UCSF

UC San Francisco Previously Published Works

Title

Cerebral vasoreactivity is impaired in treated, virally suppressed HIV-infected individuals.

Permalink

<https://escholarship.org/uc/item/3sx457qf>

Journal

AIDS (London, England), 30(1)

ISSN

0269-9370

Authors

Chow, Felicia C
Boscardin, W John
Mills, Claire
et al.

Publication Date

2016

DOI

10.1097/qad.0000000000000875

Peer reviewed

Cerebral vasoreactivity is impaired in treated, virally suppressed HIV-infected individuals

Felicia C. Chow^a, W. John Boscardin^a, Claire Mills^a, Nerissa Ko^a, Courtney Carroll^a, Richard W. Price^a, Steven Deeks^a, Farzaneh A. Sorond^b and Priscilla Y. Hsue^a

Objective: To compare cerebral vasoreactivity, a measure of cerebrovascular endothelial function, between treated, virally suppressed HIV-infected individuals and HIV-uninfected controls and to evaluate the effect of HIV-specific factors on cerebral vasoreactivity.

Methods: Cross-sectional study of 65 antiretroviral therapy-treated, virally suppressed HIV-infected individuals and 28 HIV-uninfected controls. Participants underwent noninvasive assessment of cerebral vasoreactivity using transcranial Doppler ultrasound and inhaled carbon dioxide (CO₂). We used mixed effects multivariable linear regression to determine the association of HIV infection and HIV-specific factors with cerebral vasoreactivity.

Results: Mean age was 57.2 years for HIV-infected participants and 53.5 years for HIV-uninfected controls. Most participants (95%) were men. Twenty-six per cent of HIV-infected participants were nonwhite compared to 32% of controls. Among HIV-infected participants, mean CD4⁺ cell count was 596 cells/μl, and mean duration of viral suppression was 7.8 years. Cerebral vasoreactivity in response to hypercapnia (cerebral VR_{hyper}) was lower in HIV-infected individuals compared to uninfected controls (3.23 versus 3.81%, $P = 0.010$). After adjusting for demographic and vascular risk factors, HIV infection was independently associated with lower cerebral vasoreactivity (−0.86%, 95% CI −1.30 to −0.42%, $P < 0.001$). We did not find a statistically significant effect of recent or nadir CD4⁺ cell count on cerebral vasoreactivity. There was a trend toward higher cerebral vasoreactivity for each additional year of viral suppression.

Conclusion: Treated, virally suppressed HIV infection negatively impacted cerebral vasoreactivity even after adjustment for traditional vascular risk factors. These data highlight the potential contribution of cerebrovascular endothelial dysfunction to the elevated risk of stroke observed in HIV-infected individuals.

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

AIDS 2016, **30**:45–55

Keywords: cerebral vasoreactivity, cerebrovascular disease, endothelial function, HIV, stroke, transcranial Doppler, treatment

With the transformation of HIV into a chronic, treatable disease, HIV-infected individuals face excess risk of a growing number of non-AIDS-related comorbidities more typical of an aging population [1,2]. Rates of several vascular outcomes, including stroke, are higher in

HIV-infected individuals than in age-matched uninfected controls [3–7]. Endothelial dysfunction and accelerated atherosclerosis related to HIV-associated chronic inflammation and immune activation may contribute to increased cardiovascular risk in this unique patient

^aUniversity of California San Francisco, San Francisco, California, and ^bBrigham and Women's Hospital, Boston, Massachusetts, USA.

Correspondence to Felicia C. Chow, MD, MAS, University of California San Francisco, San Francisco, San Francisco, CA, USA. E-mail: ChowF@sfg.ucsf.edu

Received: 19 February 2015; revised: 17 August 2015; accepted: 28 August 2015.

DOI:10.1097/QAD.0000000000000875

population [8]. The mechanisms underlying increased cerebrovascular risk in HIV infection, however, have not been investigated. Cerebrovascular function in individuals with treated, virally suppressed chronic HIV infection is unknown, including whether it is impaired compared with HIV-uninfected individuals.

Cerebral vasoreactivity is a measure of cerebrovascular endothelial function associated with both small vessel and severe large artery cerebrovascular injury [9–14]. Measurement of cerebral vasoreactivity with transcranial Doppler (TCD) ultrasound is noninvasive, cost-effective and has excellent temporal resolution. A vasodilatory response to inhaled carbon dioxide (CO₂) and vasoconstriction to hyperventilation, denoted by an increase and decrease in mean blood flow velocity of the cerebral arteries, respectively, is one method of assessing cerebral vasoreactivity. Changes in cerebral vasoreactivity over time and in response to interventions aimed at improving cerebrovascular function can be readily monitored. For example, cerebral vasoreactivity has been shown to improve with interventions that reduce cerebrovascular risk, including statin use and exercise [15–17].

The primary goal of the UNderstanding Cerebral VasoReactivity in HIV infection (UNCoVeR) study was to compare cerebral vasoreactivity between treated, virally suppressed HIV-infected individuals and uninfected controls and to evaluate the effect of HIV-specific factors on cerebral vasoreactivity.

Methods

Study population

We performed a cross-sectional study of a convenience sample of participants from SCOPE [18] (the Study of the Consequences of the Protease Inhibitor Era), a contemporary cohort of over 2000 HIV-infected individuals and uninfected controls followed prospectively at San Francisco General Hospital. Participants involved in a parent study evaluating cardiovascular health in HIV infection in SCOPE were contacted to gauge interest in participation in a sub-study focused on cerebrovascular risk. Original recruitment for the parent study occurred at routine SCOPE visits, where participants received information regarding the cardiovascular study and, if interested, contacted the study for additional details. Demographically matched uninfected controls were recruited for the parent study through referrals of friends and acquaintances of HIV-infected participants and were tested for HIV prior to entry. Flyers were also posted around the hospital and outpatient clinics advertising the parent study. All participants were at least 18 years of age and willing to abstain from caffeine, tobacco, alcohol or other substance use for 12 h prior to the study. The study was approved by the University of California, San Francisco Committee on Human Research.

All HIV-infected participants were on a stable antiretroviral therapy (ART) regimen with undetectable plasma HIV RNA (<40 copies/ml) for a minimum of 24 weeks prior to the study. Exclusion criteria included a history of stroke or transient ischemic attack, central nervous system (CNS) infection or traumatic brain injury in the 3 months before the study; a history of intracranial vasculitis or arteriopathy due to causes other than HIV or traditional vascular disease (e.g., primary CNS vasculitis); use of immune modulatory agents in the 3 months before the study; absent temporal acoustic windows; a history of an untreated cerebral aneurysm or other vascular malformation, severe chronic obstructive pulmonary disease, unstable angina, recent myocardial infarction or other conditions that precluded cerebral vasoreactivity testing.

