Published in final edited form as:

AIDS. 2017 February 20; 31(4): 533–537. doi:10.1097/QAD.0000000000001363.

Comprehensive assessment of the Arginine pathway and its relationship to Inflammation in HIV

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Abstract

Background—Nitric oxide helps maintain vascular function and is generated through the oxidation of arginine. Whether altered arginine metabolism may lead to elevated levels of inflammation in HIV is unclear.

Methods—We performed a cross-sectional analysis of HIV-infected adults on stable ART with HIV-1 RNA < 50 copies/mL and HIV-uninfected controls. We measured biomarkers in the arginine pathway, markers of systemic inflammation, and monocyte activation. T-tests, chi-square tests and propensity score matching analyses were used to compare markers by HIV status, and multiple linear regressions were used to assess associations of arginine metabolites with markers of inflammation.

Results—Overall, 131 participants were enrolled (93 HIV-infected and 38 HIV-uninfected controls); 70% were male; 58% African Americans; median age was 51 years, median absolute CD4 was 735cell/cm³. Lysine, arginine, citrulline, global arginine bioavailability ratio and symmetrical dimethylarginine were different between HIV-infected and HIV-uninfected adults ($p = 0.02$), but asymmetric dimethylarginine was not ($p = 0.13$). Arginine biomarkers in HIV-infected, but not in HIV-uninfected controls, were associated with all measured markers of inflammation, endothelial activation, and coagulation ($p = 0.05$).

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Author Contributions:

GM designed the study and obtained funding. SMKA and AS provided statistical support. NF assisted with the inflammation and immune activation marker assays. WHWT provided support with the arginine metabolomics profile. All authors contributed to data analysis and writing of the manuscript.

Conflicts of Interest and Sources of Funding:

GAM served as a consultant for Gilead, BMS, GSK/Viiv, and ICON, and has received research funding from Gilead, Merck, GSK/Viiv, and BMS.

Conclusions—HIV-infected participants on ART with virologic suppression have altered plasma levels of biomarkers in the arginine pathway compared to controls. These biomarkers are independently associated with markers of inflammation and monocyte activation in HIV.

Keywords

Arginine; HIV; inflammation; endothelial dysfunction

Introduction

Adults with HIV remain at higher risk of cardiovascular co-morbidities despite successful virologic control with antiretroviral therapy (ART) [1–4]. Chronic inflammation has been shown to play a key role in cardiovascular disease (CVD) in HIV infection [5] and to remain elevated after ART initiation [6]. In addition, endothelial dysfunction has been reported in HIV, and it is known in HIV-uninfected populations to precede the development of atherosclerosis [7].

Nitric oxide (NO) helps maintain normal vascular function through its anti-atherogenic and vasodilatory effects. NO is generated through the oxidation of arginine by endothelial nitric oxide synthase [8]. Asymmetric dimethylarginine (ADMA) resembles arginine and can inhibit nitric oxide synthase and has subsequently been recognized as a marker of endothelial dysfunction [9].

Arginine is the common substrate for both NO synthases and arginases and the Global Arginine Bioavailability Ratio accounts for levels of the substrate (arginine) and its major catabolic products (ornithine and citrulline). GABR has been shown to be a better predictor of CVD risk than arginine levels alone in HIV-uninfected populations [12]; however it has never been assessed in HIV infection.

We hypothesize that HIV-infected participants on suppressive ART will have increased levels of ADMA, and a decrease in GABR, which will be associated with a heightened state of inflammation and immune activation when compared to HIV-uninfected individuals.

Methods

Study Design

This is an observational cohort of HIV-infected and uninfected participants prospectively enrolled at University Hospitals Case Medical Center, Cleveland, Ohio. The study was approved by the local Institutional Review Board, and written informed consent was provided by all participants. All participants were 18 years of age, with HIV-1 infection on stable ART for at least 3 months with cumulative ART duration of at least 6 months, HIV-1 RNA <50 copies/mL in the last 4 months prior to study entry, not pregnant or lactating, or had an active infectious or inflammatory condition. Uncontrolled diabetes and known cardiovascular diseases were excluded, similarly subjects consuming arginine supplements were excluded.

