disease.
All continuous predictors are mean-centered and scaled by 1 standard deviation.

by FMD of the brachial artery, which is what can be considered a short period of time. To the best of our knowledge, this is the first study to assess endothelial function in PLHIV shortly after smoking cessation.

However, this finding is consistent with other studies that have assessed FMD and smoking cessation in the general population. For instance, in a randomized control trial comparing 5 smoking cessation pharmacotherapies, after 1 year, 36.2% quit smoking. FMD increased by 1% (6.2% [4.4%] to 7.2% [4.2%]) in those who quit smoking.[11] More recently, in another cohort study in the general population with mean age 34.4 years and smoking 23.8 cigarettes per day, after 3 months of NRT, endothelial function, arterial stiffness, and inflammatory markers significantly improved. In this scenario, 59% of the participants quit based on a self-assessment report and the end-expiratory CO concentrations and, similar to our study, participants who did not abstain from smoking reported a reduction in the number of cigarettes per day.[24] In fact, endothelial function assessed by FMD seems to ameliorate in patients who quit smoking even with new therapies such as varenicline.[25]

The mechanism of vascular dysfunction induced by smoking is initiated by reduced nitric oxide bioavailability and further by the increased expression of adhesion molecules. Smoking increases the adherence of platelets and macrophages and provokes the development of a procoagulant and inflammatory environment, therefore generating a negative impact on FMD.[26] Impaired endothelium-dependent vasodilation is a putative surrogate marker of coronary atherosclerotic disease, leading to an increased risk of CVD. PLHIV are at an increased risk of developing CVD, and smoking can increase this risk even further. Abstinence from smoking is essential for the overall health and well-being of PLHIV in the ART era. Therefore, health care providers should prioritize smoking cessation interventions for this population and provide resources and support to help them quit smoking.

Another important finding in our population study was the very common presence of alcohol consumption and major depressive symptoms associated with cigarette use; such characteristics reinforce addictive behaviors making more difficult for them to quit smoking. In this scenario, besides behavioral and pharmacotherapy treatments, an intensive approach combining multiple sessions and a multidisciplinary team is advised to increase the likelihood of abstinence end reduce relapses.[27] We also showed that smoking cessation strategies are possible to implement not only in primary care clinics at Brazilian Unified Health System as part of the National Smoking Control Policy but also works similarly on a broad scope across multiple settings such as infectious disease institutes.

There are several limitations to consider when interpreting the findings of this study. We cannot exclude the possibility of observer bias, a type of cognitive behavior that occurs when a researcher's expectations influence their interpretation of results. This bias might have occurred if the researcher had access of the smoking status of the participant at week 12 before analyzing brachial artery and their expectations could affect FMD interpretation. To minimize this bias, we used a semiautomated software to analyze FMD, making the measurements more objective. It is also important to notice that as in many other research centers across the word, because of the COVID-19 pandemic, all ongoing research became a risk in Brazil and between March and August 2020; study enrollment and inclusion had stopped as part of government lockdown strategies. Therefore, we adapted clinical visits and counseling by phone, which might have had an impact on relapse.

In addition, endothelial-independent dilation of the brachial artery, which is mediated by the smooth muscle cells in the artery wall rather than by the endothelium using nitroglycerin, was not measured because of logistics and ethical concerns. This aspect limits our ability to obtain a more comprehensive understanding of an individual's vascular health.

Abstinence rates in studies, particularly, at long-term follow-up points, are generally modest, ranging from 10% to 25%, with many participants experiencing relapse within the first year of quitting.[28] We presented preliminary 3-month results here; however, we are less certain if these rates will remain constant over time for this population. Smoking cessation success depends, among other factors, on the participant's commitment to quitting and their ability to manage triggers and cravings over time. Multiple quitting attempts may be necessary before achieving long-term success. In this study, we did not measure the cotinine levels because it was not available. Instead, we used exhaled carbon monoxide (CO) concentrations as a proxy for cigarette exposure and abstinence. Nicotine itself may be present in the blood for only

48 hours, whereas cotinine may be detectable for up to 3 weeks. Moreover, because interventions in this study included NRT, it could have produced false-positive results. Participants who were self-reported as regular smokers at week 4 were already considered as relapse. This method is commonly known as cold turkey or to quit smoking abruptly. It seems that people who adhere to this method are more likely to remain abstinent than those who quit gradually.[29]

Although we did not specifically investigate parameters such as better control of hypertension or reduced incidence of atherosclerotic CVD, the observed improvement in brachial vasoreactivity suggests a positive impact on endothelial function, a key factor in overall vascular health.

The strengths of the study were that it was conducted prospectively, which means that data were collected in real-time as the study progressed, increasing the reliability of the findings. We included a relatively large sample of 115 participants, with broad inclusion criteria, which increases the generalizability of the findings. We also used objective measures to assess smoking cessation and vascular health improvement, such as expired CO levels and FMD. This reduces the risk of measurement bias and increases the validity of the findings. We had a relatively short follow-up period of 12 weeks, which allowed the assessment of short-term effects of NRT on smoking cessation and vascular health. This is a strength because it provides insight into the potential benefits of NRT in the short-term, which can inform clinical practice.

In conclusion, we showed that the combination of brief counseling and NRT had a reasonable effect on smoking cessation, leading to short-term vascular health improvement in PLHIV. Antitobacco programs should be broadened to HIV clinics in Brazil as a public health policy to promote further reductions in the incidence of future CV events.

Declaration of competing interest

Dr. Moreira reports financial support was provided by Oswaldo Cruz Foundation. The remaining authors have no competing interest to declare.

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Rodrigo de Carvalho Moreira: Conceptualization, Investigation, Methodology, Writing − original draft, Writing − review & editing, Formal analysis, Funding acquisition. **Angela Rodrigues:** Data curation, Project administration, Writing − original draft. **Beatriz Menezes Leonardo:** Investigation, Project administration, Supervision. **Daniel Arabe:** Data curation, Project administration. **Renata Santos:** Data curation, Project administration. **Sandra Wagner Cardoso:** Investigation, Supervision, Writing − original draft. **Beatriz Grinsztejn:** Project administration, Supervision, Writing − original draft. **Valdilea Veloso:** Investigation, Methodology, Supervision, Writing − original draft. **Antonio G. Pacheco:** Methodology, Supervision, Writing − original draft.

Supplementary materials

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