Cerebral vasoreactivity

We assessed cerebral vasoreactivity with the CO₂ challenge test [19]. TCD was used to measure alterations in middle cerebral artery (MCA) mean blood flow velocity in response to changes in end-tidal CO₂ (ETCO₂). A single vascular technologist trained in the TCD CO₂ challenge test and blinded to HIV status performed all studies to minimize interrater variability. A Sonara Digital TCD machine (Natus Medical, San Carlos, California, USA) was used for all studies. The MCA signal was identified through a transtemporal approach using standard methodology [20,21]. We recorded mean blood flow velocities at rest, during inhalation of a 6% CO₂ gas mixture and during mild hyperventilation.

The primary cerebral vasoreactivity outcome was cerebral vasoreactivity in response to hypercapnia (cerebral VR_{hyper}), defined as the percentage change in mean flow velocity from baseline to induced hypercapnia per unit change in ETCO₂. As a secondary outcome, we also evaluated cerebral vasoreactivity in response to the full range of hyper and hypocapnia (cerebral VR_{hyper + hypo}), calculated as the percentage change in mean flow velocity from hypercapnia to hypocapnia per unit change in ETCO₂.

We performed repeat cerebral vasoreactivity testing within 1 month of the first procedure to assess reproducibility in five participants. For the repeat study, participants were scheduled at approximately the same time of day as the first procedure to minimize diurnal variability. Because cerebral vasoreactivity for the left and right sides were highly correlated, we compared a single value representing the mean cerebral vasoreactivity from the left and right sides between the first and second visits. We calculated the intraclass correlation coefficient (ICC) after adjusting for the change in mean arterial pressure with induced hypercapnia. We found a strong correlation between cerebral vasoreactivity from the first and second visits, although the large confidence intervals reflect the small number of repeated studies performed (ICC for

cerebral VR_{hyper} of 0.80, 95% CI 0.30–0.97; ICC for cerebral $VR_{\text{hyper} + \text{hypo}}$ of 0.93, 95% CI 0.65–0.99).

Vascular risk factors, substance use and other covariates

Prior to the TCD study, we collected data on comorbidities and other covariates that could potentially confound or mediate the association between HIV infection and cerebral vasoreactivity, including traditional vascular risk factors (defined by self-report of prior diagnosis by a medical provider with or without current use of antihypertensive, antidiabetic or lipid-lowering therapy); antiplatelet and statin use; current smoking, alcohol and substance use; and a history of migraines and sleep apnea. HIV-specific characteristics, including duration of HIV infection and viral suppression, ART use by class, most recent and nadir $CD4^+$ cell count, were also obtained. These data were supplemented and/or confirmed by available information through the parent cardiovascular study and review of electronic medical records.

Statistical analysis

We compared demographic and clinical characteristics between HIV-infected and uninfected participants using *t*-test, Chi-square test or Fisher's exact test. We used mixed effects linear regression models to determine the association between HIV and cerebral vasoreactivity and between HIV-specific factors and cerebral vasoreactivity. Because cerebral vasoreactivity is measured on the left and right sides, we included a random person effect to account for within-person correlation. All multivariable models were adjusted for age and race. In the models of HIV-infected individuals and uninfected controls, we included clinically relevant covariates of a-priori interest (e.g. hypertension, diabetes mellitus, statin use) and other covariates chosen by forward stepwise selection. To maximize parsimony in the HIV-only analyses (given fewer observations), only covariates identified through forward stepwise selection were included. *P* values were two-sided with ≤ 0.05 considered statistically significant. Statistical analyses were performed using Stata (StataCorp 2012. Stata Statistical Software: Release 12; Stata Corporation, College Station, Texas, USA).

Results

Study enrolment

We enrolled 100 individuals between January and June 2014 who underwent the TCD CO_2 challenge. Of these 100 individuals, one participant terminated the study early due to discomfort from breathing into the mouthpiece. In one HIV-infected participant, an acoustic temporal window on neither the left nor right sides could be located. In one control participant, we did not observe an adequate rise in $ETCO_2$ following inhalation of the

CO_2 gas mixture, which was hypothesized to be from a poor seal around the mouthpiece due to a preexisting neurological illness. In four participants (three HIV-infected and one control), cerebral vasoreactivity results were excluded because the expected rise or fall in mean blood flow velocities during inhalation of CO_2 or mild hyperventilation was not observed. In sum, the results from 93 participants were included in the study (65 HIV-infected and 28 uninfected control participants), on whom 175 total (87 left-sided, 88 right-sided) measurements were obtained.

Baseline demographic and clinical characteristics

Demographic and clinical characteristics of the 93 participants are included in Table 1.

HIV-infected participants were older than uninfected controls, although this did not reach statistical significance. Most participants were men, while 26% of HIV-infected participants were nonwhite compared to 32% of controls. Several vascular risk factors were more common in HIV-infected participants, including hypertension (49 versus 29%, $P = 0.065$), dyslipidemia (57 versus 21%, $P = 0.002$) and diabetes mellitus (12 versus 0%, $P = 0.10$). Statin and aspirin use were also more common among HIV-infected participants. More uninfected controls were current alcohol users, while marijuana use was more common among HIV-infected participants. Among the HIV-infected cohort, the mean $CD4^+$ cell count was 596 cells/ μ l, while the mean nadir $CD4^+$ cell count was 242 cells/ μ l. The mean duration of known HIV seropositivity was 20.2 years, and mean duration of viral suppression was 7.8 years. Nearly all participants were on an ART regimen that contained at least one nucleoside reverse transcriptase inhibitor (NRTI) and 32% were on abacavir, while over half were on a nonnucleoside reverse transcriptase inhibitor (NNRTI) compared to 42% on a protease inhibitor and 34% on an integrase inhibitor.

Baseline mean blood flow velocities

Baseline mean blood flow velocity on the right and left sides for HIV-infected individuals were 52 and 53 cm/s, respectively, compared to 51 cm/s bilaterally for uninfected controls ($P = 0.65$ for the right side, $P = 0.37$ for the left side). As expected, intra-participant right and left-sided cerebral VR_{hyper} and cerebral $VR_{\text{hyper} + \text{hypo}}$ measurements were strongly correlated [Pearson correlation coefficients 0.85 ($P < 0.001$) and 0.93 ($P < 0.001$), respectively].