Study evaluations

Blood draws were obtained for measurements of renal and lipid profiles, glucose and insulin levels. Blood was drawn after a 12-hour fast. Additionally, blood was processed and plasma and serum were cryopreserved for measurement of markers of monocyte activation, systemic inflammation, and coagulation as previously described [17, 18]. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as described [Matthews, 1985 #53].

Inflammation and soluble immune activation markers

Soluble markers of inflammation [interleukin-6 (IL-6), soluble tumor necrosis factor receptors I and II (sTNF-RI and -RII)], soluble vascular cell adhesion molecule (VCAM), soluble marker of monocyte activation soluble CD163 (sCD163), high sensitivity C-reactive protein (hsCRP) and d-dimer levels were measured by ELISA (R&D Systems, Minneapolis, MN for all and Diagnostic Stago/Asserachrome for d-dimer).

Arginine biomarkers

Measurement of arginine, ornithine, citrulline, ADMA, SDMA (symmetrical dimethyl arginine), and MMA (monomethylarginine) were performed by stable-isotope-dilution HPLC with online tandem mass spectrometry. Four volumes of methanol containing $^{13}\text{C}_6$ -Arg, d_4 -Orn, d_4 -Cit, d_7 -ADMA each 10 μM was added to plasma to precipitate proteins and the supernatant after centrifugation at 13000 rpm for 10 minutes was collected for LC/MS/MS assay. 10 μL supernatant was injected onto a HPLC column. The levels of the arginine and related metabolites were quantified by LC/ESI/MS/MS analysis using API 365 triple quadrupole mass spectrometer (Applied Biosystems, Foster, CA) with Ionics EP 10+ upgrade (Concord, Ontario, CA) and Cohesive Technologies Aria LX Series HPLC multiplexing system (Franklin, MA). Amino acids were separated with a $250 \times 4.6\text{mm}$ Rexchrom S5-100-P phenyl column (Product number 728207 Regis Chemical, Morton Grove, IL, USA) with a $1\text{mm} \times 15\text{mm}$ Optimize technologies Opti-Guard Phenyl guard column (optimize Technologies, Oregon City, OR, USA). Formic acid (0.1%) and ammonium formate (10mM) in water (solvent A) and formic acid (0.1%) and ammonium formate (10mM) in methanol (solvent B) were used as solvents. The gradient consisted of: 1) 100% solvent A at 800 $\mu\text{L}/\text{min}$ held for 0.5min after injection, 2) linear gradient run to 50% solvent B (50% solvent A) over 3min, 3) held at 50% B for 3.5min at a flow rate of 800 $\mu\text{L}/\text{min}$, 4) flow rate increased to 1000 $\mu\text{L}/\text{min}$ with solvent composition changed to 100% B in linear fashion over 1min, 5) linear gradient run to 100% A at 1000 $\mu\text{L}/\text{min}$ over 0.5min, 6) held at this composition and flow rate for 6min. Overall the 8.5min data window was started at 3.5min after injection, and mass spectrometric analyses was performed using electrospray ionization tandem mass spectrometry in the positive ion mode with multiple reaction monitoring. Intra- and inter-assay coefficients of variance $<10\%$. GABR was defined as arginine/[ornithine + citrulline].

Statistical analysis

The primary objective of this analysis was to determine the extent to which arginine biomarkers were different between HIV-infected and uninfected participants. Continuous

covariates such as age, body mass index (BMI) etc.. variables were compared using the t-test for testing the equality of means. Skewed continuous variables were transformed to zero-skewness distribution by using Box-Cox transformation. The categorical variables were compared using the chi-squared test. Spearman rank correlations quantified associations between arginine biomarkers and inflammatory markers with 10% significance level. For the mean differences in biomarkers for the two groups was compared using propensity score matching method. We used logistic regression model to predict each subject's propensity score using covariates age, sex, race, BMI, smoking status, family history of myocardial infarction, HDL and LDL cholesterol.

We performed separate pairwise correlation analyses for the HIV-infected and uninfected participants of inflammatory and arginine biomarkers and considered significance level of 0.10. With the significantly correlated pairs we performed unadjusted regression analyses of inflammatory markers for HIV-infected group. Afterwards, we performed adjusted regression analyses of inflammatory markers on arginine biomarkers based on significant correlation. Variables adjusted for included age, sex, race, body mass index (BMI), smoking status, family history of myocardial infarction, high density lipids (HDL) and low density lipids (LDL) cholesterol and HIV status. Stata 14 was used to perform this analysis.

RESULTS

Baseline Characteristics

Overall, 131 participant (93 HIV-infected and 38 controls) were included in the analysis. Demographic information and baseline characteristics are displayed in Table 1.

Among the arginine biomarkers, lysine, arginine, citrulline and GABR were significantly higher ($p = 0.02$) and sDMA was significantly lower in the HIV-infected participants ($p = 0.01$) versus uninfected controls. D-dimer was higher in the HIV-infected participants ($p < 0.01$).

Association between Arginine Biomarkers and Inflammatory Markers

In HIV-infected participants, after adjusting for the CVD risk factors age, sex, race, BMI, smoking, family history of myocardial infarction, HDL and LDL, several arginine biomarkers were negatively associated with markers of inflammation whereas ADMA and its byproduct SDMA were positively associated with markers of inflammation (see table 3). In HIV-uninfected participants, GABR was negatively associated with TNFR1, D- dimer and sCD163 ($p = 0.01$) and lysine with D-Dimer ($p = 0.03$).

Discussion

In this study, we found that HIV-infected individuals who are virologically suppressed and on stable ART do not have decreased arginine availability when compared to HIV-uninfected individuals and have similar levels of the endogenous NOS inhibitor ADMA. In addition, we found that the relationship between markers in the arginine pathway and markers of inflammation, coagulation, endothelial activation, and immune activation was more consistent in HIV-infected individuals compared to uninfected controls.

Little is known about ADMA in HIV and nothing is known about GABR. Contrary to our findings, ADMA has been found to be higher in HIV-infected subjects versus controls in separate reports [10, 19]. The difference in mean ADMA reported between HIV-infected and HIV uninfected individuals has ranged between 0.03 $\mu\text{mol/L}$ –0.18 $\mu\text{mol/L}$ [10, 19, 20]. Mean levels of ADMA in HIV-infected individuals in our study was similar to what was found in previous reports of HIV-infected individuals who were ART-naïve and were younger than our patients [11, 19], however, levels in HIV-uninfected individuals were 0.18 $\mu\text{mol/L}$ higher in our study than what has been reported [19, 4]. We hypothesize that our findings could be the result of several factors inherent to the demographics of our participants. First, the HIV-infected participants in our study may have had elevated ADMA levels pre-ART, but with viral suppression, these levels may have been reduced to levels comparable to those measured in the uninfected age matched participants. This hypothesis is supported by studies that have shown that ADMA decrease after ART initiation [11, 21]. Second, ADMA levels were higher in our uninfected controls than what has previously been reported in other HIV-uninfected cohorts. The mean plasma ADMA concentrations in the Framingham Offspring Cohort study, in participants who were free of clinical CVD, was $0.53 \pm 0.12 \mu\text{mol/L}$ [22]. This might be because our participants were older and ADMA is known to increase with age [23]. Lastly, 55% of the HIV-uninfected controls were women with a mean age of 51, and women have been shown to have higher ADMA levels with onset of menopause [23].

Global arginine bioavailability ratio or GABR accounts for arginine and its catabolic products (ornithine and citrulline) and has been shown to be a better predictor of cardiovascular disease in HIV-uninfected subjects than arginine levels alone [12], where lower GABR was associated with higher prevalence of atherosclerotic disease and increased risk of cardiac events. We report for the first time in HIV infection, a higher GABR compared to uninfected participants and a significant inverse association between GABR and several markers of inflammation and endothelial activation markers including TNFRI and II, D-Dimer and sVCAM. This finding of consistent associations between arginine and inflammatory markers in HIV-infected individuals, but not in uninfected controls, highlights a possible role of arginine in ongoing inflammation and enhanced endothelial activation among HIV-infected individuals, even those receiving ART.