Difference in cerebral vasoreactivity by HIV status

Cerebral VR_{hyper} was reduced in HIV-infected individuals compared to uninfected controls (cerebral VR_{hyper} 3.23%, 95% CI 2.98–3.47% compared with 3.81%, 95% CI 3.44–4.18%, $P = 0.010$) (Table 2). After adjustment for age, race, diabetes mellitus, hypertension, statin use,

Table 1. Demographic and clinical characteristics of treated, virally suppressed HIV-infected individuals and uninfected controls.

	HIV-infected (n = 65)	Uninfected controls (n = 28)	P value
Demographics (% of total unless noted)			
Age (years), mean (SD)	57.2 (8.5)	53.5 (10.0)	0.071
Male sex	95	96	0.80
Nonwhite race/ethnicity	26	32	0.56
Vascular and other risk factors (% of total unless noted)			
Hypertension	49	29	0.065
Dyslipidemia	57	21	0.002
Statin use	48	11	0.001
Aspirin use	49	21	0.012
Coronary heart disease	5	0	0.55
Diabetes mellitus	12	0	0.10
Hepatitis C infection	23	4	0.033
Prior stroke	3	0	1.00
Migraines	11	18	0.50
Sleep apnea	15	4	0.16
Current substance use (% of total unless noted)			
Alcohol use	55	79	0.034
0 drinks per day	45	21	0.20
Up to 1 drink per day	40	61	
Up to 2 drinks per day	9	11	
Up to 3 drinks per day	6	7	
Tobacco use	14	25	0.19
Cocaine use	5	7	0.63
Heroin use	0	4	0.30
Methadone use	2	4	0.51
Methamphetamine use	6	4	1.00
Marijuana use	48	21	0.018
HIV-specific factors			
CD4 ⁺ cell count (cells/ μ l), mean (SD)	596 (251)	—	—
Nadir CD4 ⁺ cell count (cells/ μ l), mean (SD)	242 (175)	—	—
Duration of HIV infection (years), mean (SD)	20.2 (6.8)	—	—
Duration of viral suppression (years), mean (SD)	7.8 (3.8)	—	—
Current ART regimen containing (%):			
PI	42	—	—
Any NRTI	98	—	—
Abacavir	32	—	—
Any NNRTI	55	—	—
Integrase inhibitor	34	—	—
Maraviroc	5	—	—

ART, antiretroviral therapy; PI, protease inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

aspirin use, alcohol consumption and methamphetamine use, HIV infection was independently associated with lower cerebral vasoreactivity (-0.86% , 95% CI -1.30% to -0.42% , $P < 0.001$) (Fig. 1, Table 2). Diabetes mellitus and nonwhite race were also independent predictors of lower cerebral VR_{hyper} , as was methamphetamine use (Table 2). Each additional drink per day was associated with an increase in cerebral VR_{hyper} . In the multivariate model of cerebral VR_{hyper} , we observed an interaction between statin use and HIV infection. Statin use among those with HIV infection was associated with an improvement in cerebral VR_{hyper} , while it had no statistically significant impact on cerebral VR_{hyper} among uninfected controls (Table 2). We observed a trend toward an association between hepatitis C infection and cerebral VR_{hyper} in a univariate model (Table 2) but not in the multivariate model. To check for stability of the final multivariate model, we performed adjusted models excluding several variables that affected a relatively small number of participants (e.g. diabetes mellitus,

methamphetamine and statin use). In these simpler models, the main effect of HIV infection on cerebral vasoreactivity was highly comparable to the full model.

In a secondary analysis, unadjusted cerebral $VR_{\text{hyper} + \text{hypo}}$ was lower in HIV-infected individuals compared to uninfected controls, although confidence intervals were too wide to rule out no difference in cerebral $VR_{\text{hyper} + \text{hypo}}$ by HIV status (2.23%, 95% CI 2.09–2.39% compared with 2.44%, 95% CI 2.21–2.67%, $P = 0.15$) (Table 3). After adjusting for age, race, diabetes mellitus, hypertension, statin use, aspirin use, alcohol consumption, and methamphetamine use, HIV infection was independently associated with a reduction in cerebral $VR_{\text{hyper} + \text{hypo}}$ (-0.30% , 95% CI -0.56% to -0.03% , $P = 0.030$) (Fig. 1, Table 3). In the multivariate model, statin use was strongly associated with an increase in cerebral $VR_{\text{hyper} + \text{hypo}}$ ($+0.42\%$, 95% CI $+0.13\%$ to $+0.71\%$, $P = 0.004$) as was alcohol use ($+0.19\%$ for each additional drink per day, 95% CI $+0.04\%$ to $+0.33\%$,

Table 2. Bivariate and multivariate models of the effect of HIV infection and other risk factors on cerebral vasoreactivity in response to hypercapnia (cerebral VR_{hyper}).

HIV-infected (<i>n</i> = 65) + HIV-uninfected (<i>n</i> = 28), Total <i>n</i> = 93 Total observations (left + right sides) <i>n</i> = 175				
	Difference in mean cerebral VR _{hyper} ^a (95% CI)		Difference in mean cerebral VR _{hyper} ^a (95% CI)	
	Bivariate analyses	<i>P</i> value	Multivariate model ^d	<i>P</i> value
Demographics				
Age (per 10-year increase)	+0.03 (−0.20 to +0.26)	0.81	−0.08 (−0.29 to +0.13)	0.45
Male sex	−0.26 (−1.32 to +0.80)	0.63	—	—
Race (nonwhite versus white)	−0.65 (−1.11 to −0.20)	0.005	−0.68 (−1.09 to −0.27)	0.001
Vascular and other risk factors				
Hypertension	−0.04 (−0.46 to +0.39)	0.86	−0.01 (−0.39 to +0.38)	0.97
Dyslipidemia	+0.14 (−0.28 to +0.56)	0.52	—	—
Statin use	+0.12 (−0.32 to +0.56)	0.60	Statin use in HIV-infected ^e +0.51 (+0.06 to +0.96)	0.028
			Statin use in HIV-uninfected −0.65 (−1.73 to +0.42)	0.24
Aspirin use	+0.27 (−0.15 to +0.70)	0.21	+0.39 (−0.03 to +0.81)	0.067
Coronary heart disease	+0.03 (−1.16 to +1.22)	0.96	—	—
Diabetes mellitus	−0.88 (−1.62 to −0.15)	0.019	−0.91 (−1.60 to −0.23)	0.009
Hepatitis C infection	−0.51 (−1.06 to +0.05)	0.073	—	—
Prior stroke	+0.89 (−0.55 to +2.32)	0.23	—	—
Migraines	+0.27 (−0.35 to +0.90)	0.39	—	—
Sleep apnea	+0.21 (−0.44 to +0.86)	0.53	—	—
Traumatic brain injury	−0.09 (−0.89 to +0.71)	0.83	—	—
Substance use ^b				
Alcohol use (for each additional drink per day)	+0.22 (−0.03 to +0.48)	0.083	+0.26 (+0.03 to +0.48)	0.027
Tobacco use	−0.32 (−0.88 to +0.23)	0.26	—	—
Cocaine use	−0.69 (−1.62 to +0.24)	0.15	—	—
Heroin use	+0.48 (−1.56 to +2.51)	0.65	—	—
Methamphetamine use	−1.05 (−1.96 to −0.13)	0.025	−1.13 (−1.90 to −0.36)	0.004
Marijuana use	−0.001 (−0.43 to +0.43)	1.00	—	—
HIV and HIV-specific factors ^c				
HIV infection	−0.59 (−1.03 to −0.14)	0.010	−0.86 (−1.30 to −0.42) ^f	<0.001
CD4 ⁺ cell count (per 50 cells/μl increase)	+0.001 (−0.05 to +0.05)	0.97	—	—
Nadir CD4 ⁺ cell count (per 50 cells/μl increase)	+0.04 (−0.03 to +0.11)	0.27	—	—
Duration of HIV infection (per year)	−0.004 (−0.04 to +0.03)	0.83	—	—
Duration of viral suppression (per year)	+0.02 (−0.05 to +0.08)	0.66	—	—
Current ART regimen containing:				
PI	+0.14 (−0.38 to +0.65)	0.61	—	—
Any NRTI	−1.20 (−3.25 to +0.85)	0.25	—	—
Abacavir	+0.19 (−0.36 to +0.73)	0.50	—	—
Any NNRTI	+0.08 (−0.43 to +0.60)	0.75	—	—
Integrase inhibitor	−0.24 (−0.78 to +0.30)	0.38	—	—
Maraviroc	+0.63 (−0.58 to +1.83)	0.31	—	—

ART, antiretroviral therapy; PI, protease inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor.

^aExpressed as percentage change in mean flow velocity per unit change in end-tidal carbon dioxide.

^bCurrent versus no current use unless otherwise noted.

^cEffect of HIV-specific factors on cerebral vasoreactivity among HIV-infected cohort only.

^dModel adjusted only for risk factors listed in the multivariate model column.

^e*P* value for interaction between HIV and statin use = 0.044.

^fIn the multivariate model, the difference in mean cerebral vasoreactivity shown for HIV infection compared with no HIV infection is specifically for nonstatin users.

Fig. 1. Adjusted mean cerebral vasoreactivity and 95% confidence intervals (for nonstatin users) by HIV status. Models adjusted for age, race, diabetes mellitus, hypertension, statin use, aspirin use, alcohol consumption, and methamphetamine use. *Cerebral VR-hyper*, cerebral vasoreactivity in response to hypercapnia; *cerebral VR-hyper + hypo*, cerebral vasoreactivity in response to the full range of hyper and hypocapnia.

$P=0.012$), while diabetes mellitus was associated with a reduction in cerebral $VR_{hyper+hypo}$ (-0.46% , 95% CI -0.91% to -0.01% , $P=0.047$) (Table 3).

Risk factors for impaired cerebral vasoreactivity among the HIV-infected cohort

In a multivariate model restricted to HIV-infected individuals, nonwhite race and diabetes mellitus were associated with lower cerebral VR_{hyper} , while statin use predicted higher cerebral VR_{hyper} . There was a trend toward an association between longer duration of viral suppression and higher cerebral VR_{hyper} (Table 4). We did not find a statistically significant effect of most recent or nadir $CD4^+$ cell count, duration of HIV infection or ART class on cerebral VR_{hyper} . In a multivariate model of the secondary outcome, cerebral $VR_{hyper+hypo}$, a $CD4^+$ cell count greater than 500 cells/ μl ($+0.33\%$, 95% CI $+0.03\%$ to $+0.62\%$, $P=0.032$) and longer mean duration of viral suppression ($+0.04\%$ for each additional year of suppression, 95% CI -0.001% to $+0.08\%$, $P=0.053$) were associated with higher cerebral $VR_{hyper+hypo}$. In an exploratory analysis in which we stratified the HIV-only model by statin use to investigate the effect of HIV-related and other factors (adjusted for age and race) on participants not exposed to statins, longer mean duration of HIV infection was associated with worse cerebral VR_{hyper} (-0.05% for each additional year of infection, 95% CI -0.09% to -0.01% , $P=0.026$) among nonstatin users. Current tobacco use also correlated with a reduction in cerebral VR_{hyper}

(-0.67% , 95% CI -1.33% to -0.01% , $P=0.045$) among nonstatin users.

Discussion

The primary goal of our study was to evaluate the association between well controlled HIV infection and cerebral vasoreactivity, a measure of cerebrovascular function. We found that cerebral vasoreactivity was reduced in treated, virally suppressed HIV-infected individuals independent of traditional vascular risk factors that can impact cerebral vasoreactivity, including hypertension and diabetes mellitus. These results suggest that treated HIV infection may confer additional cerebrovascular risk beyond that explained by comorbid traditional vascular risk factors.

Few studies have been performed investigating cerebral vasoreactivity in HIV-infected individuals. One study from early in the treatment era evaluated cerebral vasoreactivity using acetazolamide as the vasodilatory stimulus in 27 HIV-infected individuals and 10 uninfected controls [22]. The study participants were a heterogeneous group of HIV-infected individuals [six met criteria for Centers for Disease Control and Prevention (CDC) clinical stage A, 16 clinical stage B and seven clinical stage C] with a shorter duration of known HIV infection (mean duration 4 years) and worse

Table 3. Bivariate and multivariate models of the effect of HIV infection and other risk factors on cerebral vasoreactivity in response to hyper and hypocapnia (cerebral VR_{hyper + hypo}).