There are limitations to this study. First, there are baseline differences between the groups, including differences in gender and race, which are known to be associated with differences in arginine biomarkers and CVD risk. We adjusted for these imbalances in our analyses. The secondary objective of this study was to evaluate variables, in particular arginine biomarkers and markers of inflammation, that may account for increased risk of CVD, however, in order to control for the differences between the groups, all of our analyses adjusted for demographic factors that are known CVD risk factors such as age, sex, race, BMI, smoking, family history of myocardial infarction, HDL and LDL. The small sample size and the cross sectional design are other limitations and as such we cannot attribute cause to effect.

To our knowledge, this is the first study specifically designed to investigate the relationships among a comprehensive state-of-the art evaluation of the arginine pathway, including global arginine bioavailability ratio, and inflammation, coagulation, and endothelial activation

biomarkers in HIV-infected adults receiving ART. Our findings suggest a potential mechanism for the increased inflammation in this population, and as such, the arginine pathway could be used as a novel way to target the heightened inflammation state typically measured in HIV infection. Larger longitudinal studies are needed to further define and confirm this relationship.

Acknowledgments

The authors would like to thank the patients who participated in this research.

Source of support:

The work was supported by National Institutes of Health grant # R56HL126539 to GM. This publication was made possible by the Clinical and Translational Science Collaborative of Cleveland, 4UL1TR000439 from the National Center for Advancing Translational Sciences (NCATS) component of the National Institutes of Health and NIH roadmap for Medical Research.

References

1. 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 health care system. *J Acquir Immune Defic Syndr*. 2012; 60:351–358. [PubMed: 22580566]
2. Freiberg MS, Chang CC, Kuller LH, Skanderson M, Lowy E, Kraemer KL, et al. HIV infection and the risk of acute myocardial infarction. *JAMA Intern Med*. 2013; 173:614–622. [PubMed: 23459863]
3. Marcus JL, Leyden WA, Chao CR, Chow FC, Horberg MA, Hurley LB, et al. HIV infection and incidence of ischemic stroke. *AIDS*. 2014
4. 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. [PubMed: 17456578]
5. Nou E, Lo J, Grinspoon SK. Inflammation, immune activation, and cardiovascular disease in HIV. *Aids*. 2016; 30:1495–1509. [PubMed: 27058351]
6. Neuhaus J, Jacobs DR Jr, 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. [PubMed: 20446848]
7. Poredos P. Endothelial dysfunction in the pathogenesis of atherosclerosis. *Int Angiol*. 2002; 21:109–116. [PubMed: 12110769]
8. Antoniadou C, Antonopoulos AS, Bendall JK, Channon KM. Targeting redox signaling in the vascular wall: from basic science to clinical practice. *Curr Pharm Des*. 2009; 15:329–342. [PubMed: 19149622]
9. Antoniadou C, Shirodaria C, Leeson P, Antonopoulos A, Warrick N, Van-Assche T, et al. Association of plasma asymmetrical dimethylarginine (ADMA) with elevated vascular superoxide production and endothelial nitric oxide synthase uncoupling: implications for endothelial function in human atherosclerosis. *Eur Heart J*. 2009; 30:1142–1150. [PubMed: 19297385]
10. Kurz K, Teerlink T, Sarcelletti M, Weiss G, Zangerle R, Fuchs D. Plasma concentrations of the cardiovascular risk factor asymmetric dimethylarginine (ADMA) are increased in patients with HIV-1 infection and correlate with immune activation markers. *Pharmacol Res*. 2009; 60:508–514. [PubMed: 19651212]
11. Baker JV, Neuhaus J, Duprez D, Freiberg M, Bernardino JJ, Badley AD, et al. HIV replication, inflammation, and the effect of starting antiretroviral therapy on plasma asymmetric dimethylarginine, a novel marker of endothelial dysfunction. *J Acquir Immune Defic Syndr*. 2012; 60:128–134. [PubMed: 22421746]