HIV-infected (<i>n</i> = 65) + HIV-uninfected (<i>n</i> = 28), Total <i>n</i> = 93 Total observations (left + right sides) <i>n</i> = 175				
	Difference in mean cerebral VR _{hyper + hypo} ^a (95% CI)		Difference in mean cerebral VR _{hyper + hypo} ^a (95% CI)	
	Bivariate analyses	<i>P</i> value	Multivariate model ^d	<i>P</i> value
Demographics				
Age (per 10-year increase)	+0.06 (−0.08 to +0.20)	0.40	−0.01 (−0.15 to +0.13)	0.88
Male sex	+0.13 (−0.51 to +0.77)	0.68	—	—
Race (nonwhite versus white)	−0.09 (−0.38 to +0.19)	0.53	−0.11 (−0.38 to +0.17)	0.44
Vascular and other risk factors				
Hypertension	−0.05 (−0.31 to +0.21)	0.73	−0.12 (−0.38 to +0.14)	0.36
Dyslipidemia	+0.19 (−0.06 to +0.45)	0.14	—	—
Statin use	+0.26 (−0.005 to +0.52)	0.054	+0.42 (+0.13 to +0.71)	0.004
Aspirin use	+0.15 (−0.11 to +0.41)	0.27	+0.14 (−0.13 to +0.42)	0.31
Coronary heart disease	−0.37 (−1.09 to +0.35)	0.32	—	—
Diabetes mellitus	−0.41 (−0.87 to +0.04)	0.072	−0.46 (−0.91 to −0.01)	0.047
Hepatitis C infection	−0.15 (−0.49 to +0.19)	0.40	—	—
Prior stroke	+0.39 (−0.49 to +1.27)	0.39	—	—
Migraines	+0.14 (−0.24 to +0.52)	0.48	—	—
Sleep apnea	−0.15 (−0.55 to +0.25)	0.45	—	—
Traumatic brain injury	+0.02 (−0.47 to +0.51)	0.94	—	—
Substance use ^b				
Alcohol use (for each additional drink per day)	+0.23 (+0.08 to +0.38)	0.003	+0.19 (+0.04 to +0.33)	0.012
Tobacco use	+0.16 (−0.18 to +0.50)	0.37	—	—
Cocaine use	−0.14 (−0.71 to +0.44)	0.64	—	—
Heroin use	+0.71 (−0.52 to +1.95)	0.26	—	—
Methamphetamine use	−0.33 (−0.90 to +0.24)	0.26	−0.31 (−0.83 to +0.20)	0.23
Marijuana use	+0.04 (−0.23 to +0.30)	0.78	—	—
HIV and HIV-specific factors ^c				
HIV infection	−0.20 (−0.48 to +0.07)	0.15	−0.30 (−0.56 to −0.03)	0.030
CD4 ⁺ cell count (per 50 cells/μl increase)	+0.02 (−0.02 to +0.05)	0.34	—	—
Nadir CD4 ⁺ cell count (per 50 cells/μl increase)	+0.01 (−0.03 to +0.06)	0.60	—	—
Duration of HIV infection (per year)	+0.007 (−0.02 to +0.03)	0.56	—	—
Duration of viral suppression (per year)	+0.03 (−0.02 to +0.07)	0.22	—	—
Current ART regimen containing				
PI	−0.08 (−0.42 to +0.25)	0.62	—	—
Any NRTI	−0.11 (−1.45 to +1.23)	0.87	—	—
Abacavir	+0.08 (−0.27 to +0.43)	0.65	—	—
Any NNRTI	+0.08 (−0.25 to +0.41)	0.62	—	—
Integrase inhibitor	−0.18 (−0.53 to +0.16)	0.30	—	—
Maraviroc	+0.004 (−0.78 to +0.79)	0.99	—	—

^aExpressed as percentage change in mean flow velocity per unit change in end-tidal carbon dioxide.^bCurrent versus no current use unless otherwise noted.^cEffect of HIV-specific factors on cerebral vasoreactivity among HIV-infected cohort only.^dModel adjusted only for risk factors listed in the multivariate model column.

immunodeficiency (mean CD4⁺ cell count 217 cells/μl) compared to our cohort. Mean cerebral vasoreactivity was statistically significantly lower in the HIV-infected group compared to uninfected controls. However, minimal clinical information, such as ART use or comorbid vascular risk factors, was available for comparison.

To our knowledge, this is the first study assessing cerebral vasoreactivity in treated, virally suppressed HIV-infected individuals. Even among individuals with well controlled HIV infection, which increasingly reflects the HIV population with access to ART, we observed reduced cerebral vasoreactivity when compared with demographically matched HIV-uninfected controls. Chronic HIV infection and its long-term inflammatory and

immunologic effects may contribute to impaired cerebral vasodilatation at the arteriolar level in response to hypercapnia, which reflects intracranial endothelial dysfunction [23–25]. While few data are available regarding cerebrovascular endothelial function in HIV infection, findings from this study can be viewed in parallel with results from studies demonstrating systemic endothelial dysfunction, a marker of cardiovascular risk often evaluated with flow-mediated dilatation (FMD) of the brachial artery, in HIV-infected individuals. FMD is impaired in ART-naïve HIV-infected individuals, and rapidly improves after initiation of ART [26]. However, even among ART-treated individuals with well controlled HIV infection, systemic endothelial function remains impaired. In participants from the parent study evaluating

Table 4. Multivariate model^a of the effect of risk factors on cerebral vasoreactivity in response to hypercapnia (cerebral VR_{hyper}) and to hypocapnia (cerebral VR_{hyper} + hypo) among HIV-infected cohort only.

HIV-infected only (<i>n</i> = 65) Total observations (left + right sides) <i>n</i> = 122				
	Difference in mean cerebral VR _{hyper} ^b (95% CI)	<i>P</i> value	Difference in mean cerebral VR _{hyper} + hypo ^b (95% CI)	<i>P</i> value
Age (per 10-year increase)	−0.13 (−0.40 to +0.14)	0.35	+0.08 (−0.09 to +0.26)	0.36
Race (nonwhite versus white)	−0.88 (−1.40 to −0.35)	0.001	−0.23 (−0.59 to +0.12)	0.20
Diabetes mellitus	−0.92 (−1.60 to −0.24)	0.008	−0.53 (−1.00 to −0.07)	0.025
Statin use	+0.46 (+0.01 to +0.91)	0.046	+0.36 (+0.06 to +0.67)	0.019
Aspirin use	+0.44 (−0.03 to +0.92)	0.068	—	—
Alcohol use (for each additional drink per day)	+0.34 (+0.07 to +0.61)	0.013	+0.17 (−0.01 to +0.36)	0.070
Methamphetamine use (current versus no current use)	−1.12 (−1.99 to −0.25)	0.012	—	—
Duration of viral suppression (per year)	+0.05 (−0.004 to 0.11)	0.071	+0.04 (−0.001 to +0.08)	0.053
Most recent CD4 ⁺ cell count >500 cells/μl	—	—	+0.33 (+0.03 to +0.62)	0.032

^aModels adjusted for risk factors listed in each respective column.

^bExpressed as percentage change in mean flow velocity per unit change in end-tidal carbon dioxide.

cardiovascular health in SCOPE from which our cohort was drawn, ART-treated HIV-infected individuals with undetectable HIV viral load had worse endothelial function compared to healthy controls [27]. Persistent systemic endothelial dysfunction in well controlled HIV infection is hypothesized to be due to inflammatory and immune consequences of chronic HIV infection, which do not fully normalize despite virologic suppression and immune reconstitution on ART [28–30]. HIV ‘elite controllers’, a unique group of untreated HIV-infected individuals with undetectable viral load who lack the potential confounding effect of ART, also demonstrate increased inflammation, immune activation and atherosclerosis compared to HIV-uninfected controls, even after adjustment for traditional vascular risk factors [31,32].

Hyperemic velocity, which reflects microvascular endothelial function, has been correlated with systemic markers of inflammation in HIV-infected individuals [33]. Although we observed a trend toward an association between longer duration of viral suppression and higher cerebral vasoreactivity, markers of immune activation and inflammation, which were not available for this study, would be more informative in future studies. In an exploratory analysis, we stratified the HIV-infected cohort by statin use to examine whether the effect of HIV-specific factors on cerebral vasoreactivity was masked by statin use. Indeed, among HIV-infected nonstatin users, the duration of known HIV infection was associated with diminished cerebral vasoreactivity, independent of age, consistent with the possibility that the accumulation of immunologic and inflammatory consequences of chronic HIV infection over the long-term may drive the development of cerebral endothelial dysfunction in this population.

Statin use was consistently associated with higher cerebral vasoreactivity among HIV-infected individuals. Among their pleiotropic effects, statins improve endothelial

function by upregulating expression and activity of endothelial nitric oxide synthase resulting in increased bioavailability of nitric oxide [34]. In individuals with vascular risk factors, prior stroke and impaired cerebrovascular function, statin use may increase cerebral vasoreactivity [15,35]. However, a statistically significant increase in cerebral vasoreactivity among individuals with preserved cerebrovascular function was not found, which may explain the lack of an observed benefit of statin use on cerebral VR_{hyper} among uninfected controls in our study. The increase in cerebral vasoreactivity demonstrated with statin use argues for a unique benefit of statins among HIV-infected individuals to prevent vascular events, which is being evaluated in an ongoing trial.

Although untreated hypertension, which stimulates cerebrovascular remodelling and endothelial damage [36], has been linked to reduced cerebral vasoreactivity in the general population [37–40], we did not observe a statistically significant association between hypertension and lower cerebral vasoreactivity in our cohort. This may be at least partially accounted for by near universal use of antihypertensive therapy among individuals with hypertension in our cohort. Diabetes mellitus, however, which alters endothelial function and has been correlated with diminished cerebral vasoreactivity [23,41], was associated with reduced cerebral vasoreactivity among HIV-infected individuals. HIV-infected individuals are at high risk for diabetes mellitus [42,43], an established risk factor for cerebral small vessel disease in the general population. Diabetes mellitus has been identified as a risk factor for worse neurocognitive performance in older HIV-infected individuals [44,45]. One hypothesis that merits further investigation is whether this association between diabetes mellitus and neurocognitive dysfunction is mediated through impaired cerebral microvascular function.

While heavy alcohol use is a strong independent risk factor for stroke [46], moderate alcohol intake has been

shown to be protective against ischemic stroke and associated with better systemic endothelial function [47,48]. The majority of individuals who consumed alcohol in our study fell into the category of 'moderate use', defined as up to two drinks per day, which may explain the correlation with higher cerebral vasoreactivity. We included methamphetamine in the forward stepwise selection process to account for differences in cerebral vasoreactivity by HIV status due to discrepant methamphetamine use. However, its effect on cerebral vasoreactivity should be interpreted cautiously given few methamphetamine users in the cohort.

Although age did not affect cerebral vasoreactivity in our study, nonwhite race had a consistently negative impact on cerebral vasoreactivity in both the full cohort and in the HIV-only model. Most studies of cerebral endothelial function have not evaluated differences in cerebral vasoreactivity by race or ethnicity. Based on studies of systemic endothelial function, differences in vascular biology by race/ethnicity may result in more impaired endothelium-dependent and microvascular vasodilatory function in blacks compared to whites, independent of vascular and metabolic risk factors [49,50]. Although stroke incidence in the general population is strongly related to black race [51], none of the large cohort studies of cerebrovascular events in HIV-infected individuals have demonstrated nonwhite race as a risk factor for either ischemic or hemorrhagic stroke.

Our study has several limitations that bear mention. First, this was a small, cross-sectional study, limiting our ability to prospectively assess changes in cerebral vasoreactivity over time. Secondly, we lacked markers of systemic inflammation or immune activation obtained at the time of TCD with which to compare cerebrovascular function. Additionally, the overwhelming majority of our study participants were men, which reflects the general demographic make-up of the SCOPE cohort. Thus, we are not able to generalize our results to HIV-infected women. Given our data that the relative risk of stroke conferred by HIV infection in women is greater than men [3,4], studies aimed specifically at evaluating cerebrovascular function in women should be a top priority. Furthermore, use of one technologist in a single vascular laboratory precluded us from assessing interrater agreement for cerebral vasoreactivity, although data on interrater reliability of TCD-measured cerebral vasoreactivity are available [52]. Lastly, there were differences at baseline between the HIV-infected cohort and the uninfected control group, which may have affected the association between HIV status and cerebral vasoreactivity. However, the differences in cerebral vasoreactivity observed by HIV status persisted even after adjusting for these baseline differences.

The findings from this study advance our current knowledge of the impact of treated, virally suppressed

HIV infection on vascular dysfunction by extending its negative effect to the cerebral vasculature. Importantly, these results provide one possible mechanism to explain increased cerebrovascular risk in HIV-infected individuals. Future studies should compare longitudinal changes in cerebrovascular function between HIV-infected individuals and uninfected controls, in addition to correlating markers of immune activation and inflammation with cerebrovascular function in HIV infection. Assessment of cerebral vasoreactivity has potential applications for use to identify individuals with cerebrovascular dysfunction at greater risk for stroke and to monitor response to interventions intended to reduce cerebrovascular risk in HIV infection.

Acknowledgements

The authors are grateful to Janet Browne (Vascular Laboratory, University of California, San Francisco, San Francisco, California, USA), Colleen Douville (Vascular Ultrasound Laboratory, Swedish Medical Center, Seattle, Washington, USA), Sarah LaRose (Cerebrovascular Laboratory, Brigham and Women's Hospital, Boston, Massachusetts, USA), John Kelly (Respiratory Care Services, San Francisco General Hospital, San Francisco, California, USA) and Mark Siobal (Respiratory Care Services, San Francisco General Hospital, San Francisco, California, USA) for their assistance in administration and set-up for the TCD CO₂ challenge test.