12. Tang WH, Wang Z, Cho L, Brennan DM, Hazen SL. Diminished global arginine bioavailability and increased arginine catabolism as metabolic profile of increased cardiovascular risk. *J Am Coll Cardiol.* 2009; 53:2061–2067. [PubMed: 19477356]
13. Hattab S, Guiguet M, Carcelain G, Fourati S, Guihot A, Autran B, et al. Soluble biomarkers of immune activation and inflammation in HIV infection: impact of 2 years of effective first-line combination antiretroviral therapy. *HIV Med.* 2015; 16:553–562. [PubMed: 25944318]
14. Rudy BJ, Kapogiannis BG, Worrell C, Squires K, Bethel J, Li S, et al. Immune Reconstitution but Persistent Activation After 48 Weeks of Antiretroviral Therapy in Youth With Pre-Therapy CD4 >350 in ATN 061. *J Acquir Immune Defic Syndr.* 2015; 69:52–60. [PubMed: 25942459]
15. McKibben RA, Margolick JB, Grinspoon S, Li X, Palella FJ Jr, Kingsley LA, et al. Elevated levels of monocyte activation markers are associated with subclinical atherosclerosis in men with and those without HIV infection. *J Infect Dis.* 2015; 211:1219–1228. [PubMed: 25362192]
16. 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 anti-retroviral therapy. *J Infect Dis.* 2011; 204:154–163. [PubMed: 21628670]
17. Eckard AR, Jiang Y, Debanne SM, Funderburg NT, McComsey GA. Effect of 24 weeks of statin therapy on systemic and vascular inflammation in HIV-infected subjects receiving antiretroviral therapy. *J Infect Dis.* 2014; 209:1156–1164. [PubMed: 24415784]
18. Funderburg NT, Jiang Y, Debanne SM, Storer N, Labbato D, Clagett B, et al. Rosuvastatin treatment reduces markers of monocyte activation in HIV-infected subjects on antiretroviral therapy. *Clin Infect Dis.* 2014; 58:588–595. [PubMed: 24253250]
19. Hudson CL, Zemlin AE, Ipp H. The cardiovascular risk marker asymmetric dimethylarginine is elevated in asymptomatic, untreated HIV-1 infection and correlates with markers of immune activation and disease progression. *Ann Clin Biochem.* 2014; 51:568–575. [PubMed: 24142400]
20. Jang JJ, Berkheimer SB, Merchant M, Krishnaswami A. Asymmetric dimethylarginine and coronary artery calcium scores are increased in patients infected with human immunodeficiency virus. *Atherosclerosis.* 2011; 217:514–517. [PubMed: 21549379]
21. Kurz K, Teerlink T, Sarcletti M, Weiss G, Zangerle R, Fuchs D. Asymmetric dimethylarginine concentrations decrease in patients with HIV infection under antiretroviral therapy. *Antivir Ther.* 2012; 17:1021–1027. [PubMed: 22892398]
22. Luneburg N, Xanthakis V, Schwedhelm E, Sullivan LM, Maas R, Anderssohn M, et al. Reference intervals for plasma L-arginine and the L-arginine:asymmetric dimethylarginine ratio in the Framingham Offspring Cohort. *J Nutr.* 2011; 141:2186–2190. [PubMed: 22031661]
23. Schulze F, Maas R, Freese R, Schwedhelm E, Silberhorn E, Boger RH. Determination of a reference value for N(G), N(G)-dimethyl-L-arginine in 500 subjects. *Eur J Clin Invest.* 2005; 35:622–626. [PubMed: 16178881]
24. Madden E, Lee G, Kotler DP, Wanke C, Lewis CE, Tracy R, et al. Association of antiretroviral therapy with fibrinogen levels in HIV-infection. *Aids.* 2008; 22:707–715. [PubMed: 18356600]
25. Reingold J, Wanke C, Kotler D, Lewis C, Tracy R, Heymsfield S, et al. Association of HIV infection and HIV/HCV coinfection with C-reactive protein levels: the fat redistribution and metabolic change in HIV infection (FRAM) study. *J Acquir Immune Defic Syndr.* 2008; 48:142–148. [PubMed: 18344877]
26. Baker JV, Neuhaus J, Duprez D, Kuller LH, Tracy R, Belloso WH, et al. Changes in inflammatory and coagulation biomarkers: a randomized comparison of immediate versus deferred antiretroviral therapy in patients with HIV infection. *J Acquir Immune Defic Syndr.* 2011; 56:36–43. [PubMed: 20930640]
27. Hwang SJ, Ballantyne CM, Sharrett AR, Smith LC, Davis CE, Gotto AM Jr, et al. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. *Circulation.* 1997; 96:4219–4225. [PubMed: 9416885]
28. Ross AC, Rizk N, O’Riordan MA, Dogra V, El-Bejjani D, Storer N, et al. Relationship between inflammatory markers, endothelial activation markers, and carotid intima-media thickness in HIV-infected patients receiving antiretroviral therapy. *Clin Infect Dis.* 2009; 49:1119–1127. [PubMed: 19712036]