Author contributions: F.C.C. participated in the study concept and design, performed the data analysis and interpretation and drafted and revised the manuscript. W.J.B. performed the data analysis and interpretation and preparation and revision of the manuscript. C.M. and C.C. participated in data collection and preparation and revision of the manuscript. N.K., R.W.P. and S.D. contributed to data interpretation and preparation and revision of the manuscript. F.A.S. contributed to study design, data interpretation and preparation and revision of the manuscript. P.Y.H. supervised study design, data analysis and interpretation, and preparation and revision of the manuscript.

Funding: This work was supported by grants from the National Institutes of Health, 5T32MH090847 (F.C.C.), R01NS085002 (F.A.S.), K24AI112393 and R01HL095130 (P.Y.H.) and from the National Institutes of Health, University of California, San Francisco-Gladstone Institute of Virology & Immunology Center for AIDS Research, P30-AI027763 (F.C.C.). Support for statistical analysis provided through the UCSF Clinical and Translational Science Institute (UL1TR000004).

Conflicts of interest

There are no conflicts of interest.

References

- Neuhaus J, Angus B, Kowalska JD, Rosa AL, Sampson J, Wentworth D, et al. **Risk of all-cause mortality associated with nonfatal AIDS and serious non-AIDS events among adults infected with HIV.** *AIDS* 2010; **24**:697–706.
- Hasse B, Ledergerber B, Furrer H, Battegay M, Hirschel B, Cavassini M, et al. **Morbidity and aging in HIV-infected persons: The Swiss HIV cohort study.** *Clin Infect Dis* 2011; **53**:1130–1139.
- Chow FC, Regan S, Feske S, Meigs JB, Grinspoon SK, Triant VA. **Comparison of ischemic stroke incidence in HIV-infected and non-HIV-infected patients in a US healthcare system.** *J Acquir Immune Defic Syndr* 2012; **60**:351–358.
- Chow FC, He W, Bacchetti P, Regan S, Feske SK, Meigs JB, et al. **Elevated rates of intracerebral hemorrhage in individuals from a US clinical care HIV cohort.** *Neurology* 2014; **83**:1705–1711.
- Marcus JL, Leyden WA, Chao CR, Chow FC, Horberg MA, Hurley LB, et al. **HIV infection and incidence of ischemic stroke.** *AIDS* 2014; **28**:1911–1919.
- Triant VA, Lee H, Hadigan C, Grinspoon SK. **Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease.** *J Clin Endocrinol Metab* 2007; **92**:2506–2512.
- Freiberg MS. **HIV infection and the risk of acute myocardial infarction HIV infection and the risk of AMI.** *JAMA Intern Med* 2013; **173**:614–622.
- Hsue PY, Deeks SG, Hunt PW. **Immunologic basis of cardiovascular disease in HIV-infected adults.** *J Infect Dis* 2012; **205**:S375–S382.
- Markus H, Cullinane M. **Severely impaired cerebrovascular reactivity predicts stroke and TIA risk in patients with carotid artery stenosis and occlusion.** *Brain* 2001; **124**:457–467.
- Silvestrini M, Vernieri F, Pasqualetti P, Matteis M, Passarelli F, Troisi E, et al. **Impaired cerebral vasoreactivity and risk of stroke in patients with asymptomatic carotid artery stenosis.** *JAMA* 2000; **283**:2122–2127.
- Molina C, Sabin JA, Montaner J, Rovira A, Abilleira S, Codina A. **Impaired cerebrovascular reactivity as a risk marker for first-ever lacunar infarction: a case-control study.** *Stroke* 1999; **30**:2296–2301.
- Cupini LM, Diomedì M, Placidi F, Silvestrini M, Giacomini P. **Cerebrovascular reactivity and subcortical infarctions.** *Arch Neurol* 2001; **58**:577–581.
- Panczel G, Bönöczk P, Voko Z, Spiegel D, Nagy Z. **Impaired vasoreactivity of the basilar artery system in patients with brainstem lacunar infarcts.** *Cerebrovasc Dis* 1999; **9**:218–223.
- Deplanque D, Lavallée PC, Labreuche J, Góngora-Rivera F, Jaramillo A, Brenner D, et al. **Cerebral and extracerebral vasoreactivity in symptomatic lacunar stroke patients: a case-control study.** *Int J Stroke* 2013; **8**:413–421.
- Forteza A, Romano JG, Campo-Bustillo I, Campo N, Haussen DC, Gutierrez J, et al. **High-dose atorvastatin enhances impaired cerebral vasomotor reactivity.** *J Stroke Cerebrovasc Dis* 2012; **21**:487–492.
- Sterzer P, Meintzschel F, Rosler A, Lanfermann H, Steinmetz H, Sitzer M. **Pravastatin improves cerebral vasomotor reactivity in patients with subcortical small-vessel disease.** *Stroke* 2001; **32**:2817–2820.
- Vicente-Campos D, Mora J, Castro-Piñero J, González-Montesinos JL, Conde-Caveda J, Chicharro JL. **Impact of a physical activity program on cerebral vasoreactivity in sedentary elderly people.** *J Sports Med Phys Fitness* 2012; **52**:537–544.
- Gange SJ, Kitahata MM, Saag MS, Bangsberg DR, Bosch RJ, Brooks JT, et al. **Cohort profile: the North American AIDS cohort collaboration on research and design (NA-ACCORD).** *Int J Epidemiol* 2007; **36**:294–301.
- Douville CM. **Vasomotor reactivity testing of the cerebral circulation using the transcranial Doppler carbon dioxide challenge test.** *J Vasc Technol* 2000; **24**:43–48.
- Aaslid R, Markwalder TM, Nornes H. **Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries.** *J Neurosurg* 1982; **57**:769–774.
- Alexandrov AV, Sloan MA, Wong LKS, Douville C, Razumovsky AY, Koroshetz WJ, et al. **Practice standards for transcranial Doppler ultrasound: part I—test performance.** *J Neuroimaging* 2007; **17**:11–18.
- Brilla R, Nabavi DG, Schulte-Altdorneburg G, Kemény V, Reichelt D, Evers S, et al. **Cerebral vasculopathy in HIV infection revealed by transcranial Doppler: a pilot study.** *Stroke* 1999; **30**:811–813.
- Lavi S, Gaitini D, Milloul V, Jacob G. **Impaired cerebral CO₂ vasoreactivity: association with endothelial dysfunction.** *Am J Physiol Heart Circ Physiol* 2006; **291**:H1856–H1861.
- Thompson BG, Pluta RM, Gorton ME, Oldfield EH. **Nitric oxide mediation of chemoregulation but not autoregulation of cerebral blood flow in primates.** *J Neurosurg* 1996; **84**:71–78.
- Zimmermann C, Haberl RL. **L-arginine improves diminished cerebral CO₂ reactivity in patients.** *Stroke* 2003; **34**:643–647.
- Torriani FJ, Komarow L, Parker RA, Cotter BR, Currier JS, Dubé MP, et al. **Endothelial function in human immunodeficiency virus-infected antiretroviral-naïve subjects before and after starting potent antiretroviral therapy.** *J Am Coll Cardiol* 2008; **52**:569–576.
- Hsue PY, Hunt PW, Wu Y, Schnell A, Ho JE, Hatano H, et al. **Association of abacavir and impaired endothelial function in treated and suppressed HIV-infected patients.** *AIDS* 2009; **23**:2021–2027.
- Neuhaus J, Jacobs DR, Baker JV, Calmy A, Duprez D, La Rosa A, et al. **Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection.** *J Infect Dis* 2010; **201**:1788–1795.
- Wada NI, Jacobson LP, Margolick JB, Breen EC, Macatangay B, Penugonda S, et al. **The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation.** *AIDS* 2015; **29**:463–471.
- Burdo TH, Lentz MR, Autissier P, Krishnan A, Halpern E, Letendre S, et al. **Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after antiretroviral therapy.** *J Infect Dis* 2011; **204**:154–163.
- Hsue PY, Hunt PW, Schnell A, Kalapus SC, Hoh R, Ganz P, et al. **Role of viral replication, antiretroviral therapy, and immunodeficiency in HIV-associated atherosclerosis.** *AIDS* 2009; **23**:1059–1067.
- Pereyra F, Lo J, Triant VA, Wei J, Buzon MJ, Fitch KV, et al. **Increased coronary atherosclerosis and immune activation in HIV-1 elite controllers.** *AIDS* 2012; **26**:2409–2412.
- Longenecker CT, Jiang Y, Orringer CE, Gilkeson RC, Debanne S, Funderburg NT, et al. **Soluble CD14 is independently associated with coronary calcification and extent of subclinical vascular disease in treated HIV infection.** *AIDS* 2014; **28**:969–977.
- Endres M. **Effects of statins on endothelium and signaling mechanisms.** *Stroke* 2004; **35**:2708–2711.
- Pretar-Oblak J. **Influence of atorvastatin treatment on l-arginine cerebrovascular reactivity and flow-mediated dilatation in patients with lacunar infarctions.** *Stroke* 2006; **37**:2540–2545.
- Pires PW, Ramos CMD, Matin N, Dorrance AM. **The effects of hypertension on the cerebral circulation.** *Am J Physiol Heart Circ Physiol* 2013; **304**:H1598–H1614.
- Maeda H, Matsumoto M, Handa N, Hougaku H, Ogawa S, Itoh T, et al. **Reactivity of cerebral blood flow to carbon dioxide in hypertensive patients: evaluation by the transcranial Doppler method.** *J Hypertens* 1994; **12**:191–197.
- Magyar MT, Valikovics A, Bereczki D, Ficzer A, Czuriga I, Csiba L. **Transcranial Doppler monitoring in hypertensive patients during physical exercise.** *Cerebrovasc Dis* 2001; **12**:186–191.
- Settakis G, Páll D, Molnár C, Bereczki D, Csiba L, Fülesdi B. **Cerebrovascular reactivity in hypertensive and healthy adolescents: TCD with vasodilatory challenge.** *J Neuroimaging* 2003; **13**:106–112.
- Wijnhoud AD, Koudstaal PJ, Dippel DWJ. **Relationships of transcranial blood flow Doppler parameters with major vascular risk factors: TCD study in patients with a recent TIA or nondisabling ischemic stroke.** *J Clin Ultrasound* 2006; **34**:70–76.

41. Last D, Alsop DC, Abduljalil AM, Marquis RP, de Bazelaire C, Hu K, *et al.* **Global and regional effects of type 2 diabetes on brain tissue volumes and cerebral vasoreactivity.** *Diabetes Care* 2007; **30**:1193–1199.
42. Tien PC, Schneider MF, Cole SR, Levine AM, Cohen M, DeHovitz J, *et al.* **Antiretroviral therapy exposure and incidence of diabetes mellitus in the Women's Interagency HIV Study.** *AIDS* 2007; **21**:1739–1745.
43. Brown TT, Cole SR, Li X, Kingsley LA, Palella FJ, Riddler SA, *et al.* **Antiretroviral therapy and the prevalence and incidence of diabetes mellitus in the multicenter AIDS cohort study.** *Arch Intern Med* 2005; **165**:1179–1184.
44. Valcour VG, Shikuma CM, Shiramizu BT, Williams AE, Watters MR, Poff PW, *et al.* **Diabetes, insulin resistance, and dementia among HIV-1-infected patients.** *J Acquir Immune Defic Syndr* 2005; **38**:31–36.
45. McCutchan JA, Marquie-Beck JA, Fitzsimons CA, Letendre SL, Ellis RJ, Heaton RK, *et al.* **Role of obesity, metabolic variables, and diabetes in HIV-associated neurocognitive disorder.** *Neurology* 2012; **78**:485–492.
46. Gill JS, Zezulka AV, Shipley MJ, Gill SK, Beevers DG. **Stroke and alcohol consumption.** *N Engl J Med* 1986; **315**:1041–1046.
47. Elkind MSV, Sciacca R, Boden-Albala B, Rundek T, Paik MC, Sacco RL. **Moderate alcohol consumption reduces risk of ischemic stroke: the Northern Manhattan Study.** *Stroke* 2006; **37**:13–19.
48. Suzuki K, Elkind MSV, Boden-Albala B, Jin Z, Berry G, Di Tullio MR, *et al.* **Moderate alcohol consumption is associated with better endothelial function: a cross sectional study.** *BMC Cardiovasc Disord* 2009; **9**:8.
49. Lteif AA, Han K, Mather KJ. **Obesity, insulin resistance, and the metabolic syndrome: determinants of endothelial dysfunction in whites and blacks.** *Circulation* 2005; **112**:32–38.
50. Morris AA, Patel RS, Binongo JNG, Poole J, Mheid Al I, Ahmed Y, *et al.* **Racial differences in arterial stiffness and microcirculatory function between Black and White Americans.** *J Am Heart Assoc* 2013; **2**:e002154.
51. Koton S, Schneider ALC, Rosamond WD, Shahar E, Sang Y, Gottesman RF, *et al.* **Stroke incidence and mortality trends in US communities, 1987 to 2011.** *JAMA* 2014; **312**:259–268.
52. McDonnell MN, Berry NM, Cutting MA, Keage HA, Buckley JD, Howe PRC. **Transcranial Doppler ultrasound to assess cerebrovascular reactivity: reliability, reproducibility and effect of posture.** *Peer J* 2013; **1**:e65DOI 10.7717/peerj.65.