29. Longenecker CT, Funderburg NT, Jiang Y, Debanne S, Storer N, Labbato DE, et al. Markers of inflammation and CD8 T-cell activation, but not monocyte activation, are associated with subclinical carotid artery disease in HIV-infected individuals. *HIV Med.* 2013; 14:385–390. [PubMed: 23332012]
30. Barbour JD, Jalbert EC, Chow DC, Gangcuangco LM, Norris PJ, Keating SM, et al. Reduced CD14 expression on classical monocytes and vascular endothelial adhesion markers independently associate with carotid artery intima media thickness in chronically HIV-1 infected adults on virologically suppressive anti-retroviral therapy. *Atherosclerosis.* 2014; 232:52–58. [PubMed: 24401216]
31. Aderka D. The potential biological and clinical significance of the soluble tumor necrosis factor receptors. *Cytokine Growth Factor Rev.* 1996; 7:231–240. [PubMed: 8971478]
32. Brown TT, Tassiopoulos K, Bosch RJ, Shikuma C, McComsey GA. Association between systemic inflammation and incident diabetes in HIV-infected patients after initiation of antiretroviral therapy. *Diabetes Care.* 2010; 33:2244–2249. [PubMed: 20664016]
33. Dube MP, Shen C, Greenwald M, Mather KJ. No impairment of endothelial function or insulin sensitivity with 4 weeks of the HIV protease inhibitors atazanavir or lopinavir-ritonavir in healthy subjects without HIV infection: a placebo-controlled trial. *Clin Infect Dis.* 2008; 47:567–574. [PubMed: 18636958]

Table 1

Baseline characteristics comparison between HIV-infected and HIV-uninfected groups

Variables	HIV- infected (N = 93)	HIV-uninfected (N = 38)	p-value ^a
Age (years)	51.20 (7.30)	51.06 (8.82)	0.929
Female (%)	18 (19.4)	21 (55.3)	<0.001
African Am (%)	59 (63.4)	17 (44.7)	0.049
Current Smoking (%)	51 (54.8)	20 (55.6)	0.135
Alcohol (%)	45 (48.4)	17 (47.2)	0.752
Family history of myocardial infarction (%)	28 (31.8)	16 (48.5)	0.116
Body Mass Index (kg/m ²) ^b	27.41 (5.64)	27.38 (4.91)	0.847
Systolic Blood Pressure (mm Hg)	132.20 (16.01)	130.71 (15.62)	0.846
Diastolic Blood Pressure (mm Hg)	82.94 (7.60)	82.58 (9.58)	0.860
Framingham Score ^b	6.85 (5.62)	5.69 (5.87)	0.002
HDL cholesterol (mg/dL)	50.74 (16.60)	55.84 (17.18)	0.344
LDL cholesterol (mg/dL)	93.20 (32.03)	111.16 (30.67)	0.175
Insulin (uIU/dL) ^b	14.09 (11.36)	10.00 (4.25)	0.103
Homa-IR ^b	3.41 (2.96)	2.29 (1.05)	0.149
CD4+ cell count (/mm ³)	781.13 (373.47)		
HIV-1 RNA (copies/mL)	29.52 (32.98)		
ARV duration (months)	140.72 (72.31)		
Protease Inhibitor duration (months)	79.43 (68.25)		
Non nucleoside inhibitor duration (months)	59.69 (58.98)		
Ornithine ^b (μmol/L)	60.80 (17.49)	60.47 (15.44)	0.354
Lysine (μmol/L)	168.69 (37.67)	156.23 (35.57)	0.000
Arginine (μmol/L)	98.74 (29.97)	91.91 (30.29)	0.017
Citrulline ^b (μmol/L)	40.52 (10.63)	37.57 (10.73)	0.024
sDMA ^b (μmol/L)	0.42 (0.10)	0.45 (0.09)	0.014
ADMA (μmol/L)	0.67 (0.10)	0.66 (0.09)	0.128
GABR	0.99 (0.27)	0.95 (0.24)	0.001
IL6 ^b (μg/mL)	3.91 (10.42)	2.71 (2.78)	0.150
hsCRP ^b (μg/mL)	3688.31 (8022.11)	2682.18 (2809.14)	0.647
TNFR1 ^b (pg/mL)	795.04 (319.98)	805.41 (282.74)	0.065
TNFR2 ^b (pg/mL)	2767.64 (1128.48)	2548.85 (1074.91)	0.782
D-dimer ^b (μg/mL)	858.66 (1839.83)	461.19 (194.57)	0.007
VCAM ^b (pg/mL)	842.69 (373.47)	828.44 (272.57)	0.098
sCD163 ^b (ng/mL)	896.67 (739.19)	889.39 (511.50)	0.371

^aHIV-infected and HIV-uninfected groups are compared by performing hypotheses tests: t-tests or chi-square tests for the variables, age, female, African American, current smoking, alcohol, family history of MI, and BMI as appropriate. For the rest of the variables/biomarkers (systolic blood pressure to Scd163) in this table we performed propensity score matching analyses for testing significant mean differences, and the corresponding p-values are reported.

^bSkewed continuous variables are transformed to zero-skewness distribution by applying Box-Cox transformation. Values are mean (SD) or N(%)

Table 3

Multipredictors regression analysis of inflammatory markers for HIV-infected group (regression coefficient (β), 90% Confidence Interval for β ; and p-values)

	Ornithine	Lysine	Arginine	Citrulline	sDMA	ADMA	GABR
IL6					1.6415 (0.432, 2.852; p=0.027)		
hsCRP			-0.131 (-0.022, -0.005; p=0.014)				
TNFR1		-0.150 ^c (-0.027, -0.003; p=0.045)	-0.228 ^c (-0.036, -0.009; p=0.007)		.3192 (0.208, 0.431; p=0.000)		-0.0862 (-0.124, -0.048; p=0.000)
TNFR2					.0152 (0.009, 0.022; p=0.000)	.0161 (0.010, 0.023; p=0.000)	-0.0041 (-0.007, -0.002; p=0.005)
Ddimer		-0.111 ^c (-0.016, -0.006; p=0.001)	-0.183 ^c (-0.023, -0.013; p=0.000)	-0.0080 ^c (-0.015, -0.002; p=0.044)	.1354 (0.085, 0.186; p=0.000)		-0.0354 (-0.055, -0.016; p=0.004)
VCAM	.0028 ^c (0.000, 0.005; p=0.064)				.0495 (0.026, 0.073; p=0.001)	.0373 (0.012, 0.063; p=0.017)	-0.0133 (-0.022, -0.005; p=0.010)
sCD163		-1.784 ^c (-0.282, -0.075; p=0.006)					

Adjusted for Age, Sex, Race, BMI, Smoking, Family History of Heart Attack, HDL, LDL, Viral Load, CD4abs, Nadirabs, PI Use, Abacavir, NRTI Durations, PI Durations, NNRTI Durations, HIV Durations, ARV Durations, INT Inhibitor Durations, Abacavir Durations and TDF Durations in step-wise regression analyses.

^c Re-scaled covariate was used in the regression (rescaled variable = variable/standard deviation).